American Journal of Animal and Veterinary Sciences

Research Article

Optimizing Boar Epididymal Sperm Preservation: Comparative Analysis of Slicing Float-Up vs. Flushing and BTS vs. Andromed® Extenders on Sperm Motility and Morphometry Traits

Mamonene Angelinah Thema[1, 2], Ntuthuko Raphael Mkhize[2], Gerhard van der Horst[3], Liana Maree[3], Maleke Dimpho Sebopela[2], Mahlatsana Ramaesela Ledwaba[1] and Masindi Lottus Mphaphathi[1,4]

[1]Germplasm Conservation and Reproductive Biotechnologies, Agricultural Research Council, Animal Production, Private Bag X2, Irene, 0062, South Africa
[2]School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, 3209, Pietermaritzburg, South Africa
[3]Department of Medical Bioscience, University of the Western Cape, Private Bag X17, Bellville, 7530, South Africa
[4]Department of Agriculture and Animal Health, University of South Africa, Private Bag X6, Florida, 1710, South Africa

Article history
Received: 06-03-2025
Revised: 17-04-2025
Accepted: 01-08-2025

Corresponding Author:
Masindi Lottus Mphaphathi
Germplasm Conservation and Reproductive Biotechnologies, Agricultural Research Council, Animal Production, Private Bag X2, Irene, 0062, South Africa
Email: masindim@arc.agric.za

Abstract: Epididymal sperm collection represents a valuable strategy for preserving genetics from deceased or castrated boars of superior breeding value. The two primary collection methods slicing float-up (high sperm yield) and flushing (low blood contamination risk) differ in sperm recovery efficiency, while semen extenders provide cryoprotective nutrients that protect against cold shock during preservation. This study compared the effects of slicing float-up versus flushing collection methods and Beltsville Thawing Solution (BTS) versus Andromed® extenders on motility and morphometric characteristics of boar epididymal sperm. Epididymides were collected from 40 testes obtained at a local slaughterhouse and processed within 30 minutes post-slaughter at 5°C. Sperm retrieved from the cauda epididymis were diluted with BTS or Andromed® and analyzed using computer-assisted sperm analysis (CASA) for motility assessment and morphometric evaluation. The flushing-Andromed® combination produced the lowest total (29.7%), progressive (4.9%), and rapid (1.24%) motility values. Conversely, slicing float-up with BTS yielded the highest total (85.0%), progressive (57.7%), and rapid (31.8%) motility. Andromed® treatment induced decreased sperm head diameter and acrosomal morphology alterations, consistent with premature acrosome reaction, thereby reducing sperm head dimensions. Sperm head morphometric parameters remained within acceptable reference ranges across all treatment groups (length: 8.2-8.9 µm; perimeter: 18.2-19.9 µm; width: 4.1-4.6 µm; area: 34.8-38.6 µm²). These findings recommend slicing float-up with BTS extender as the optimal protocol for boar epididymal sperm collection and short-term preservation, with implications for swine genetic resource conservation.

Keywords: Boar Epididymal Sperm, Slicing Float-Up, Semen Extender, Sperm Motility, Sperm Morphometry, Acrosome Reaction, Semen Preservation, Swine Reproduction

Introduction

Semen is commonly collected through ejaculation for breeding and preservation. However, that cannot be possible in every species or in every circumstance, such as in cases of unforeseen mortality or subsequent to castration (Strand et al., 2016). Animals may die suddenly, far away from the laboratory where the semen sample could be handled and

preserved (Turri et al., 2012). As a result, semen collection from the epididymis may be one technique accessible for conserving male gametes from animals with superior traits (Bertol, 2016). Therefore, determining the most effective retrieval method could help obtain the epididymal semen of boars possessing superior traits. Several semen retrieval methods have been reported, and they differ based on the authors, species, and semen extenders.

Semen can be retrieved from the post-mortem distal epididymis in a few different ways, including flushing, slicing float-up, or mincing (Stout, 2012). Flushing method is commonly characterized by low blood contamination risk (Podico and Canisso, 2022) and may be challenging in obtaining a high sperm yield for procedures such as artificial insemination (Auer et al., 2022). The flushing method commonly works best, and it is used to retrieve bull epididymal semen (Monaco et al., 2024). In some cases, the cuts method might be more practical, but to improve sample condition, caution must be taken to ensure a quick extension of the sample and to prevent contamination (Bertol, 2016). Epididymal slicing float-up (Ali Hassan et al., 2023) and mincing (Podico and Canisso, 2022) have been proposed as alternatives when the flushing procedure proves impractical. Conversely, slicing float-up presents high sperm yield and may result in high blood contamination if not done accordingly (Ellerbrock et al., 2017). The slicing float-up is commonly used to retrieve epididymal semen in the equidae species (Huijsmans et al., 2023). The mincing method involves cutting both the cauda epididymis and the ductus deferens several times in a petri dish to retrieve semen (Hori et al., 2015). Semen samples retrieved by mincing often characterised by blood and cell debris, potentially causing negative effects on the quality and cryo-tolerance of the sperm (Monaco et al., 2024).

Dilution of semen extracted from the epididymides may provide the sperm with additional protection during the cooling process or preservation (Fernandez-Novo et al., 2021). Semen extenders are biological substances that fulfil numerous tasks, including reducing the impact of sudden pH fluctuations, preventing sperm against oxidative stress and damage during preservation (Bustani and Baiee, 2021). Boar semen extenders, like Beltsville Thawing Solution (BTS), has been widely used in boar semen because they contain a high concentration of antibiotics (penicillin and streptomycin) (2.20 g/L) that is capable of treating a wide range of infections, lessen, and regulate bacterial contamination upon preservation (Thema et al., 2022). The BTS extender provide the sperm with protection against oxidative damage, maintains quality and minimise the bacterial contamination during preservation (Schulze et al., 2017). The study by Basumatar et al. (2024) reported, BTS extender to had negative effect on DNA integrity of the boar sperm. Andromed® is a soya lecithin-based extender typically used as a bull semen diluent and has yielded an increased percentage of sperm exhibiting higher superoxide levels. Andromed® extender provide additional energy and nutrients that may fortify sperm against cold shock during preservation (Pérez-Durand et al., 2024). The Andromed® is currently identified as a non-permeable cryoprotectant and a substitute for egg yolk in the freezing of cattle sperm (Akhter et al., 2012). The existence of BTS and Andromed® extenders with such characteristics could significantly enhance the swine artificial insemination sector and improve protocol for cryopreserving boar sperm.

Sperm morphometric evaluation offers comprehensive understanding into the dimensions, shape, function, and structural features of sperm, which are associated with fertility capability (Yáñez et al., 2015). The advent of advanced systems and instruments, such as computer-assisted sperm analysis (CASA), has enabled more rapid and objective assessment of sperm morphometry and motility (Amann and Waberski, 2014). The CASA system has become the favoured approach owing to its decreased subjectivity, enhanced repeatability, and greater reliability (Yániz et al., 2015). Male sperm morphometry, motility, and fertility have all been successfully correlated using the CASA systems (Maroto-Morales et al., 2015). The CASA system makes accurate sperm measurements and automatically identifies the acrosome, head, and midpiece of the sperm (Mortimer et al., 2015). According to Dziekońska et al. (2022), sperm obtained from the epididymis cauda exhibit distinct sperm motility, morphometry, membrane integrity, and DNA integrity. Sperm motility is a crucial characteristic associated with the capacity of sperm to achieve fertilization, signifying their structural integrity and viability (Nagy et al., 2015).

While slicing float-up and flushing method have been studied in stallions, bulls, and dogs, only few studies have compared these two semen retrieval method on boar epididymal sperm quality utilizing CASA system. Although the BTS and Andromed®extender have been widely evaluated in boar ejaculated semen during liquid preservation respectively, limited studies have been done on boar epididymal sperm. To our knowledge this is the first study to compare the sperm motility and morphometry of boar epididymal semen retrieved with slicing float-up vs flushing and diluted with BTS vs Andromed® extenders. Therefore, understanding the morphometric and motility traits of boar epididymal sperm is essential for assessing their fertility potential and optimizing reproductive outcomes.

This study aimed to compare slicing float-up vs. flushing and BTS vs. Andromed® extenders on boar epididymal sperm motility and morphometry traits features to predict reproductive success.

Materials and Methods

Study Area and Duration

The data (testes) was collected from the local abattoir (Renbro Abattoir), and the study was conducted at the

Germplasm Conservation and Reproductive Biotechnologies section (Agricultural Research Council), located in Pretoria, Gauteng Province. The data and study area are located at 25º 53′ 59.6″ South latitude and 28º 12′ 51.6″ longitude in Pretoria, South Africa. The entire collection took a period of 2 months. Four testes each day from diverse boars were obtained biweekly from the local slaughterhouse. The collection was replicated 10 times/treatments (4x10 = 40).

Study Design and Sampling

Based on the statistical advice regarding the number of treatments (2 semen retrieval methods x 2 extenders) and availability of the testes at the abattoir, a total of 40 testicles from 20 heterogeneous boars were used in the current study. The sample size was further supported by the few studies recorded on the comparison of retrieval methods on the epididymis sperm quality in various species. The sample size corresponds with the research conducted by Mogheiseh et al. (2022) which worked on the impact of epididymal sperm retrieval techniques on the properties of fresh and frozen-thawed sperm in twenty adult big mixed-breed male dogs. Further corresponds with the research by Neuhauser et al. (2018), who worked on the impact of four distinct commercial semen extenders on epididymal sperm motility of twenty testes (10 healthy stallions).

Composition and Preparation of Boar Semen Extenders

The semen extenders [BTS (IMV Technologies, France) and Andromed® (Minitube, Germany)] were prepared as per manufactures' instructions and stored at 5 °C. Chemical composition of the BTS and Andromed® extenders is shown in Table 1.

Table 1: Chemical composition of the semen extenders per litre

Beltsville Thawing Solution	Andromed®
Glucose37g/L	Fructose 1.25 g/L
Tri-sodium citrate 6.0 g/L	Citric acid 1.34 g/L
EDTA 1.25g/L	Buffer 1 g/L
Sodium bicarbonate 1.25g/L	Phospholipids 1 g/L
Spectinomycin 0.75 g/L	Spectinomycin 0.343 g/L
Gentamycin -	Gentamycin 0.286 g/L
	Lincomycin 0.172 g/L
Penicillin 1.10 g/L	Tylosin 0.57 g/L
Potassium chloride 1.10 g/L	-

EDTA: Ethylenediamine Tetra Acetic

Boar Testes Collection and Semen Retrieval from the Epididymis

A total of 40 testicles from diverse boars were collected (4 testes per day) from the local abattoir and brought to the laboratory at 5 °C within 30 minutes post-slaughter. The testicles were thereafter preserved at 5 °C for a further 2 hours prior to processing. Subsequent to storage, the cauda epididymis was excised from the testes and rinsed with warm saline solution (NaCl 0.9% at 37 °C). To prevent blood contamination, superficial blood arteries were perforated to facilitate the removal of the majority of the blood (Turri et al., 2012). Prior to semen extraction, a total of 20 cauda epididymides were evenly distributed for flushing versus slicing and BTS versus Andromed® extender in distinct petri plates warmed at 37 °C. The semen was extracted from the cauda region of the epididymis with either the flushing or slicing float-up technique and subsequently diluted with either BTS or Andromed® slicing float-up and evaluated for sperm motility and morphometry. The flushing method involves inserting a sharpened needle through the ductus deferens and pushing the extender in the opposite direction of the physiological sperm through the cauda epididymis (Esteves and Agarwal, 2011). The slicing float-up procedure entails employing a scalpel blade to incise the caudal part of the epididymis to release the sperm in a petri dish, suspend it with 5 mL of a warm extender (37 °C), and gently agitate the mixture for approximately 10 minutes (Turri et al., 2012; Falomo et al., 2016).

Evaluations of the Boar Epididymal Sperm Motility

A total of 5 µL of retrieved semen with the flusing vs. slicing float-up diluted with BTS vs. Andromed® applied on a preheated microscope glass slide (76x26x1 mm-WadmarKnittel, Germany) and covered with a warm coverslip (22x22 mm-Wadmar-Knittel, Germany) on a CASA (Microptin, Spain) phase contrast microscope (Nikon®, Japan), warm plate (Omron®, Japan) adjusted to 37 °C. Sperm motility traits [Total Motility (TM), Progressive Motility (PM), Rapid (RAP), Medium (MED), static] and velocity traits [Curvilinear Velocity (VCL), Straight Line Velocity (VSL), Average Pathway Velocity (VAP), Linearity (LIN), Straightness (STR), Wobble (WOB), the Amplitude of Lateral Head (ALH) and beat cross frequency (BCF)] were analyzed under 10X magnification (Mphaphathi, 2017). Two fields with an averaging of 250 sperm and a final sperm concentration of approximately 34.3×10^6/mL/treatment/replication were observed at 10X magnification (Nikon®, Japan) during analysis using the CASA system.

Boar Epididymal Sperm Staining

Following semen retrieval with the use of flushing and slicing float-up method, samples were transferred to 15 mL tubes as per different semen extenders namely BTS and Andromed®. For sperm morphometry analysis, semen samples were diluted at a ratio of 1:10 with BTS or Andromed® extenders. Diluted semen samples were further fixed with 4% Formaldehyde for 10 minutes. Furthermore, a total of 10 µL of each fixed semen sample

per retrieval method and extender was smeared on a distinct slide and left to air dry overnight at ambient temperature. The dried samples on the slide were delicately immersed in SpermBlue® for staining for 2 minutes and submerged in pure water for 3 seconds to eliminate particles and guarantee a pristine background. A paper towel was utilised to clean the reverse side of the slide, which was then allowed to air dry at ambient temperature. Subsequent to drying, the slide was affixed utilising Dibutylphthalate Polystyrene Xylene., covered with the coverslips and evaluated for sperm morphometry traits.

Boar Epididymal Sperm Morphometry Analysis

Dried slides were evaluated for sperm morphometry using CASA software (Nikon, Tokyo, Japan). This software involved capturing the sperm from a stained sperm smear using brightfield microscopy, under 40X magnification, and various morphometric traits relating to the sperm head, acrosome, and midpiece. At least a total of 1600 sperm were analyzed per semen retrieval method and extender treatments. Overall, a total of 3200 sperm were measured/replicated 10 times (80 x 10 = 800 sperm per retrieval technique/extender). The sperm head area (A), head perimeter (P), head length (L), head width (W), head ellipticity (length/width), head elongation (L-W/L+W), head regularity (πx (L.W/4.A), head roughness [4 π x (A/P^2)], midpiece width and the shape indices (head shape type, type of the sperm and midpiece size) were measured.

Statistical Analysis

Sperm morphometric data were categorised into two groups. The initial group comprised the measurements of basic characteristics of sperm heads (length, L; area, A; width, W, and perimeter, P), derived traits (ellipticity, L/W; rugosity, 4 πA/P2; elongation, L−W/L+W and regularity, πLW/4A). Descriptive statistics were utilized in the initial sperm morphometric data group to determine the minimum and maximum values (threshold) for each morphometric feature using the 10-90 percentiles.

The data relates to the initial sperm morphometric group and sperm motility characteristics (TM, PM, RAP, MED, static, VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF) were analyzed using factorial analysis of variance (2 sperm retrieval techniques x 2 semen extenders). The main effect of independent variables (semen retrieval methods and extenders) and their interaction were measured based on the sperm morphometric and motility changes (dependant variables). Student's t-LSDs (Least significant differences) were calculated at a 5% significance level to compare means of significant source effect of semen extenders and semen retrieval on the sperm morphometric first group and motility traits.

The subsequent set of sperm morphometric data was analysed using a Chi-square test to assess the effect of flushing vs. slicing and BTS vs. Andromed® on the differences in sperm proportion concerning normal head, acrosome, midpiece, and abnormal head morphology (amorphous, pyriform, tapering). The Anova and Chi-Square analysis was done using statistical analysis system (SAS) version 9.4 statistical software.

Table 2: Minimum and maximum values for morphometric parameters in order to define normal boar epididymal sperm morphometry

	Boar Minimum	**Maximum**
Length (µm)	8.20	9.70
Width (µm)	3.50	5.50
Perimeter (µm)	16.20	26.20
Area (µm)	28.49	44.00
Ellipticity	1.81	2.16
Regularity	0.70	1.00
Elongation	0.29	0.37
Roughness	0.62	1.50
Mid piece width (µm)	0.50	1.60

Results

The effect of semen retrieval methods and semen extender on boar epididymal sperm motility is presented in Table 4. Semen diluted with Andromed® extender had the lowest total (47.4; 29.7%), progressive (13.8; 4.9%), and rapid (3.9; 1.2%) motility, regardless of the retrieval technique employed. Nonetheless, semen diluted with BTS extender exhibited a higher sperm count (85.0%), progressive motility (57.7%), and rapid motility (31.8%) when obtained by the slicing float-up technique.

Figure 1 represent the effect of the semen retrieval method and extenders on the type of sperm percentage using CASA. The semen retrieved with the slicing float-up method and diluted with BTS extender exhibited over 70% normal sperm. An average of 31.9% abnormal sperm was recorded in the semen retrieved with flushing method irrespective of the specific semen extender utilized. The normal sperm percentage in the current study was affected by several factors such as the shape of the sperm head, the type of sperm acrosome and the midpiece (Figures 2-4).

Figure 2 represents the effect of the semen retrieval method and extenders on the type of sperm head shape using CASA. Greater than 70% of sperm were recorded as normal shape and matured irrespective of the semen retrieval method. The range between 10-22.5% sperm with pyriform shape was recorded irrespective of the semen extender and retrieval method. Less than 6.0% of sperm with tapered and amorphous shapes were recorded irrespective of the semen retrieval method or extender.

Moreover, an average of 0.9% of sperm with a round shape was recorded irrespective of semen retrieval method and extenders.

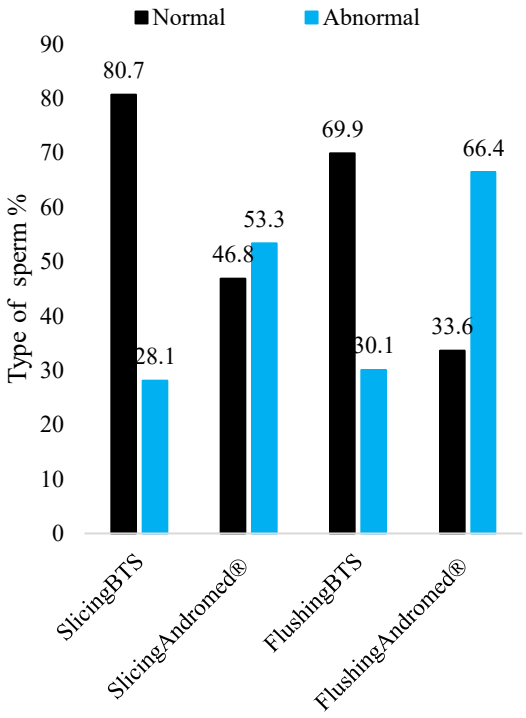

Fig. 1: The effect of semen retrieval method and extenders on the type of sperm percentage using CASA. BTS: Beltsville Thawing Solution

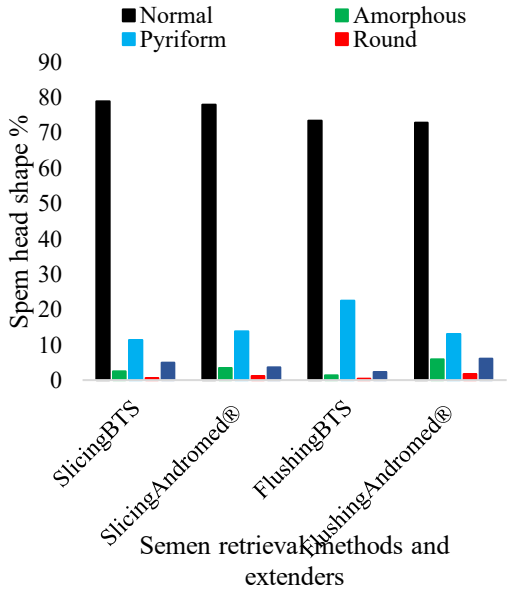

Fig. 2: The effect of semen retrieval method and extenders on the type of sperm head shape using CASA. BTS: Beltsville Thawing Solution

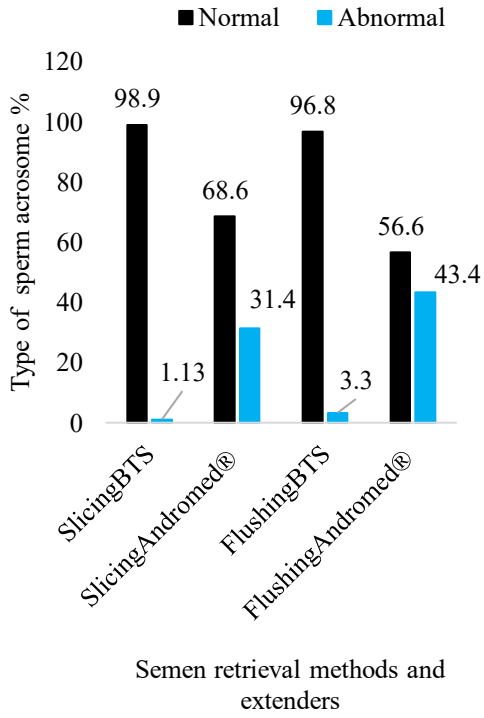

Fig. 3: The effect of semen retrieval method and extenders on the type of sperm acrosome using CASA

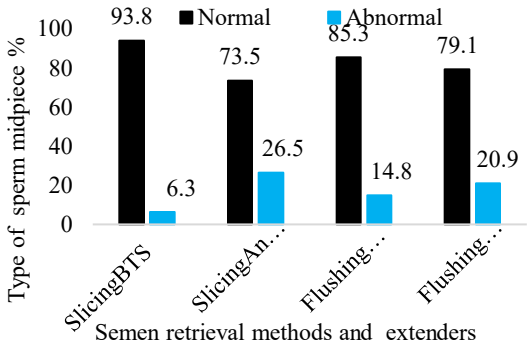

Fig. 4: Effect of boar epididymal semen retrieval method and extenders on the sperm midpiece size using CASA

The effect of the semen retrieval method and extenders on the type of sperm acrosome using CASA is represented in Figure 3. A decrease in sperm head size and a modification in acrosome morphology was linked to the type of semen extender used. The sperm diluted with Andromed® extender showed an acrosome response, resulting in a reduced sperm head length. The sperm diluted with Andromed® extender observed lower than 70 % of sperm showing a normal acrosome,

irrespective of the semen retrieval technique. Over 90 % of sperm showed a normal acrosome in the semen diluted with BTS irrespective of the semen retrieval method.

Figure 4 represents the effect of the boar epididymal semen retrieval method and extenders on the sperm midpiece size using CASA. Less than 30% of sperm had aberrant midpiece size, regardless of the semen retrieval method and extenders. Semen diluted with Andromed® extender showed more than 20% sperm with abnormal midpiece size irrespective of the semen retrieval method. Greater than 80% of sperm with normal midpiece size were recorded in the semen diluted with BTS irrespective of the semen retrieval method. The more sperm undergo acrosome reaction, the higher the chances of midpiece size being affected. Additionally, a large occurrence of cytoplasmic droplet distribution was apparent in the sperm underwent acrosome reaction and immature sperm, which causes abnormal midpiece size.

Table 3 represents the effect of boar epididymal semen retrieval methods and extenders on sperm morphometry using CASA. Overall, the semen diluted with BTS and Andromed®, showed an average sperm head size within the acceptable parameters (length: 8.2-8.9 µm; perimeter: 18.2-19.9 µm; width: 4.1–4.6 µm and area: 34.8-38.6 µm2), midpiece and shape indices that were within the acceptable range, irrespective of the semen retrieval method.

Table 3: Effect of boar epididymal semen retrieval methods and extenders on sperm morphometry using CASA (mean ± SD)

Semen retrieval methods	Slicing float-up		Flushing	
Semen extenders	BTS	Andromed®	BTS	Andromed®
Sperm head trait				
Length (µm)	8.8±1.2^{a}	8.6±1.7^{b}	8.9±1.9^{a}	8.2±2.9^{c}
Width (µm)	4.5±0.7^{b}	4.6±0.9^{c}	4.4±0.9^{a}	4.1±1.4^{d}
Area (µm^2)	37.3±6.9^{a}	38.6±7.5^{b}	36.9±10.0^{a}	34.8±13.6^{c}
Perimeter (µm)	19.9±2.8^{a}	19.8±3.8^{b}	19.3±4.1^{a}	18.2±6.4^{c}
Sperm midpiece trait				
Width (µm)	1.1±0.4^{c}	1.2±0.5^{a}	1.3±0.6^{b}	1.2±0.7^{b}
Sperm shape indices				
Ellipticity	2.0±0.6^{a}	1.9±1.1^{b}	2.0±0.8^{a}	1.9±0.8^{b}
Elongation	0.3±0.1^{a}	0.3±0.1^{b}	0.3±0.1^{a}	0.3±0.1^{c}
Regularity	0.8±0.1^{a}	0.8±0.1^{b}	0.8±0.2^{a}	0.8±0.3^{c}
Roughness	1.5±1.7^{a}	1.4±4.7^{a}	1.2±0.3^{a}	1.5±6.3^{a}

$^{a-d}$ Values with different superscripts in a row differ significantly (P<0.05). BTS: Beltsville thawing Solution

Table 4: Effect of boar epididymal semen retrieval methods and extenders on sperm motility using CASA (mean ± SD)

Sperm motility trait	Slicing float-up		Flushing	
	BTS	Andromed®	BTS	Andromed®
TM (%)	85.0±10.3^{a}	47.4±5.6^{c}	60.8±5.4^{b}	29.7±9.7^{d}
PM (%)	57.7±19.2^{a}	13.8±8.4^{bc}	22.1±9.2^{b}	4.9±3.8^{c}
NPM (%)	27.3±10.4^{bc}	33.6±7.7^{ab}	38.7±6.6^{a}	24.7±7.7^{c}
RAP (%)	31.8±16.8^{a}	3.9±5.5^{b}	9.3±6.3^{b}	1.2±2.2^{b}
MED (%)	29.5±7.7^{a}	13.9±5.4^{c}	21.8±4.9^{b}	7.8±5.4^{d}
SLW (%)	20.4±8.7^{b}	29.6±9.1^{a}	29.8±7.2^{a}	20.6±5.2^{b}
Static (%)	14.9±10.3^{d}	52.6±5.6^{b}	39.2±5.4^{c}	70.3±9.7^{a}
VAP (µm/sec)	21.1±4.5^{a}	13.2±5.5^{b}	15.1±4.6^{b}	11.2±3.8^{b}
VCL (µm/sec)	42.1±9.3^{a}	25.1±6.6^{bc}	29.9±5.6^{b}	23.3±4.6^{c}
VSL (µm/sec)	12.6±3.6^{a}	7.7±3.7^{b}	7.6±3.1^{b}	4.1±1.6^{c}
STR (%)	59.0±7.6^{a}	54.4±11.5^{a}	50.7±8.6^{a}	40.9±9.8^{b}
LIN (µm/sec)	31.6±7.5^{a}	29.1±6.9^{a}	26.3±8.9^{a}	17.4±6.8^{b}
WOB (%)	51.6±5.6^{a}	51.2±11.8^{a}	51.3±9.9^{a}	46.9±10.2^{a}
BCF (Hz)	6.5±1.8^{a}	3.2±1.8^{b}	3.6±1.1^{b}	2.7±1.8^{b}
ALH (µm/sec)	1.5±0.2^{a}	0.9±0.1^{c}	1.1±0.1^{b}	0.9±0.2^{c}

$^{a-d}$ Values with different superscripts differ significantly in the same row (P<0.05). TM: Total motility, PM: Progressive motility, RAP: Rapid motility, MED: Medium motility, SLW: Slow motility, VCL: Curvilinear velocity, VSL: Straight line velocity, VAP: Average pathway velocity, LIN: Linearity, STR: Straightness, Sec: Second, WOB: Wobble, ALH: Amplitude of lateral head and beat cross frequency (BCF). BTS: Beltsville Thawing Solution

Discussion

A comparison of two semen extenders in the current study showed an alteration in sperm motility and morphometry traits, and an effect between the two semen retrieval methods. The current study recorded sperm motility to be higher when semen was retrieved with the slicing float-up technique and diluted with a BTS extender. Similar findings were recorded in our earliest study (Thema et al., 2022), which reported BTS to perform better than Kobidil^{+} extender on the Windsnyer boar sperm motility and morphology, due to the presence of potassium chloride in its chemical composition that is crucial for preserving sperm quality characteristics. The present of potassium chloride in the BTS extender assist in regulating the semen intra cellular pH level, calcium level and stimulate the sperm motility while supporting the acrosome reaction (Vílchez et al., 2017). The pH is one of the important requirements for extenders, due to its ability to limit bacterial development while maintaining the sperm quality (Ho et al., 2023). Moreover, the research by Ho et al. (2023), suggested pH values range between 6.80-7.87to be suitable for long- and short-term extenders, during liquid preservation. Furthermore, the current results align with the research conducted by Kumar et al. (2023, which found BTS extender to have maintained higher sperm motility rates in the Large White Yorkshire ejaculated semen compared to the other contemporary extender. The presence of citrate and ethylenediamine tetraacetic acid in BTS extender played a significant role in providing energy to sperm, which sustains a higher percentage of motile sperm (Kumar et al., 2023). Slicing float-up BTS treatments did not induce major changes in the proportions of motile sperm.

The current study differs with the research by Turri et al. (2012), which establish that the Limousine bull sperm quality was more affected by the slicing float-up than flushing technique. The reason for the dissimilarity in their findings was that, utilizing the slicing float-up method could expose the sperm to blood that may occur during cutting. However, the blood contamination can, however, be reduced by quickly combining the sperm with a medium or extender (Canissoa et al., 2021). The use of BTS in the current study was valuable to minimize the risk of blood contamination and improve motile sperm. The BTS extender contains 2.20 g/L amounts of antibiotic capable of treating various conditions and minimising bacterial contamination in the retrieved semen sample (Thema et al., 2022). Moreover, to prevent blood contamination, superficial blood vessels were first pierced with a needle, and their contents were subsequently cleansed and cleaned off before semen retrieval.

The present results demonstrate that the extender and retrieval methods have a clear influence on the quality of sperm. The lowest values of sperm motility were recorded when semen was retrieved with the flushing method and diluted with Andromed® followed by the BTS extender. Furthermore, the current study differs from the study by Podico and Canisso (2022) on the equidae, which reported a greater yield of the total motile epididymal sperm retrieved using the flushing method and recorded lower yield with the slicing float-up method. Additionally, it was discovered that when using the flushing method, semen samples may be washed off by semen extenders and other fluids, which may limit the ability to protecting the survivability of the sperm may be limited due to the few cuts performed. However, according to Lamglait (2014), flushing has been demonstrated to be a better method in most species due to its lower risk of contamination. Whereas, according to Nichi et al. (2017), flushing is the recommended method for retrieving bull semen.

In terms of sperm morphometry, a higher proportion of sperm showing normal acrosomes, midpieces, size, and morphology was observed in the semen diluted with BTS extender irrespective of the retrieval method. The current findings are consistent with the research conducted by Kumar et al. (2023) which recorded BTS extender to have protected the sperm against acrosomal impairment. According to Govindasamy et al., (2016), the presence of tri-sodium citrate in the chemical composition of BTS extender assists in contributing a higher proportion of sperm-intact acrosomes. Overall, the semen diluted with BTS and Andromed® showed an average of satisfactory sperm head morphology, midpiece and shape indices which fell within the normal range, irrespective of the semen retrieval method. However, the current research found a decrease in sperm head size and alterations in the form of the sperm head and acrosome linked with Andromed®. Furthermore, the sperm head shrank, and the acrosome's shape changed in relation to the Andromed® extender. The sperm diluted with the Andromed® irrespective of the semen retrieval method showed sperm to have undergone a premature acrosome reaction. The acrosome reaction observed in the Andromed® extender treatments could explain the decreasing percentage of motile, normal head, acrosome, and midpiece of the sperm recorded in the current study.

One of the key elements that influenced the retrieved sperm quality in the present research was the composition of the semen extenders used. According to Hernández-Corredor et al. (2016), Andromed® extender is thought to be protein-free and contains a high phospholipid content derived from soybean lecithin. Whereas it is known that the lack of seminal plasma in the epididymal semen necessitates the use of a semen extender with protein content. As a result, Andromed® extender was discovered unsuitable for use with the boar's epididymal semen in the current study. The current study differs from the reports from other studies on bull (Abdussamad et al., 2016) and

bucks (Quintero-Elisea et al., 2023) semen which, recorded the Andromed® extender to exert a higher protective influence on the motility and viability of ejaculated sperm. Additionally, a study by Ansari et al., (2017) reported Andromed® to be capable of protecting the buffalo bull post-thawed sperm and further reported that it might be properly utilized for regular substitution of other extenders. Semen retrieval methods utilized in the current research had no influence on the sperm morphometry traits. Therefore, the current study fails to reject the null hypothesis when it comes to the sperm retrieval methods' effect on sperm morphometry traits.

Conclusion

In conclusion, the slicing float-up samples had more desirable quality sperm possibly attributable to higher yield than flushing method. Furthermore, the addition of BTS has demonstrated a substantial positive impact on protecting epididymal sperm retrieved in slicing float-up method. Overall, the semen diluted with BTS and Andromed® showed average acceptable sperm head, midpiece and shape indices which fell within the normal range, irrespective of the semen retrieval method. The current findings indicate that the application of BTS extender may positively influence sperm during the stage of recovery using slicing float-up, before cooling and during freezing process. Therefore, it is recommended that slicing float-up method should be used to recover boar epididymal sperm at post-mortem using BTS extender. Future studies should implement the use of BTS extender on boar epididymal sperm during liquid preservation, transportation, holding and cryopreservation. Additionally, the standard protocol should be developed for post-mortem sperm recovery, preservation, and fertilization to support the commercial use and conservation of boar epididymal sperm.

Acknowledgment

The authors wish to convey their heartfelt appreciation to the Agricultural Research Council-Animal Production, University of KwaZulu-Natal and the Agricultural Sector Education and Training Authority (AgriSETA) for the financial assistance and facilities. The authors would like to express gratitude and appreciation to a statistician, Mr E. Mathebula, for analyzing the experimental data.

Funding Information

This study was supported by the Agricultural Research Council (API012403000057), University of KwaZulu-Natal AgriSETA and the Department of Agriculture.

Author's Contributions

Mamonene Angelinah Thema: Conceptualization, methodology, data curation, formal analysis, writing original draft, project administration.

Masindi Lottus Mphaphathi, Ntuthuko Raphael Mkhize, Gerhard van der Horst, and Liana Maree: Conceptualization, methodology, supervision, writing review and edited, validation.

Ramaesela Mahlatsana Ledwaba and Maleke Dimpho Sebopela: Data curation, formal analysis, investigation, writing review and edited.

Ethics

The experimental procedures received approval and were conducted in compliance with the ethical standards set forth by the Agricultural Research Council-Animal Production Ethics Committee [APIEC 23/05], the University of KwaZulu-Natal Research Ethics Committee [AREC/00007100/2024], and Section 20 of the Animal Diseases Act no 35 of 1984 [12/11/1/18 (6269BM)].

Conflict of Interest

The authors confirm that there are no conflicts of interest regarding this article.

References

Abdussamad, A. M., Detterer, J., Gauly, M., & Holtz, W. (2016). Comparison of various semen extenders and addition of prostaglandin F2 on pregnancy rate in cows. *Animal*, *10*(4), 655–659. https://doi.org/10.1017/s1751731115002256

Akhter, S., Ansari, M. S., Andrabi, S. M. H., Rakha, B. A., Ullah, N., & Khalid, M. (2012). Soya-lecithin in Extender Improves the Freezability and Fertility of Buffalo (*Bubalus bubalis*) Bull Spermatozoa. *Reproduction in Domestic Animals*, *47*(5), 815–819. https://doi.org/10.1111/j.1439-0531.2011.01973.x

Ali Hassan, H., Banchi, P., Domain, G., El Khoury, R., Chaaya, R., Wydooghe, E., Smits, K., & Van Soom, A. (2023). A comparative study of canine epididymal sperm collection techniques and cryopreservation. *Frontiers in Veterinary Science*, *10*, 1181054. https://doi.org/10.3389/fvets.2023.1181054

Amann, R. P., & Waberski, D. (2014). Computer-assisted sperm analysis (CASA): Capabilities and potential developments. *Theriogenology*, *81*(1), 5-17.e3. https://doi.org/10.1016/j.theriogenology.2013.09.004

Ansari, M. S., Rakha, B. A., & Akhter, S. (2017). Cryopreservation of Nili-Ravi buffalo (*Bubalus bubalis*) semen in AndroMed® extender; in vitro and in vivo evaluation. *Reproduction in Domestic Animals*, *52*(6), 992–997. https://doi.org/10.1111/rda.13008

Auer, M., Wagner, H., Failing, K., & Wehrend, A. (2022). Epididymis incision as a method to collect epididymal sperm cells in alpacas. *Veterinary Medicine and Science*, *8*(1), 157–163. https://doi.org/10.1002/vms3.681

Bertol, M. A. F. (2016). Cryopreservation of Epididymal Sperm. *Cryopreservation in Eukaryotes*, 121–135. https://doi.org/10.5772/65010

Bustani, G. S., & Baiee, F. H. (2021). Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Veterinary World*, *14*(5), 1220–1233. https://doi.org/10.14202/vetworld.2021.1220-1233

Canisso, I. F., & Teixeira Martini Segabinazzi, L. G. (2021). Diagnosis and management strategies for urospermia in stallions. *Revista Brasileira de Reprodução Animal*, *45*(4), 443–452. https://doi.org/10.21451/1809-3000.rbra2021.059

Dziekońska, A., Neuman, N. M., Burdal, K. K., Wiszniewska-Łaszczych, A., & Bogdaszewski, M. (2022). The Effect of Different Extenders on the Quality Characteristics of European Red Deer Epididymal Sperm Stored at 5 °C. *Animals*, *12*(19), 2669. https://doi.org/10.3390/ani12192669

Ellerbrock, R. E., Prell, M. J., Stewart, J. L., Bojko, M. S., Lima, F. S., & Canisso, I. F. (2017). Comparison of centrifugation and noncentrifugation methods to cryopreserve stallion epididymal semen. *Journal of Equine Veterinary Science*, *50*, 27-32.

Esteves, S. C., Miyaoka, R., & Agarwal, A. (2011). Sperm retrieval techniques for assisted reproduction. *International Braz j Urol*, *37*(5), 570–583. https://doi.org/10.1590/s1677-55382011000500002

Falomo, M. E., Rossi, M., & Mantovani, R. (2016). Collection, storage and freezability of equine epididymal spermatozoa. *Italian Journal of Animal Science*, *15*(3), 386–389. https://doi.org/10.1080/1828051x.2016.1210485

Fernandez-Novo, A., Santos-Lopez, S., Barrajon-Masa, C., Mozas, P., de Mercado, E., Caceres, E., Garrafa, A., Gonzalez-Martin, J. V., Perez-Villalobos, N., Oliet, A., Astiz, S., & Perez-Garnelo, S. S. (2021). Effects of Extender Type, Storage Time, and Temperature on Bull Semen Parameters. *Biology*, *10*(7), 630. https://doi.org/10.3390/biology10070630

Govindasamy, K., Ponraj, P., Thulasiraman, S., Andonissamy, J., Naskar, S., Das, A., Hasin, D., & Baishya, S. K. (2016). Efficacy of different extenders on sperm characteristics and fertility in crossbred pigs of north-eastern India. *Veterinarski Arhiv*, *86*(4), 515–528.

Hernández Corredor, L., Páez Barbosa, J. C., Quintero Moreno, A., & Rubio Parada, J. A. (2016). Efecto de diferentes niveles de Prostaglandina (Dinoprost y Cloprostenol) y Glicosaminoglicanos (Ácido hialurónico) en la motilidad, postdescongelación de espermatozoides bovinos. *Revista Agropecuaria y Agroindustrial La Angostura*,*3*(1), 25–34. https://doi.org/10.23850/raaa.v3i1.504

Ho, T. K., Vo, C. T., Lam, T. N., Nguyen, T. K. K., & Nguyen, T. T. (2023). Effect of difference extender on extended boar semen quality in the long-term storage. *Journal of Animal Husbandry Science and Technic (JAHST*, *9*, 14–23.

Hori, t., atago, t., kobayashi, m., & kawakami, E. (2015). Influence of different methods of collection from the canine epididymides on post-thaw caudal epididymal sperm quality. *Journal of Veterinary Medical Science*, *77*(5), 625–630. https://doi.org/10.1292/jvms.14-0421

Huijsmans, T. E. R. G., Hassan, H. A., Smits, K., & Van Soom, A. (2023). Postmortem Collection of Gametes for the Conservation of Endangered Mammals: A Review of the Current State-of-the-Art. *Animals*, *13*(8), 1360. https://doi.org/10.3390/ani13081360

Kumar, s., Singh, a. K., Dhindsa, s. S., & Singh, P. (2023). Effect of certain extenders on semen quality of boars during preservation at 17°C. *The Indian Journal of Animal Sciences*, *93*(8), 783–787.

Lamglait, B. (2014). Longevity of sperm cells retrieved by post-mortem epididymal aspiration in wild bovids in zoo conditions. *Journal of Zoo and Aquarium Research*, *2*(4), 92–100. https://doi.org/https://doi.org/10.19227/jzar.v2i4.47

Maroto-Morales, A., Ramón, M., García-Álvarez, O., Montoro, V., Soler, A. J., Fernández-Santos, M. R., Roldan, E. R. S., Pérez-Guzmán, M. D., & Garde, J. J. (2015). Sperm head phenotype and male fertility in ram semen. *Theriogenology*, *84*(9), 1536–1541. https://doi.org/10.1016/j.theriogenology.2015.07.038

Mogheiseh, A., Divar, M. R., Vesal, N., & Mahdivand Moradloo, F. (2022). Effects of epididymal sperm recovery methods on fresh and frozen–thawed sperm characteristics in dogs. *Reproduction in Domestic Animals*, *57*(9), 1038–1045. https://doi.org/10.1111/rda.14171

Monaco, D., Rota, A., Carbonari, A., Lillo, E., Lacalandra, G. M., & Rizzo, A. (2024). Collection of epididymal semen in the tomcat (Felix catus) by stereomicroscope-aided retrograde flushing (SARF) improves sample quality. *Animal Reproduction Science*, *261*, 107388.

https://doi.org/10.1016/j.anireprosci.2023.107388

Mortimer, D., Mortimer, S., & Horst, G. der. (2015). The future of computer-aided sperm analysis. *Asian Journal of Andrology*, *17*(4), 545. https://doi.org/10.4103/1008-682x.154312

Nagy, Á., Polichronopoulos, T., Gáspárdy, A., Solti, L., & Cseh, S. (2015). Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. *Acta Veterinaria Hungarica*, *63*(3), 370–381. https://doi.org/10.1556/004.2015.035

Neuhauser, S., Gösele, P., & Handler, J. (2018). The Effect of Four Different Commercial Semen Extenders on the Motility of Stallion Epididymal Sperm. *Journal of Equine Veterinary Science*, *62*, 8–12. https://doi.org/10.1016/j.jevs.2017.10.015

Nichi, M., Rijsselaere, T., Agostini Losano, J. D. de, Ramos Angrimani, D. de S., Vechiato Kawai, G. K., Goovaerts, I. G. F., Van Soom, A. V., Barnabe, V. H., De Clercq, J. B. P., & Bols, P. E. J. (2017). Evaluation of epididymis storage temperature and cryopreservation conditions for improved mitochondrial membrane potential, membrane integrity, sperm motility and *in vitro* fertilization in bovine epididymal sperm. *Reproduction in Domestic Animals*, *52*(2), 257–263. https://doi.org/10.1111/rda.12888

Pérez-Durand, M. G., Bustamante, C. W., Machaca, P. P., García, W., Condori, E. A., Macedo, R., Fernández, E., Manrique, Y. P., Gutiérrez-Reinoso, M. A., Perez-Guerra, U. H., & García-Herreros, M. (2024). Effect of Three Semen Extenders on Sperm Quality and In Vitro Fertilization Rates of Fresh and Cryopreserved Sperm Collected from Llama (Lama glama) Vas Deferens. *Animals*, *14*(11), 1573. https://doi.org/10.3390/ani14111573

Podico, G., & Canisso, I. F. (2022). Retrograde Flushing Followed by Slicing Float-Up as an Approach to Optimize Epididymal Sperm Recovery for the Purpose of Cryopreservation in Equids. *Animals*, *12*(14), 1802. https://doi.org/10.3390/ani12141802

Quintero-Elisea, J. A., Clemente-Sánchez, F., Cortez-Romero, C., Velázquez-Morales, J. V., Olguín-Arredondo, H. A., & Orozco-Lucero, E. (2023). Increase of the motility of buck semen with Andromed® extender and the addition of HTF. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2532

Schulze, M., Grobbel, M., Riesenbeck, A., Brüning, S., Schaefer, J., Jung, M., & Grossfeld, R. (2017). Dose rates of antimicrobial substances in boar semen preservation-time to establish new protocols. *Reproduction in Domestic Animals*, *52*(3), 397–402. https://doi.org/10.1111/rda.12921

Stout, M. A. (2012). *Comparison of epididymal and ejaculated sperm collected from the same holstein bulls*.

Strand, J., Ragborg, M. M., Pedersen, H. S., Kristensen, T. N., Pertoldi, C., & Callesen, H. (2016). Effects of post-mortem storage conditions of bovine epididymides on sperm characteristics: investigating a tool for preservation of sperm from endangered species. *Conservation Physiology*, *4*(1), cow069. https://doi.org/10.1093/conphys/cow069

Thema, M. A., Mphaphathi, M. L., Ledwaba, M. R., & Nedambale, T. L. (2022). Investigation of the efficacy of different semen extenders and in vitro storage period on Windsnyer boar sperm quality equilibrated at 18°C. *Reproduction in Domestic Animals*, *57*(12), 1644–1648. https://doi.org/10.1111/rda.14256

Turri, F., Madeddu, M., Gliozzi, T. M., Gandini, G., & Pizzi, F. (2012). Influence of Recovery Methods and Extenders on Bull Epididymal Spermatozoa Quality. *Reproduction in Domestic Animals*, *47*(5), 712–717. https://doi.org/10.1111/j.1439-0531.2011.01948.x

Vílchez, M. C., Morini, M., Peñaranda, D. S., Gallego, V., Asturiano, J. F., & Pérez, L. (2017). Role of potassium and pH on the initiation of sperm motility in the European eel. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *203*, 210–219. https://doi.org/10.1016/j.cbpa.2016.09.024

Yániz, J. L., Soler, C., & Santolaria, P. (2015). Computer assisted sperm morphometry in mammals: A review. *Animal Reproduction Science*, *156*, 1–12. https://doi.org/10.1016/j.anireprosci.2015.03.002