Comparative Efficacy of Soybean Milk and OptiXcell Extenders for Cryopreservation of Goat Semen

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SUMMARY

Semen quality is of paramount importance for the success of any artificial insemination program. The objective of this study was to compare the efficiency of OptiXcell® and Soy-bean milk (SBME) extenders for cryopreservation of semen from Boer and Malya bucks. Semen collection was performed using artificial vagina technique. 144 fresh ejaculates were visually assessed for volume, color and foreign bodies. Eosin-Nigrosin and Giemsa stain techniques used for assessments of sperm morphology and acrosome integrity. CASA used to assess for sperm motions from both fresh and frozen semen samples. Fresh semen assessment revealed breed differences in sperm viability, membrane integrity, curvilinear velocity, beat cross frequency, straightness index, and linearity. Whereas, semen volume, pH, concentration, progressive motility, morphology, acrosomal integrity, velocity straight line, velocity average pathway, and Amplitude of lateral head did not vary between bucks and breeds. Analysis revealed decrease in sperm kinetics after cryopreservation. Cryopreservation using both OptiXcell and SBME resulted into decline in sperm kinetic parameters. However, semen samples extended with OptiXcell had higher post-thawing values for progressive motility, normalcy, viability, velocity average pathway, velocity straight line, curvilinear velocity, amplitude of lateral head, beat cross frequency, straightness and linearity than semen diluted using SBME-Glycerol and SBME-DMSO. No significant difference ($P > 0.05$) was noted while comparing the effects of cryopreservation between buck breeds on sperm parameters while using OptiXcell and SBME. Although a remarkable decrease in sperm variables was noted after cryopreservation with the two extenders and cryoprotectants, OptiXcell showed superiority in cryopreserving buck semen compared with SBME.

Keywords: Goats, Malya and Boer breeds, semen cryopreservation, sperm kinetics.

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INTRODUCTION

Goats (*Capra hircus*) were the first herbivores to be domesticated in agricultural-based societies, fulfilling a vital role in economic, cultural, and religious in human civilizations (Zeder, 2006). In Tanzania, the population of goats is estimated at 24.8 million majority of which are the Small East African (SEA) breed distributed in most of the agro-ecological zones, expressly kept for the livelihood of many resource-poor people in rural areas (Nguluma *et al.,* 2022). Although goats are kept for multipurpose uses including red meat, milk and socio-cultural functions, their productivity is constrained by low genetic potential, which is partly associated with inbreeding and shortage of

quality breeding stock (Kichamu *et al*., 2024). Assisted reproductive biotechnologies such as artificial insemination (AI) and semen cryopreservation are afore-mentioned methods for improving the performance of goats in poor communities (Rege *et al*., 2011). Despite the major advantages of spreading elite genetic material, elimination of the need for importation of good live bucks on the farm and reduction of inbreeding risk, goat AI technology is not common in Tanzania (Kifaro *et al*., 2007). For successful AI, semen cryopreservation is mandatory allowing for long-term storage of insemination doses and securing for insemination at a desired time (Purdy, 2006; Oldenhof *et al*., 2021). However, the cryopreservation process is associated with reduced sperm post-thaw motility and membrane integrity mainly due to cold shock and osmotic stresses (Nisfimawardah *et al*., 2023). Successful buck semen cryopreservation technologies require in-depth knowledge of semen extenders, cryoprotectants and cryopreservation protocol (Sariözkan *et al*., 2010).

Currently, extenders commonly used for cryopreservation of goat semen are egg yolk-based and plant phospholipid-based extenders (Saratsi *et al*., 2024). The low density of lipoproteins present in egg yolk protects the spermatozoa from cold shock and cryodamage while preserving spermatozoa motility and mitochondrial function (Sieme *et al*., 2016). However, egg yolk phospholipids react with bulbourethral gland secretion present in goat seminal plasma to form egg yolk coagulating enzyme (EYCE) which has cytotoxic effects to sperm cells (Ngoma *et al*., 2016). Furthermore, egg yolk extenders are implicated in increased risk of microbial contamination, which is linked to reduced fertilization and increased risk of disease transmission while transporting stored semen (Bustani and Baiee, 2021). Nevertheless, new compounds have been formulated as an alternative to replace the components of animal origin in semen extenders, including plant phospholipids such as soybean lecithin (Vidal *et al*., 2013; Sorongbe *et al*., 2019). Soy lecithin, which contains phosphatidylcholine and several fatty acids such as stearic, oleic, and palmitic, etc., has been extensively researched as a substitute for cryoprotectants of animal origin (Bustani and Baiee, 2021). Researchers have reported that the use of Soybean extenders improve post thawing sperm motility, viability, acrosome integrity and sperm membrane structure in human (Reed *et al*., 2009), ram (Forouzanfar *et al*., 2010; Zhao *et al*., 2021), cat (Vick *et al*., 2012), dog (Kmenta *et al*., 2011), goat (Sorongbe *et al*., 2019) and bovine (El–Keraby *et al.*, 2010). Alternatively, OptiXcell®, a liposome-based semen extender, is commercially available and has expressed a better cryoprotective ability compared to soya-lecithin in bovine (Kumar *et al*., 2015), and it has been recommended for use in the extension of semen from some breeds of goats (Vidal *et al*., 2013).

Goat semen cryopreservation technologies require in-depth knowledge of the properties of different extenders and cryoprotectants. At present, a variety of membrane-permeable cryoprotectants (glycerol, dimethyl sulfoxide, ethylene glycol, and propylene glycol) and their combinations have been tested to suit for the cryopreservation of buck sperm (Sharafi *et al*., 2022; Silva *et al*., 2022). Although goat sperm is sensibly cryotolerant, significant variations in cryotolerance have been reported among breeds and between individual bucks within the breeds (Saratsi *et al*., 2024). Limited information on cryopreservation and cryotolerance of Boer and Malya buck semen is available. This warrants the search for new formulations of sperm cryoprotectants which have different chemical natures and lower molecular weights and viscosity but not causing cellular damage during cryopreservation and thawing processes. Furthermore, most AI stations have adopted computer-assisted semen analysis (CASA) for increased objectivity in the determination of semen quality (Anand and Yadav, 2016; Nisfimawardah *et al*., 2023). CASA helps to characterize the patterns of motion of the goat sperm cryopreserved and detect subtle differences between cryopreservatives and cryoprotectants. Therefore, the current study aimed to compare the effects of two semen extenders (Soybean milk and OptiXcell®) and two cryoprotectants (Glycerol and dimethyl sulfoxide: DMSO) in cryopreservability of Boer and Malya semen based on post-thaw sperm viability and kinetic characteristics evaluated objectively by CASA.

METHODOLOGY

Ethical Clearance

The study was conducted after obtaining ethical clearance (REF NO. TLRI/CC.21/009) from the Tanzania Livestock Research Institute (TALIRI). Furthermore, the research was conducted in accordance with the laws and regulations governing experiments on live animals in Tanzania, with the primary aim of minimizing unnecessary stress during the experiments.

Study Area and Animals Management

Six healthy mature bucks (3 Malya and 3 Boer breeds) maintained at TALIRI West Kilimanjaro station were used for this study. TALIRI West Kilimanjaro is situated at a latitude of 3°S and longitude of 39°E, with an elevation of approximately 1270 meters above sea level. It is located in a warm (11.7°C to 27.8°C) semi-arid tropical condition with an annual average precipitation ranging from 450 to 700 mm. The research bucks were reared under a semi-intensive management system consisting of daily grazing for about 7 hours. After returning from pasture feeding, each buck was confined in individual pen with an average size of 2.0 to 2.5 m^2, supplemented with 350gm of home-made concentrates/animal/day (comprising of crude protein content: 120g/kg DM and energy content: 10.4 MJ ME/kg DM), and given overnight feeding of about 5.0 kg of green grass (Napier grasses). Throughout the study, clean fresh water was provided *ad libitum* to each buck. In addition, bucks were regularly inspected for health, received anti-helminthic drugs, sprayed to control ectoparasites, and vaccinated against major endemic diseases such as Contagious Caprine Pneumonia (CCPP), Peste des petits ruminants (PPR), and Foot and Mouth disease. A brucellosis test was performed to all animals before the commencement of this experiment.

Extenders and cryoprotectants used in the experiment

Two extenders (Soybean milk and OptiXcell®) and two cryoprotectants (Glycerol and dimethyl sulfoxide; DMSO) were used in the experiment. The soybean extender (SBME) was prepared as previously outlined by El–Keraby *et al.* (2010). Briefly, 10g of soybean grains were measured, washed, and then soaked in 100ml of distilled water before being boiled at 40 ^{o}C for 30 min. After boiling, the soybean grains were separated, washed and immersed to cool in 50ml distilled water containing 0.25% $NaHCO_3$. The soybean grains were separated, blended into a fine powder, and the slurry kept cool. Soybean milk was prepared through a filtration process involving a series of sieving using clean cotton cloth and centrifugation, boiling for 10 minutes, and cooling at room temperature. Two antibiotics: 500 IU Penicillin and 500 µg Streptomycin were added to each milliliter of soybean slurry before ready for use. The OptiXcell® Extenders were commercially available (IMV Technologies, L'Aigle, France) and prepared for use as per the manufacturer's instructions. The main ingredients in the OptiXcell® were carbohydrates, antioxidants, buffers, phospholipids, glycerol, mineral salts, water, gentamicin, Lincomycin, Spectomycin, and Tylosin. The two semen cryoprotectants (Glycerol and DMSO) used in this study were commercially obtained and used at 10% levels during soybean milk-based semen cryopreservation.

Semen Collection and Evaluation

A total of one hundred and forty-four (144) fresh semen samples (24 collections from each of six buck) were collected at weekly interval (once on Tuesday between 8.00 and 10.00 hours) using artificial vagina (IMV, France), and estrous does as teasers for the bucks to mount. The collection of semen was carried out for six months from November 2023 to May 2024. Immediately after semen collection, the ejaculates were macroscopically evaluated for volume, color, presence of foreign bodies, and thereafter placed in a warm water-filled thermo-flask maintained at 37°C until further evaluation within 1 hour of collection (Gacitua and Arav, 2005). Semen pH was assessed with the aid of Neutralit® pH indicator strips (Merck KGaA). The percentage of live spermatozoa and sperm morphology was determined using the Eosin-Nigrosin staining technique (Agarwal *et al.,* 2016). The percentage of sperm acrosome integrity was determined using a Giemsa stain procedure and viewing under the oil-immersion microscopic view. The intactness of

spermatozoa membrane was assessed using the Hypo-osmotic Swelling Test (HOST) as previously described by Zubair *et al.* (2015).

Spermatozoa concentration and kinetic parameters were evaluated using Computer Assisted Sperm Analysis (CASA) system (SCA Microptic SL, Barcelona, Spain) with the following basic components: bright field phase-contrast (negative phase contrast) microscope - Olympus BX 51 microscope (Olympus, Japan), digital camera to capture image (Minitüb, Tiefenbach, Germany), and a computer with SCA® software (SCA Microptic, SL, Barcelona, Spain). The analyses were conducted following the instrument's standardization and validation procedure as described by Palacín et al. (2013). The semen variables analyzed included concentration (million sperm/ml); percentage of total and progressively motile sperms (% all sperms moving with average path velocity >60 mm/s and straightness >90%, respectively); average path velocity – the length of a derived ''average'' path of sperm head movement per unit time (VAP; µm/s), curvilinear velocity - total distance travelled by the sperm head per unit time (VCL; µm/s), straight line velocity - net distance gain of the sperm head per unit time (VSL; µm/s), amplitude of lateral head displacement - width of the head movement envelope (ALH; µm], beat cross frequency - number of times the curvilinear path crosses the average path per unit time; approximation to the flagellar beat frequency for seminal spermatozoa (BCF; Hz), straightness index - ratio of straight line velocity/average path velocity (STR; %), and linearity - ratio of VSL/VCL (LIN; %).

Semen dilution, cryopreservation and post-thawing sperm evaluation

Good ejaculates were processed for cryopreservation using two different extenders and two cryoprotectants. Briefly, fresh semen from each buck was divided into three proportions; one semen aliquot was mixed with an equal volume of Soybean milk extender and glycerol; the second vial of semen sample was mixed with Soybean milk extender and DMSO; the third semen vial was mixed with an equal volume of OptiXcell®. The final semen concentration for each mixture was adjusted to 1.0 x 10^6 sperm/mL. Extended semen was first packed in 0.25 ml straws using an automatic filling, sealing, and printing machine. The straws were kept in a cooled handing cabinet and underwent through steps of the freezing using a programmable freezing unit (IMV, Technologies-Digitcool L'Aigle, France) to reach -140°C within 10 to 15 minutes and finally plugged into liquid nitrogen (LN) tank for storage at -196°C until further evaluated. During semen post-cryopreservation evaluation, frozen straws were picked from the -196°C LN2 container, wiped with a paper towel, and immediately placed in a thawing water bath maintained at 37°C for 30 seconds. Thawed semen was analyzed for sperm viability using the Eosin-Nigrosin staining technique and kinetic characteristics using the CASA system.

Statistical analysis

Semen analytical data were first stored in a spreadsheet (MS Excel) and then exported to Statistical Package for Social Science (SPSS) version 10.0 (SPSS Institute, Chicago, IL, USA) for analysis. Analysis of variance (ANOVA) was performed to compare quantitative sperm characteristics (sperm viability, acrosomal integrity, membrane intactness and kinetics). The quantitative data are presented as the means ± SDs. The variations in parameters were considered significant at the $P<0.05$ level. Correlations were measured with Pearson's correlation coefficient.

RESULTS

The quality examinations of fresh semen from Boer and Malya bucks were carried out to determine the suitability of the semen for further cryopreservation. The results of fresh semen examinations are presented in Table 1. Statistical analysis revealed significant differences ($p < 0.5$) between the two breeds in six parameters, namely sperm viability, membrane integrity, curvilinear velocity, beat cross frequency, straightness index, and linearity. Whereas, the other nine parameters (volume, pH, concentration, progressive motility, morphology, acrosomal integrity, Velocity straight line, Velocity average pathway, and Amplitude of lateral head), although varied between bucks, had no statistical differences ($p < 0.05$) between bucks and breeds.

Table 1: Fresh semen characteristics (mean ± standard deviation) of Malya and Boer bucks

	Buck breed		
Parameters	Malya (Mean ± S.E)	Boer (Mean ± S.E)	P-Values
Ejaculate Volume (ml)	1.84±0.15	1.67±0.10	0.642
pH	6.99±0.58	7.04±0.62	0.364
Concentration (billion sperm/mL)	1.59±0.92	1.67±0.19	0.732
Progressive sperm motility (%)	92.67±1.91	89.22±0.60	0.161
Normal Sperm Morphology (%)	92.54±0.47	88.42±1.94	0.061
Live Sperm Viability (%)	93.42±0.52[a]	88.42±1.94[b]	0.002
Acrosomal Integrity	87.98±2.93	87.36±1.05	0.849
Membrane Integrity	89.87±0.79[a]	83.19±1.09[b]	0.008
Velocity straight line (µm/s)	54.59±2.49	58.47±0.78	0.213
Velocity average pathway (µm/s)	71.21±2.46	70.53±2.15	0.079
Curvilinear velocity (µm/s)	91.49±1.42[a]	83.66±2.79[b]	0.046
Amplitude of lateral head (µm)	3.42±0.02	3.41±0.31	0.972
Beat cross frequency (Hz)	24.99±0.64[a]	37.61±11.46[b]	0.033
Straightness index (%)	76.63±1.78[a]	82.98±1.59[b]	0.045
Linearity (%)	59.76±3.54[a]	69.98±1.55[b]	0.045

[a,b] Values within rows with different superscripts are different; $p < 0.05$.

The cryopreservation process greatly affected sperm parameters, as most sperm quality parameters assessed in fresh semen were higher than those evaluated post-thaw in both extenders. However, the effects of cryopreservation were not equal for both extenders. The percentages of post-thawing in most sperm variables were significantly ($P<0.05$) higher when using OptiXcell® extenders than when using SMBE plus Glycerol and SBME with DMSO cryoprotectant (Tables 2, 3 and 4). Specifically, midpiece abnormalities, loose heads and coiled tails were slightly, though not significantly increased post–thawing. Nevertheless, there was no significant difference ($P>0.05$) while comparing the use of SBME with either Glycerol or DMSO cryoprotectants. In particular, the semen samples with OptiXcell® extender showed higher values of quality parameters (progressive motility, normalcy and viability) and sperm kinetics (Velocity average pathway, Velocity straight line, Curvilinear velocity, Amplitude of lateral head, Beat cross frequency, Straightness and Linearity) after freezing and thawing than spermatozoa extended with Soy milk in both breeds (Figure 1 and 2). With the exception of two kinetic parameters (Straightness and Linearity), which showed significant differences ($P < 0.05$), other parameters were not significantly different ($P > 0.05$) between OptiXcell® and Soy-milk extended semen. Interestingly, no significant difference ($P > 0.05$) was noted while comparing the effects of cryopreservation between buck breeds (Malya versus Boer) on sperm parameters while using OptiXcell® and SBME extenders.

Table 2: The effects of cryopreservation of Semen parameters (Mean ± S.E) using OptiXcell® extender

Parameter	Malya bucks		Boer bucks	
	Pre-freezing	Post-freezing	Pre-freezing	Post-freezing
Progressive sperm motility (%)	92.67±1.91	80.54 ± 2.31	89.22±0.60	78.77 ± 4.20
Sperm normalcy (%)	92.54±0.47	87.03 ± 2.86	88.42±1.94	79.89 ± 1.23
Live Sperm (%)	93.42±0.52	78.15 ± 3.65	88.42±1.94	76.02 ± 7.21
Velocity average pathway (VAP)	71.21±2.46	69.01 ± 3.42	70.53±2.15	67.07 ± 4.18
Velocity straight line (VSL)	54.59±2.49	50.40 ± 2.61	58.47±0.78	51.14 ± 7.04
Curvilinear velocity (VCL)	91.49±1.42	84.88 ± 3.93	83.66±2.79	78.16 ± 6.63
Amplitude of lateral head (ALH)	3.42±0.02	3.11 ± 0.07	3.41±0.31	2.84 ± 0.21
Beat cross frequency (BCF)	24.99±0.64	22.71 ± 0.45	37.61±11.46	33.64 ± 0.83
Straightness (STR)	76.63±1.78	68.70 ± 1.34	82.98±1.59	71.96 ± 6.47
Linearity (LIN)	59.76±3.54	52.20 ± 1.58	69.98±1.55	51.34 ± 5.50

Table 3: The effects of cryopreservation of Semen parameters (Mean ± S.E) using a combination of SBME and Glycerol cryoprotectant.

Parameter	Malya bucks		Boer bucks	
	Pre-freezing	Post-freezing	Pre-freezing	Post-freezing
Progressive sperm motility (%)	92.67±1.91	72.01 ± 1.18	89.22±0.60	74.82 ± 1.49
Sperm normalcy (%)	92.54±0.47	81.79 ± 1.49	88.42±1.94	75.13 ± 1.65
Live Sperm (%)	93.42±0.52	75.38 ± 1.23	88.42±1.94	75.67 ± 1.27
Velocity average pathway (VAP)	71.21±2.46	63.99 ± 1.50	70.53±2.15	64.68 ± 1.56
Velocity straight line (VSL)	54.59±2.49	48.30 ± 1.45	58.47±0.78	48.99 ± 1.56
Curvilinear velocity (VCL)	91.49±1.42	88.60 ± 1.87	83.66±2.79	75.09 ± 2.00
Amplitude of lateral head (ALH)	3.42±0.02	2.52 ± 0.063	3.41±0.31	2.12 ± 0.072
Beat cross frequency (BCF)	24.99±0.64	19.30 ± 0.13	37.61±11.46	27.27 ± 0.54
Straightness (STR)	76.63±1.78	66.40 ± 1.45	82.98±1.59	68.66 ± 1.55
Linearity (LIN)	59.76±3.54	48.07 ± 0.92	69.98±1.55	49.04 ± 1.01

Table 4: The effects of cryopreservation of Semen parameters (Mean ± S.E) using a combination of SBME and DMSO cryoprotectants.

Parameter	Malya bucks		Boer bucks	
	Pre-freezing	Post-freezing	Pre-freezing	Post-freezing
Progressive sperm motility (%)	92.67±1.91	71.68 ± 1.26	89.22±0.60	71.68 ± 1.59
Sperm normalcy (%)	92.54±0.47	80.47 ± 1.59	88.42±1.94	73.91 ± 1.76
Live Sperm (%)	93.42±0.52	73.49 ± 1.31	88.42±1.94	68.80 ± 1.36
Velocity average pathway (VAP)	71.21±2.46	59.41 ± 1.61	70.53±2.15	63.09 ± 1.67
Velocity straight line (VSL)	54.59±2.49	40.93 ± 1.54	58.47±0.78	43.80 ± 1.67
Curvilinear velocity (VCL)	91.49±1.42	74.02 ± 1.99	83.66±2.79	69.07 ± 2.14
Amplitude of lateral head (ALH)	3.42±0.02	2.36 ± 0.067	3.41±0.31	2.27 ± 0.076
Beat cross frequency (BCF)	24.99±0.64	21.92 ± 0.48	37.61±11.46	24.52 ± 0.58
Straightness (STR)	76.63±1.78	61.71 ± 1.54	82.98±1.59	62.99 ± 1.65
Linearity (LIN)	59.76±3.54	44.66 ± 0.98	69.98±1.55	44.03 ± 1.08

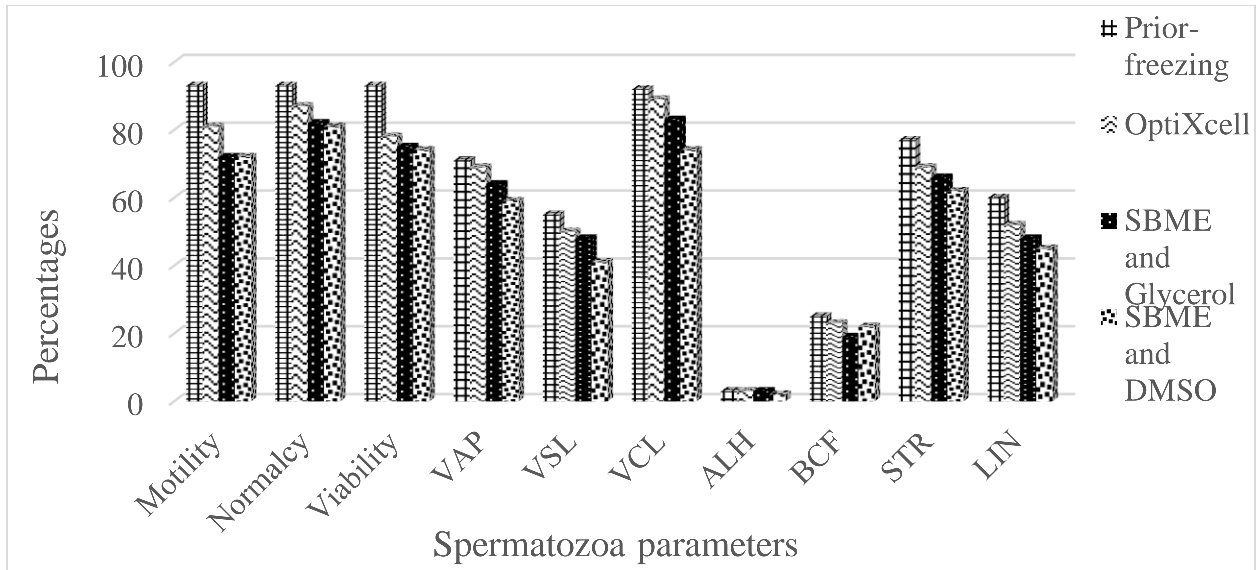

Figure 1: The effects of OptiXcell extender on Malya buck semen parameters.

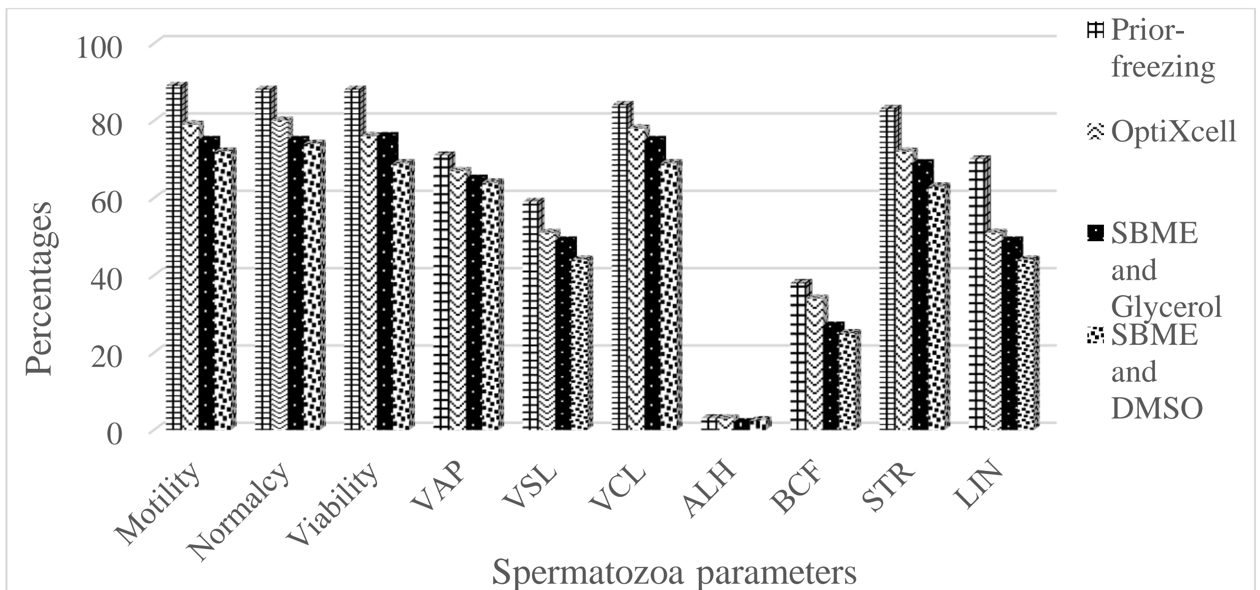

Figure 2: The effects of OptiXcell® on Boer buck semen parameters

DISCUSSION

Semen parameters such as sperm plasma membrane integrity, kinetics and acrosomal membrane integrity are the key indicators of male fertility (Utt, 2016). The present study investigated the effects of cryopreservation on semen quality collected from Malya and Boer buck breeds. The values of fresh semen parameters were evaluated, including ejaculate volume, sperm concentration, and kinetics varied with the breed of the bucks. Similar variations have been reported for ejaculate volume as it has been found to vary between breeds, age, method of collection, and between the seasons of the year (Silvestre *et al.,* 2004). The average ejaculate volumes of 1.84 ml and 1.67 ml from Malya and Boer bucks, respectively, obtained in this study are almost similar to the semen volume of 1.7 ml collected from bucks of non-descript genotypes in South Africa (Monau *et al.,* 2018). However, the ejaculated are higher than the volumes of ejaculates collected from East African

goat bucks (Gororo *et al.,* 2019) Toggernburg and Saanen bucks kept under tropical conditions (Gore *et al.,* 2020), but lower than that obtained from Saanen bucks (Nisfimawardah *et al.,* 2023) and Boer buck kept in South Africa (Monau *et al.,* 2018).

Sperm motility and kinetics are good indicators for post-coital sperm quality and sperm fertilizing capability (Robayo *et al.,* 2008). The average ALH values of fresh semen from both bucks recorded in the present study were slightly lower than the value reported in bulls (Kathiravan *et al.,* 2011) but higher than ejaculates collected from Murciano-Granadina bucks (Mocé *et al.,* 2022). The average BCF values from both Boer and Malya bucks were almost similar to those reported from Florida bucks (Dorado *et al.,* 2010), showing the strong frequency of spermatozoa movement along their path in the female reproductive system. The average values for Straightness (79.81±2.93%) reported in our study are almost similar to other researchers (Nisfimawardah *et al*., 2023). Linearity findings are higher than reported in adult Murciano-Granadina male goats (Sadeghi *et al.,* 2020). The differences in sperm kinetics could be resulting from storage conditions and extension media used before CASA examination.

Although cryopreservation is the most important procedures for success in assisted reproductive biotechnology, the process has a significant structural and molecular alterations in mammalian spermatozoa (Yanez-Ortiz *et al.,* 2022). Cryopreservation strongly alters the acrosomal integrity, obliterates spermatozoa function, and changes in the membrane fluidity, phospholipid and protein patterns, all of which are associated with the decrease in spermatozoa enzymatic activity, motility, viability, and capacity to fertilize the ovum (Shadan *et al.,* 2004). In this study, the process of cryopreservation affected the buck sperm characteristics through a reduction in the percentages of sperm motility, viability, and kinetic variables. Decrease in sperm motility after cryopreservation has also been reported elsewhere (Dorado *et al.,* 2010; Guthrie *et al.,* 2014) as it is articulated that the cooling process suppresses spermatozoa's metabolic activity, leading to a significant decrease in energy consumption and thus reduction in post-thawing motility activities. We also report post-thawing decreases in the percentage of sperm viability and slight increase in morphology anomalies, similar observations have been reported by other researchers (Bintara *et al.,* 2021; Khalil *et al.,* 2018). Post-freezing decrease in sperm viability is related to intracellular changes following cryopreservation, leading to the formation of intracellular ice-crystals which cause destruction of sperm membrane, increased permeability and finally death of the spermatozoa (Khan *et al.,* 2021).

Sperm kinetics/movements are important components in the fertilizing capacity of spermatozoa (İnanç *et al.,* 2018). Both VSL, VAP and VCL measure the sperm velocity over specific paths, whereby high values indicate the speed of sperm within the female reproductive system (King *et al*., 2020). In the present study, sperm kinetics were of higher values in pre-freezing, although they showed a slight reduction in motility post-thawing. Decreased sperm kinetics post-freezing has also been recorded in dairy goats (Sun *et al.,* 2023) and dairy cattle (Khalil *et al.,* 2018). However, when comparing the buck breeds, semen collected from Malya bucks performed better in all kinetic parameters than Boer semen. The observed differences between breeds have also been reported in dairy bulls (Khalil *et al*., 2018) and dairy bucks (Sun *et al.,* 2023), and the variations may be linked with genetic and environmental tolerance of the particular breed.

Goat semen cryopreservation is a distinctly different process as compared to semen from other livestock such as cattle or buffalo (Sharma and Sood, 2020). In goats, mostly used sperm cryopreservation medium includes egg yolk-based and plant phospholipid-based extenders (Saratsi *et al*., 2024) with the addition of cryoprotectants such as glycerol, ethylene glycol, or dimethyl sulfoxide (Sharma and Sood, 2020) and antibiotics such as penicillin and streptomycin (Vickram *et al*., 2017). Recently, OptiXcell®, a liposome-based semen extender, which has shown an excellent cryoprotection in bovine semen (Kumar *et al*., 2015), has been recommended for use in goat semen of some breeds (Vidal *et al*., 2013). In this study, OptiXcell® extender showed superiority against the soybean-based extenders for

cryopreservation of buck semen. Our results are in agreement with the previous studies showing that OptiXcel®l is better than the egg yolk-based extenders in cryoprotection of spermatozoa from white tailed deer (Stewart *et al*., 2016), boar (Zhang *et al*., 2009), stallion (Mariana *et al*., 2024) and ram (Kasimanickam *et al*., 2011). OptiXcell®, a liposome-based extender, has been reported to have better cryoprotective ability compared to soya-lecithin extenders for buffalo semen (Kumar et al., 2015), and better than egg yolk-based extenders in post-thawing quality of bull spermatozoa (Salman etal., 2017). Similarly, OptiXcell® extender has been reported to be superior to tris-citric egg yolk and egg yolk citrate extenders in cryopreservation of bull semen and providing higher fertility rates following AI under field conditions (Ansari *et al*., 2017).

CONCLUSION

In conclusion, OptiXcell® and Soybean milk are potential extenders for cryopreservation of goat semen as they can maintain the quality of frozen spermatozoa for over 24 hours. However, OptiXcell® is superior compared to the extenders prepared from the mixture of Soybean milk and DMSO as well as a combination of Soybean milk and Glycerol in protecting the spermatozoa viability and kinetics of buck semen during cryopreservation. Nevertheless, future studies should be carried out to investigate the in vivo fertility rate of the liposome-containing commercially available OptiXcell® extender in the field.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any conflict of interest.

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