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Buck Semen Does Not Easily Succumb to Oxidative Stress

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Abstract

Semen processing and manipulation generally result in loss of sperm motility and sperm velocity due in part to oxidative stress. In this study we investigated the vulnerability of South African indigenous unimproved buck semen to oxidative stress induced by an oxidative stress inducing agent, namely, hydrogen peroxide (H_2O_2). Semen ejaculates were collected from four superior South African indigenous unimproved bucks in a total of ten collections and then each duplicate was treated with different concentrations of H_2O_2 in presence or absence of Dithiothreitol (DTT). Sperm motility and velocities were determined using the computer aided sperm class analyser (CASA). SYBR-14 and propidium iodide (PI) Live/Dead assay kit was used to determine cell viability and Yo-Pro-1 plus PI Apoptosis kit was used to determine apoptosis. Statistical analysis was performed on the data using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL). South African indigenous unimproved buck raw semen motility was between 97% with 98% viability and 0% apoptotic cells. Comparisons of the untreated controls at 0 and 3 hrs incubations revealed that after 3 hrs there was overall a decrease in the number viable cells with the majority of remaining cells exhibiting circular movements accompanied by high progressive (PM) and rapid (RAP) motilities. In treated South African indigenous unimproved buck semen, H_2O_2 marginally increased total motility (TM) with few apoptotic sperm cells while retaining high viability. Also, H_2O_2 increased straight line distances travelled of more than 4 fold as compared to untreated controls with no circularly moving cells. Moreover, inclusion of DTT, an antioxidant, had minimal effects on TM, RAP, curvilinear velocity (VCL), straight line velocity (VSL), linearity (LIN)

and wobble (WOB) but positively affected PM, average path velocities (VAP), apoptosis and viability. Our Pearson's correlation data revealed that only straightness (STR) was highly positively affected by H_2O_2. Overall, the South African indigenous unimproved buck semen resisted deterioration in TM, RAP, VCL, VAP, VSL, LIN, WOB, viability and apoptosis under oxidative stress conditions. These data suggest that the South African indigenous unimproved buck semen does not easily succumb to oxidative stress.

Keywords

South African Indigenous Bucks, Oxidative Stress, Motility, Semen

1. Introduction

There is growing interest in the cryopreservation of the buck semen worldwide [1] [2]. Cryopreservation is the technique of freezing cells and tissues at very low temperatures at which the biological material remains genetically stable but metabolically inert. In South Africa, cryopreservation of gametes from indigenous breeds has been recognised as a national priority due to their superior traits that are suitable for the local conditions [3] [4]. In particular, the South African indigenous unimproved bucks are currently enjoying maximum attention as they have been previously ignored [5] [6].

Previously published data indicates that raw semen from this breed has TM above 83%, high PM, and high RAP accompanied by high viability [5]. Accompanying these features were high VAP, LIN, STR and WOB, characteristic of superior quality semen [5]. These experiments have also revealed that following cryopreservation, the South African indigenous unimproved buck semen showed decreased TM to less than 40% with low PM and RAP, accompanied by lower VAP, LIN, STR and WOB, indicating deterioration of semen quality [5].

Hydrogen peroxide (H_2O_2) is often used as an experimental source of oxygen-derived free radicals such as superoxide radical (O^{2-}.) and hydroxyl radical ($^{\cdot}OH$) which induces oxidative stress [7]. Oxidative stress has been implicated as the main culprit for the decreased semen motility and semen velocity parameters in males leading to infertility [8]. However, a cocktail of antioxidants can effectively improve semen parameters in infertile males [9]. Other studies have indicated that the addition of antioxidants to cryopreservation medium can improve post-thaw sperm quality [10], while other antioxidants, mainly thiols, like DTT can prevent H_2O_2 mediated loss of sperm motility [11].

Our previously published data indicated that in boars, the semen motility and semen velocity parameters were compromised under H_2O_2 induced oxidative stress in the presence or absence of an anti-oxidant like DTT [12]. Notably, TM, PM, RAP plus VAP, LIN, STR and WOB were all drastically decreased under oxidative stress, irrespective of the presence or absence of an antioxidants like DTT. The decreases in these parameters, accompanied by high rate of sperm cell

death via apoptosis and decreased viability, indicated that boar semen is highly susceptible to oxidative stress and DTT fail to rescue them [12]. We have also shown recently that other antioxidants like *α*-tocopherol, taurine and cysteine do not restore boar semen motility and velocity parameters following oxidative stress associated with liquid preservation [13]. Others have shown that post-thaw buck semen exhibited decreased DNA damage and lipid peroxidation accompanied by increased antioxidant enzyme activities with no changes in progressive motilities and sperm velocity parameters in the presence of antioxidants [14].

The South African unimproved buck semen has never been directly exposed to an oxidative stress inducing agent to test their susceptibility to oxidative stress. Hence, this study was conducted to assess whether the South African unimproved buck semen can easily succumb to oxidative stress induced by H_2O_2, in the presence or absence of an antioxidant like DTT.

2. Materials and Methods

The study was conducted at the Small Stock Research Unit of Agricultural Research Council, Germplasm Conservation & Reproductive Biotechnologies Unit, Irene, South Africa. The Agricultural Research Council-Irene campus is located at 25˚55'South; 28˚12'East. The institute is located in the Highveld region of South Africa and situated at an altitude of 1525 meters above sea level. Four superior South African indigenous bucks were stratified based on age (2 years) and weight (25 - 45 kg). The South African indigenous unimproved bucks were in good health condition throughout the duration of the study. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Animals under the guidelines of the Agricultural Research Council, Animal Production Institute Animal Ethics Committee (APIEC2011/38). Water was given *ad libitum* throughout the duration of the study.

2.1. Bucks Semen Collection and Processing

Semen was collected from four South African indigenous unimproved bucks using an electro-ejaculator, each with a total of ten collections. After collections, the semen samples were placed into the thermo-flask at 37˚C and transported to the laboratory. In the laboratory semen evaluations were performed within 1 h. Semen volume was measured by using the graduated falcon tube, pH was measured using the pH meter (Oaklon, EW35614-30, Cole-Parmer, USA), the sperm concentration was measured using the spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England), the semen motility rates were assessed using the CASA system (Sperm Class Analyzer® [SCA] 5.0, Microptic, Barcelona, Spain), semen viability and apoptosis were evaluated using the SYBR/PI live/dead kit (Invitrogen, Molecular probes, USA) and Yo-Pro-1/PI apoptosis kit (Invitrogen, Molecular probes, USA) respectively.

2.2. Treatments

For semen treatment, H_2O_2 stock solution was prepared in pre-warmed BO-Wash

medium and kept at 4˚C until use. During the experiment, semen at equal concentration and volume was treated with pre-warmed H_2O_2 stock to make 0, 5 µM, 50 µM and 200 µM concentrations in pre-warmed BO-Wash medium. The treated semen samples were then incubated at 37˚C for three hours in a humidified 5% CO_2 and 95% atmospheric air incubator (Sanyo, Japan). After three hours the samples were evaluated for total motility, progressive motility, rapid motility, semen velocities, sperm cell viability and sperm cell apoptosis.

2.3. Sperm Motility Rate

About 10 µl of raw semen were placed into 500 µl of BO-Wash medium in 15ml tube (Falcon® 352099, USA). The tube was then kept in CO_2 incubator (Sanyo, Japan) adjusted to 39˚C. Five micro litres of semen was placed on the warm glass slide (~76 × 26 × 1 mm, Germany) and placed with a warmed cover slip (22 × 22 mm, Germany) over the microscope-warm plate (Omron) adjusted at 39˚C. The sperm motility rates were evaluated by computer assisted sperm analysis system (Sperm Class Analyzer® [SCA] 5.0, CASA, Microptic, Barcelona, Spain) at the 10× magnification (Nikon, China). The semen TM, PM, RAP and semen velocity parameters were then determined.

2.4. Viability Assay

For cell viability, SYBR-14 and PI Live/Dead kit was used and the cells were treated according to the manufacturer's recommendation (Invitrogen, Molecular probes, USA). Briefly, 50 µl of semen was diluted with pre-warmed BO-Wash to 1 ml and 5 µl of a 50 times diluted SYBR-14 was added to the cells followed by incubation at 37˚C for 10 minutes. After 10 minutes, 5 µl of PI was added to the cells followed by incubation for another 10 minutes. After 10 minutes 5 µl of cells was immediately placed on pre-warmed glass slide and observed under a fluorescent microscope at 10× magnifications (Olympus, model BX51). For this analysis, viable cells appeared green in colour while non-viable cells appeared red in colour. The percent cell viability was determined by counting the number of green cells out of three hundred cells in a field, divided by three. A correlation was then established between viability and H_2O_2 treatment in the presence or absence of DTT.

2.5. Apoptosis Assay

To determine cell apoptosis, cells were treated with the Yo-Pro-1/PI staining kit solutions according to the manufacturer's recommendations (Invitrogen, Molecular probes, USA). In brief, cells were treated with 5 µl of Yo-Pro-1 and PI at the same time and incubated for 10 minutes. After incubation, 5 µl of the stained cells was placed on a slide and viewed under a fluorescent microscope at 60× magnification (Olympus, model BX51). Four populations of cells were obtained, the light green or clear (live cells), the dark green (apoptotic cells), and red (dead/necrotic cells) and the red plus green cells (dead cells). The percent cell

apoptotic cells were determined by counting the number of dark green cells out of three hundred cells in a field. A correlation was then established between the apoptosis and H_2O_2 treatment in the presence or absence of DTT.

2.6. Data Analysis

Pearson's correlation coefficients were calculated to test the relationship between the motility and velocity rates parameters, namely, TM, PM, RAP, VCL, VSL, VAP, LIN, STR, WOB, apoptosis and viability against the treatments. Data were examined using the Kolmogorov-Smirnov test to determine their distribution, a multivariate analysis of variance was performed (ANOVA) and when significant differences were found, and the non-parametric Mann-Whitney U-test was used to compare pairs of values directly if data did not adjust to a normal distribution. All analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL). Significance was set at $p < 0.05$.

3. Results

The analysis of the sperm motility and velocity parameters, by definition, gives an excellent insight into the sperm quality profile (Table 1). The raw semen obtained from the South African indigenous unimproved bucks showed an average sperm concentration of $0.876 \pm 321.55 \times 10^9$ cells/ml, with a pH of 7.5 ± 0.5, viability of 98 ± 1.5 and no apoptotic spermatozoa (Table 2). The analysed sperm quality profile of this raw semen revealed the percentage TM value of97.0 ± 3.95, PM of 29.3 ± 1.3 and RAP of 8.37 ± 8.01 accompanied by VCL of 70.3 ± 1.56, VSL of 30.83 ± 4.32, VAP of 44.0 ± 7.21 and LIN plus STR of 64.6 ± 5.32 and 67.73 ± 5.28, respectively (Table 3).

The analysis of sperm cell viability using the SYBR-14/PI revealed a clear distinction between the viable and non-viable cells, with viable cells fluorescing

Table 1. Definitions of the sperm motility and velocity parameters.

Parameter	Definition	Unit
Total Motility (TM)	Percent of sperm showing any movement	%
Progressive Motility (PM)	Percent of sperm moving rapidly and in a straight path	%
Rapid Motility (RAP)	Percent of sperm travelling at a speed of 25 μm/sec or faster	%
Curvilinear Velocity (VCL)	Time-average velocity of sperm head along its actual path	μm/s
Straight Line Velocity (VSL)	Time-average velocity of sperm head projected along straight line	μm/s
Average Path Velocity (VAP)	Time-average velocity of sperm head projected along its spatial trajectory	μm/s
Linearity (LIN)	Ratio of projected length to total length of curvilinear trajectory; LIN = VSL/VCL	%
Straightness (STR)	Ratio of projected length to average velocity of sperm head along a spatial trajectory, STR = VSL/VAP	%
Wobble (WOB)	Expression of the degree of oscillation of the curvilinear path about its spatial average path; WOB = VAP/VCL	%

Table 2. South African indigenous unimproved buck raw semen viability, apoptosis and macroscopic evaluations represented as mean ± SD.

Raw semen	Concentration (1×10^9 sperm cells/ml)	pH	Volume (ml)	Viability (%)	Apoptosis (%)
Buck	0.876 ± 321.55[a]	7.5 ± 0.5[a]	0.45 ± 0.20[a]	98.5 ± 1.5[a]	0.00[a]

[a]Values with different subscripts with the same column differ significantly ($p \leq 0.05$).

Table 3. The motility and velocity rates of the South African indigenous unimproved buck raw semen represented as mean ± SD.

TM%	PM%	RAP%	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)	LIN%	STR%	WOB%
97.0 ± 3.95[a]	29.3 ± 1.3[b]	8.37 ± 8.01[a]	70.3 ± 1.56[b]	30.83 ± 4.32[a]	44.0 ± 7.21[a]	64.6 ± 5.32[a]	67.73 ± 5.28	63.5 ± 0.5[b]

[a,b]Values with different subscripts with the same column differ significantly ($p \leq 0.05$).

green due to SYBR-14 absorption and non-viable cells absorbing SYBR-14 and PI and appearing as dominant red fluorescence (Figure 1). The class distribution of sperm cells according to average speed and distance travelled indicated untreated controls at 0 hrs and 3 hrs revealed a decrease in the number of motile cells as the viability decreased (Figure 2). At 0 hrs the RAP and PM motilities appears to be dominant however, after 3 hrs, NPM become dominant with more cells moving in circles or semi-circles (Figure 2) This class distribution of sperm cells according to average speed and distance travelled also revealed that while the H_2O_2 treatment decreased the overall number of viable cells, the progressive and rapid motilities remained high accompanied by the elimination of circular moving cells as compared to untreated controls (Figure 3). After 3 hrs of incubation, DTT, H_2O_2 and H_2O_2 plus DTT appeared to activate sperm cells to travel longer distances in excess of 100 μm as compared to untreated controls (Figure 3). Interestingly, all H_2O_2 concentrations used increased TM, but 50 μM H_2O_2 increased both TM and RAP in the presence of DTT (Figure 3, Table 4).

The untreated control semen had TM = 50.0 ± 33.1, PM = 43.6 ± 1.3 and RAP = 21.6 ± 5.1. Treatment with H_2O_2 revealed that 50 μM H_2O_2 decreased PM (29.95 ± 1.95) and RAP (23.7 ± 12) but increased TM (68.8 ± 5.75). 200 μM H_2O_2 also decreased RAP (12.8 ± 1.1) and PM (32.95 ± 11.05) with no changes in TM (56.85 ± 3.85) (Table 3). Also, in the presence of DTT, only 50 μM H_2O_2 increased PM (from 29.95 ± 1.95 to 42.8 ± 1.5) and RAP (from 11.7 ± 9.6 to 23.7 ± 12.0) while 200 μM H_2O_2 increased PM (from 32.95 ± 11.05 to 57.17 ± 15.92) and decreased TM (from 56.85 ± 3.85 to 37.4 ± 24.9) with no change in RAP (from 12.8 ± 1.1 to 12.6 ± 10.1) (Table 3). The TM Pearson's correlation coefficient with H_2O_2 concentration remained low in the presence (−0.0719) or absence (0.182) of DTT, while in the presence of DTT, PM Pearson's correlation improved from r = 0.104 to r = 0.752 and the RAP correlation changed from moderate but negative correlation (−0.557) to low positive correlation (0.0477) (Table 4).

Interestingly, H_2O_2 increased LIN (24.89 ± 2.86) significantly at 5 μM, 50 μM and 200 μM H_2O_2 to 67.25 ± 13.15, 65.05 ± 11.45, and 68.35 ± 9.15 respectively

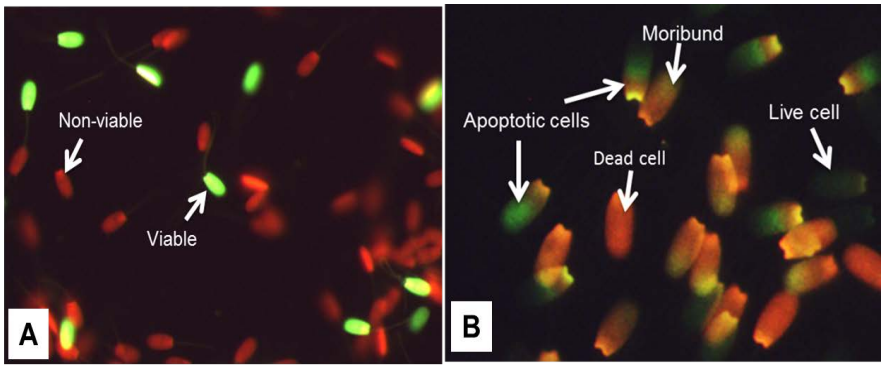

Figure 1. (A) The SYBR-14/PI cell viability staining of buck semen where red fluorescence represent non-viable sperm cells and green fluorescence represent viable sperm cells (10× magnification); (B) The Yo-Pro-1/PI apoptosis staining of buck semen showing apoptotic sperm cells in green fluorescence, moribund/dying sperm cells with dual fluorescence, dead sperm cells in red fluorescence while live cells had clear/light green fluorescence (60× magnification).

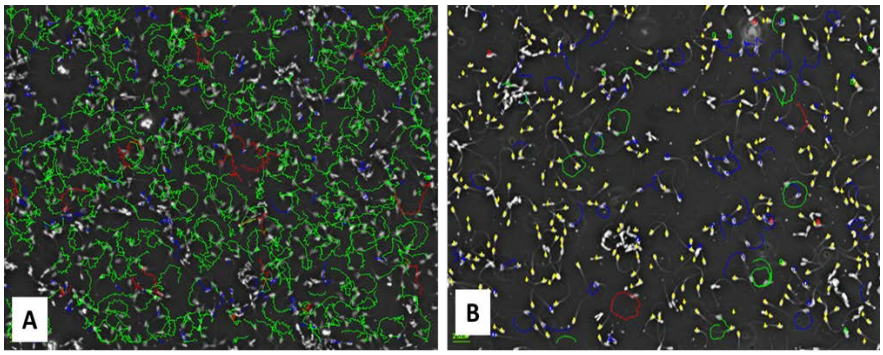

Figure 2. The class distribution of sperm cells according to their average speed showing the rapidly moving spermatozoa (RAP, red), Progressive motility (PM, green), Non-progressive motility (NPM, blue) and static (yellow) for untreated controls at (A) 0 hrs and (B) 3 hrs, bar = 25 µm.

Table 4. The sperm motility and velocity rates of treated South African indigenous unimproved buck semen represented as mean ± SD.

	%TM	%PM	%RAP	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	%LIN	%STR	%WOB
Control	50.0 ± 33.1^{a}	43.6 ± 1.3^{a}	21.6 ± 5.1^{a}	65.7 ± 29.5^{a}	37.7 ± 28.4^{a}	49.3 ± 32.5^{a}	24.8 ± 2.86^{a}	73.2 ± 8.87^{a}	68.43 ± 14.6^{a}
5 µM H_2O_2	54.8 ± 14.0^{a}	41.1 ± 0.9^{a}	16.15 ± 5^{a}	86.6 ± 17.7^{a}	60.5 ± 23.5^{a}	75.8 ± 20.5^{a}	67.2 ± 13.1^{b}	77.25 ± 9.75^{a}	86.3 ± 6.12^{a}
50 µM H_2O_2	68.8 ± 5.75^{a}	29.95 ± 1.9^{b}	11.7 ± 9.6^{a}	75.05 ± 4.2^{a}	49.3 ± 11.4^{a}	63.6 ± 17.8^{a}	65.0 ± 11.4^{b}	77.25 ± 8.55^{a}	83.55 ± 5.65^{a}
200 µM H_2O_2	56.85 ± 3.8^{a}	32.9 ± 11.0^{b}	12.8 ± 1.1^{b}	78.6 ± 9.3^{a}	52.7 ± 0.7^{a}	66.4 ± 4.65^{a}	68.3 ± 9.15^{b}	79.80 ± 6.70^{a}	85.05 ± 4.15^{a}
DTT	47.0 ± 20.3^{a}	41.15 ± 1.1^{a}	12.7 ± 8.9^{a}	82.4 ± 6.21^{a}	46.0 ± 21.4^{a}	66.8 ± 15.9^{a}	54.3 ± 21.9^{b}	65.2 ± 17.08^{a}	80.1 ± 13.97^{a}
5 µM H_2O_2 + DTT	31.9 ± 5.1^{a}	15.3 ± 1.3^{c}	8.4 ± 5.4^{a}	85.45 ± 5.1^{a}	55.2 ± 12^{a}	74.7 ± 8.53^{a}	64.1 ± 11.6^{b}	72.18 ± 8.6^{a}	87.2 ± 6.78^{a}
50 µM H_2O_2 + DTT	70.35 ± 9.9^{a}	42.8 ± 1.5^{a}	23.7 ± 12^{a}	70.2 ± 6.2^{a}	23.5 ± 8.7^{a}	51.0 ± 11.6^{a}	33.1 ± 11.9^{a}	44.53 ± 11.1^{a}	72.20 ± 11.6^{a}
200 µM H_2O_2 + DTT	37.4 ± 24.9^{a}	57.17 ± 15.9^{a}	12.6 ± 10^{a}	65.1 ± 15.4^{a}	71.8 ± 14.0^{a}	89.7 ± 3.85^{a}	71.8 ± 14.0^{b}	86.8 ± 3.92^{a}	89.75 ± 3.85^{a}

^{a,b,c}Values with different subscripts with the same column differ significantly $p \leq 0.05$).

while the other semen velocity parameters of treated semen were not significantly different from their corresponding untreated controls (Table 3). In the

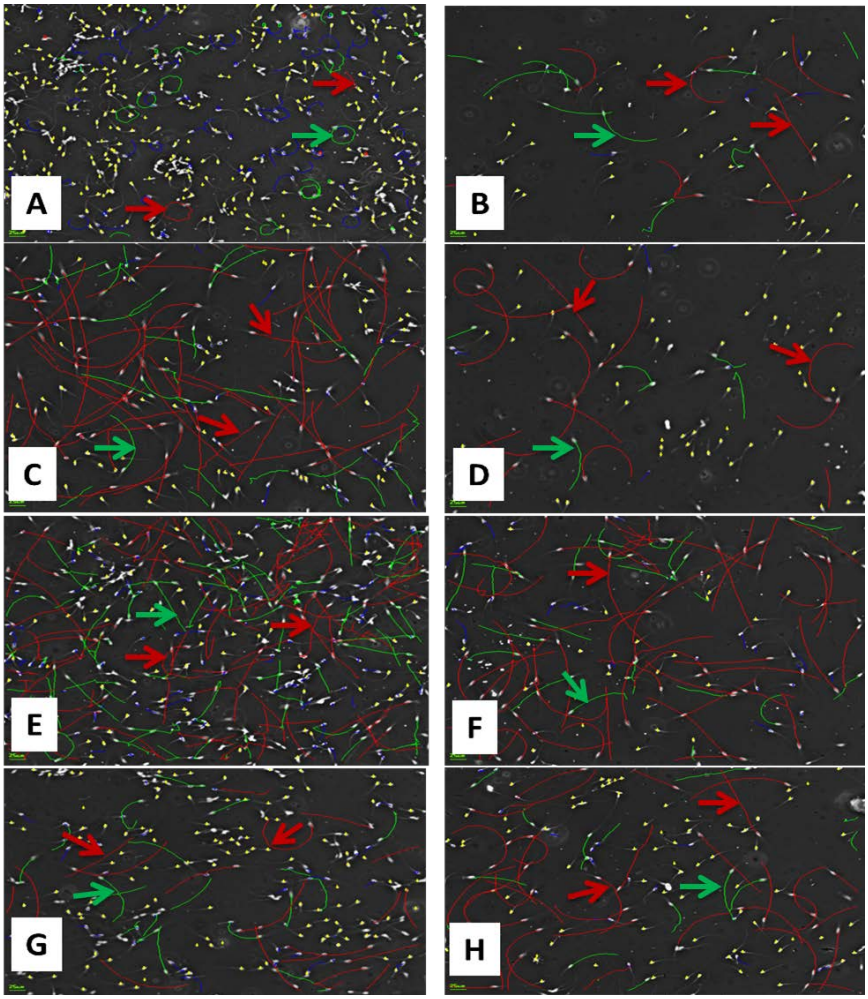

Figure 3. The class distribution of sperm cells according to their average speed showing the rapidly moving spermatozoa (RAP, red), Progressive motility (PM, green), Non-progressive motility (NPM, blue) and static (yellow). Sperm cells were treated for 3 hrs (A) Untreated controls; (B) 5 μM DTT; (C) 5 μM H_2O_2; (D) 5 μM H_2O_2 plus 5 μM DTT; (E) 50 μM H_2O_2; (F) 50 μM H_2O_2 plus 5 μM DTT, (G) 200 μM H_2O_2; (H) 200 μM H_2O_2 plus 5 μM DTT. DTT treatment alone show comparable effects to H_2O_2 treatments. The arrows indicate the typical length showing the distance travelled by RAP motile sperm cell (red arrow) and progressive motility (green arrow), bar = 25 μm.

presence of DTT, 50 μM H_2O_2 induced a decrease in VSL (from 49.3 ± 11.4 to 23.5 ± 8.71), VAP (from 63.63 ± 17.86 to 51.07 ± 11.6), LIN (from 65.05 ± 11.45 to 33.1 ± 11.98), STR (from 77.25 ± 8.55 to 44.53 ± 11.12) and WOB (from 83.55 ± 5.65 to 72.20 ± 11.6). The presence of DTT did not improve the Pearson's correlation coefficient of all the semen velocity parameters except for VCL, which indicated a high negative correlation in the presence of DTT (r = −0.829) and a low correlation in the absence of DTT (r = 0.165), and VAP which indicated a moderately high correlation in the presence of DTT (r = 0.644) and a low correlation in the absence of DTT (r = 0.184), plus STR which indicated a high moderate correlation in the presence of DTT (r = 0.791) and a low moderate correlation in the absence of DTT (r = 0.424). The correlation for the other semen

velocity parameters were VSL (r = 0.581 from r = 0.562), LIN (r = 0.446 from r = 0.489) and WOB (r = 0.455 from r = 0.235). In addition, H_2O_2 had a low correlation with buck semen apoptosis(r = 0.305) while the presence of DTT increased the buck semen apoptosis correlation (r = 0.9886) and also the buck semen viability correlation (from r = 0.128 to r = 0.7299) (**Table 4**).

4. Discussions

This study indicates that H_2O_2 induces oxidative damage to the South African indigenous unimproved buck semen which is accompanied by changes in the semen velocity parameters, semen motility parameters and semen viability after 3 hours of treatment. Interesting, after 3 hours of H_2O_2 treatment there was minimal changes in the pH which accompanied decreases in cell viability. H_2O_2 increased TM but the presence of DTT decreased it, except at 50 μM were TM was increased by 20%. In a similar study, Griveau *et al.* [15] demonstrated that 50 μM H_2O_2 accelerated the hyperactivation and acrosome reaction of semen after incubation for 3 hrs. In this study, at the highest H_2O_2 concentration used (200 μM), PM was increased by 4%. Amazingly, DTT further increases PM of buck semen by a further 16%. For RAP, H_2O_2 induced an increase of 4% above the raw semen RAP value. These observations indicated that buck semen was more robust in dealing with ROS effects on motility parameters, as opposed to boars under similar conditions [12]. This is in agreement with de Lamirande *et al.* [16] who concluded that, the deleterious effects of ROS on the semen motility parameters, depends on the species being investigated. Also, Sarangi *et al.* [17] reported that buck semen liquid preserved in tris-based extender in the presence of vitamin E and glutathione antioxidants revealed high PM, viability, acrosome integrity and antioxidant enzymes with low lipid peroxidation.

Also, 200 μM H_2O_2 increased VCL, VAP, VSL, LIN, STR and WOB in South African indigenous unimproved buck semen. In the presence of DTT, VAP, VSL, LIN, STR and WOB were even further increased but not VCL. Also, semen treated with 200 μM H_2O_2, DTT had no effect on VCL. The decrease in semen VCL is due to a reduction in both the angle of lateral head displacement (ALH) and the beat cross frequency (BCF). A decrease in ALH means the sperm head is moving less from side to side while a decrease in BCF means these actions occur slower. The VSL is increased by H_2O_2 and further increased by DTT. Since VSL is improved, this indicates that the buck semen could still be used for fertilization of an oocytesince increases in VSL is indicative of high semen fertilizing potential, as shown previously in rats [18] and hamsters [19]. VAP was increased even further by DTT in H_2O_2 induced semen. Also, H_2O_2 had a minimal increase in LIN that was further improved by about 10% by presence of DTT. STR and WOB were increased by 10% by H_2O_2 and further increased by 18% in the presence of DTT as compared to buck raw semen values. This data demonstrates that buck semen can resist and remain viable under moderate to high oxidative stress.

5. Conclusion

South African indigenous unimproved buck semen characterisation is currently been addressed and knowledge of their vulnerability to oxidative stress is necessary. Interestingly South African unimproved buck semen shows robust resistance to ROS effects, as shown by improved or unaffected motility and velocity parameters. Currently, in livestock, semen motility parameters are being used as a measure of their fertility potential. In South African unimproved buck semen, fertilizing potential might not be an issue since they show resistance to ROS, however the use of antioxidant should still be considered. While these data show buck semen resistance to ROS, more research is required to investigate how and why buck semen has resistance to ROS as compared to semen from boars.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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