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Evaluation of Tomato Juice-Supplemented Extender Formulations for Liquid Preservation of Red Sokoto Buck Semen

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Abstract

Efficient liquid preservation of buck semen is fundamental to successful artificial insemination programmes and the dissemination of superior genetics in indigenous goat populations. This study evaluated the effects of tomato juice supplementation in different extender formulations on the quality of pooled Red Sokoto buck semen during short-term liquid storage. Semen was collected weekly from five healthy Red Sokoto bucks using an electro-ejaculator, pooled to minimise individual buck variation, and allocated to 11 extender formulations (5%D, EYC, NS, SC, TJ, TJD, TJDEY, TJEYNS, TJNS, TJSC, and TJSCEY). Extended semen samples were stored at 4–5°C and evaluated after 24, 48, and 72 h for sperm motility, live sperm percentage, and dead sperm percentage. Data were analysed using linear mixed-effects models with extender formulation, storage duration, and their interaction as fixed effects and experimental batch as a random effect. Semen quality declined progressively with increasing storage duration, with significant reductions in sperm motility and viability and corresponding increases in sperm mortality. Extender formulation, storage duration, and their interaction significantly affected sperm motility ($p < 0.001$), live sperm percentage ($p \leq 0.049$), and dead sperm percentage ($p \leq 0.049$). Egg yolk citrate maintained the highest sperm motility after 72 h (45.0 ± 8.7%), whereas the tomato juice-sodium citrate-egg yolk (TJSCEY) formulation achieved the best overall preservation performance by maintaining high sperm viability (81.9 ± 3.6%), low sperm mortality (18.1 ± 3.6%), and the highest composite preservation ranking. These findings demonstrate that tomato juice enhances semen preservation when incorporated into appropriate extender combinations and identify TJSCEY as a promising extender for short-term liquid preservation of Red Sokoto buck semen, warranting further evaluation through fertility trials and assessment of oxidative stress biomarkers.

Keywords: Artificial insemination; buck semen; Red Sokoto goat; semen extender; tomato juice; sperm viability.

1. Introduction

1.1 Importance of goat production and Red Sokoto goats

Goats represent an important component of livestock production systems, particularly in tropical and subtropical regions where they contribute substantially to meat, milk, skin production, income generation, and household livelihoods. Their small body size, efficient feed utilization, reproductive efficiency, and ability to survive under harsh environmental conditions make them especially valuable to smallholder and resource-limited farming systems (Peacock, 2005; Elzaki *et al.,* 2019; Desta, 2024). In Nigeria, goats constitute a major livestock resource and play important socioeconomic roles by providing animal protein, financial security, and employment opportunities across rural communities.

The Red Sokoto goat, also known as the Maradi goat, is one of the most economically important indigenous goat breeds in Nigeria. The breed is particularly recognized for its adaptability to the semi-arid environment, tolerance to nutritional and climatic stress, reproductive potential, and high-quality skin production, which contributes to the internationally valued "Morocco leather" industry (Dossa *et al*., 2007; Adejuwon, 2019). Despite these desirable characteristics, productivity remains largely constrained by traditional breeding systems, limited genetic improvement programmes, and inadequate application of assisted reproductive technologies. Improving reproductive efficiency and facilitating dissemination of superior genetics are therefore essential for enhancing the productivity and conservation of indigenous goat genetic resources.

1.2 Artificial insemination and semen preservation challenges

Artificial insemination (AI) is an important reproductive biotechnology that enables rapid genetic improvement through wider utilization of genetically superior males, improved disease control, and efficient management of breeding programmes (Foote, 2002). Successful implementation of AI depends largely on effective semen collection, processing, preservation, and maintenance of sperm fertilizing capacity during storage. Semen extenders

are therefore essential because they provide nutrients, maintain osmotic balance, regulate pH, and protect spermatozoa against structural and functional damage during preservation (Bustani & Baiee, 2021).

However, preservation of buck semen remains challenging because sperm cells undergo progressive deterioration during storage. Cooling and prolonged preservation can induce cold shock, reduce membrane fluidity, impair mitochondrial function, and decrease sperm motility and viability (Heydari *et al.,* 2021). Buck spermatozoa are particularly sensitive to preservation-induced injury because of the high proportion of polyunsaturated fatty acids in the sperm plasma membrane, which increases vulnerability to lipid peroxidation and oxidative damage (Qamar *et al*., 2023; Soria-Meneses *et al.,* 2025). Therefore, improvement of extender formulations remains necessary to enhance sperm survival and maintain semen quality during storage.

1.3 Oxidative stress and antioxidants in semen preservation

Oxidative stress is considered one of the major mechanisms responsible for reduced sperm quality during semen preservation. Although low physiological concentrations of reactive oxygen species (ROS) participate in normal sperm functions such as capacitation and fertilization, excessive ROS production overwhelms antioxidant defence mechanisms, leading to lipid peroxidation, protein oxidation, DNA damage, and reduced sperm motility (Wang *et al*., 2025).

The sperm plasma membrane contains high concentrations of polyunsaturated fatty acids that are essential for membrane flexibility but highly susceptible to oxidative attack. Consequently, antioxidant supplementation of semen extenders has gained increasing attention as a strategy to improve sperm resistance against preservation-associated stress. Various natural antioxidants have been investigated for their potential ability to reduce oxidative injury, stabilize sperm membranes, and improve semen quality during storage (Martin-Hidalgo *et al.,* 2019; Dimitriadis *et al*., 2023).

1.4 Potential role of tomato juice supplementation in semen preservation

Plant-derived antioxidant sources have recently attracted interest as alternative or complementary additives in semen preservation systems because of their availability, affordability, and bioactive properties. Tomato (*Solanum lycopersicum*) is rich in several antioxidant compounds, including lycopene, vitamin C, phenolic compounds, and other carotenoids, which have demonstrated free radical scavenging and membrane-protective activities (Ros-Santaella & Pintus, 2021; Shayestehyekta *et al.,* 2026).

Lycopene, the predominant carotenoid in tomato, is recognized for its strong antioxidant potential and ability to neutralize reactive oxygen species. Previous studies have suggested that lycopene and related antioxidants may contribute to improved sperm quality by reducing lipid peroxidation, supporting membrane integrity, and protecting sperm cells against oxidative damage (Durairajanayagam *et al.,* 2014; Tvrdá *et al.,* 2016; Babaei *et al*., 2021; Bouhadana *et al.,* 2025). These characteristics suggest that tomato-derived supplementation could potentially improve semen preservation, particularly in production systems where affordable and locally available extender additives are desirable.

However, the effectiveness of tomato juice incorporation into buck semen extenders, particularly in combination with conventional extender components such as egg yolk citrate, remains insufficiently investigated.

1.5 Knowledge gap and study justification

Egg-yolk-based extenders remain widely used for semen preservation because of their ability to protect spermatozoa against cold shock and membrane damage during cooling and storage (Anzar *et al.,* 2019; Bustani & Baiee, 2021). However, reliance on conventional egg-yolk-containing extenders presents some limitations, including biological variability, undefined composition, potential microbial contamination risks, and the need for improved protection against preservation-induced oxidative stress (Forouzanfar *et al*., 2010; Layek *et al*., 2016). Consequently, increasing attention has focused on identifying natural antioxidant additives that can enhance sperm resistance to oxidative damage while supporting more accessible semen preservation strategies (Walke *et al*., 2023; Hungerford *et al*., 2024; Maapola *et al.,* 2026).

Despite the antioxidant composition of tomato juice, particularly its richness in lycopene, vitamin C, and phenolic compounds (Agarwal & Rao, 2000; Ali *et al*., 2020), limited information exists regarding its effectiveness as an extender supplement for preserving Red Sokoto buck semen. Furthermore, comparative evaluation of tomato juice-based formulations, either alone or combined with conventional components such as egg yolk citrate, remains limited. Evaluating these formulations may provide useful information for developing affordable extender alternatives suitable for indigenous goat genetic improvement programmes.

1.6 Study aim and objectives

The aim of this study was to evaluate the effects of incorporating tomato juice into different extender formulations on the preservation quality of pooled Red Sokoto buck semen during liquid storage.

The specific objectives were:

1. To compare sperm motility changes among different extender formulations during semen storage.
2. To evaluate the effects of extender formulations on sperm viability and mortality during preservation.
3. To determine the interaction between extender formulation and storage duration on semen quality characteristics.
4. To evaluate the relative preservation efficiency of extender formulations based on maintenance of semen quality during storage.

2. Materials and Methods

2.1 Study location

The study was conducted at the Andrology Laboratory, Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Sokoto State is located in the extreme north-western region of Nigeria, within the Sudan Savannah ecological zone. The area is characterized by a semi-arid climate with distinct dry and rainy seasons. The rainy season typically occurs between

June and October, while the dry season extends from November to May. The region experiences high ambient temperatures, particularly during the hot dry months, with livestock production dominated by goats, sheep, cattle, camels, and poultry (Mamman & Oyebanji, 2000; SMANR, 2007).

2.2 Experimental animals and management

Five clinically healthy Red Sokoto bucks aged between 1 and 2 years were used as semen donors for the study. The bucks had an average body condition score of approximately 3 on a 5-point scale and were maintained under an intensive management system. Animals were housed under similar environmental and management conditions throughout the study period.

The bucks were fed wheat bran and hay, with clean drinking water provided. Prior to semen collection, animals were examined to ensure good general health status and suitability for semen collection.

2.3 Ethical approval

Ethical approval for the use of animals in this study was obtained from IACUC Institutional Animal Care and Use Committee (UDUS/IACUC/2026/R09). All procedures were conducted in accordance with institutional guidelines for animal care and use.

2.4 Semen collection procedure

Semen was collected from each buck once weekly for three consecutive weeks using an electro-ejaculator. Electrical stimulation was performed using a direct current output of approximately 12 V to induce ejaculation. Immediately after collection, ejaculates were protected from environmental stress and transported promptly for laboratory evaluation and processing.

Only ejaculates considered suitable following initial evaluation were processed further for extender allocation and preservation studies.

2.5 Semen pooling procedure

Following collection and preliminary assessment, ejaculates obtained from the five Red Sokoto bucks were pooled before allocation into extender treatment groups. Pooling was performed to minimize the influence of individual buck variation and to provide a uniform semen source for evaluating extender-level preservation effects. Consequently, individual buck identity was not considered an experimental factor because extender comparisons represented responses of pooled semen samples rather than individual animal variation.

2.6 Preparation of tomato juice antioxidant supplement

Fresh ripe tomatoes were obtained and processed for use as a natural antioxidant supplement. The tomatoes were disinfected using methylated spirit, rinsed thoroughly with distilled water, chopped into smaller pieces, and blended until a homogeneous mixture was obtained.

The blended tomato preparation was filtered to obtain tomato juice and subsequently centrifuged at 10,000 rpm for 5 minutes to remove solid residues. The clarified tomato juice fraction was collected, and 0.5 ml of the prepared juice was supplemented with 500 IU crystalline penicillin before incorporation into the respective extender formulations.

2.7 Preparation of semen extender formulations

Eleven semen extender formulations were evaluated in this study: 5% dextrose saline (5%D), egg yolk citrate (EYC), normal saline (NS), sodium citrate (SC), tomato juice (TJ), tomato juice–dextrose saline (TJD), tomato juice–dextrose saline–egg yolk (TJDEY), tomato juice–egg yolk–normal saline (TJEYNS), tomato juice–normal saline (TJNS), tomato juice–sodium citrate (TJSC), and tomato juice–sodium citrate–egg yolk (TJSCEY).

Sodium citrate extender was prepared by dissolving 2.9 g sodium citrate in 100 ml distilled water and mixing thoroughly until complete dissolution. Normal saline extender consisted of 0.9% physiological saline solution. Prepared extender solutions were supplemented with 500 IU crystalline penicillin.

Egg yolk was prepared from fresh eggs after shell disinfection using methylated spirit. The eggs were rinsed with distilled water, broken aseptically, and the yolk separated from albumen before incorporation into the appropriate extender formulations.

Egg yolk citrate, which served as the conventional control extender, consisted of 20% egg yolk prepared in sodium citrate solution. Tomato-containing extender formulations were prepared by combining tomato juice with the respective extender components according to the treatment groups.

The composition of all extender formulations evaluated in the study is presented in Table 1.

Table 1. Composition of semen extender formulations used for preservation of pooled Red Sokoto buck semen

Extender code	**Extender formulation**
5%D	5% dextrose saline
EYC	Egg yolk citrate (20% egg yolk in sodium citrate solution)
NS	0.9% normal saline
SC	Sodium citrate solution
TJ	Tomato juice-based extender

TJD	Tomato juice + 5% dextrose saline
TJDEY	Tomato juice + dextrose saline + egg yolk
TJEYNS	Tomato juice + egg yolk + normal saline
TJNS	Tomato juice + normal saline
TJSC	Tomato juice + sodium citrate
TJSCEY	Tomato juice + sodium citrate + egg yolk

Footnote: *EYC = egg yolk citrate; TJ = tomato juice; NS = normal saline; SC = sodium citrate. All extender formulations were supplemented with crystalline penicillin before semen dilution*.

2.8 Semen dilution and storage protocol

Following pooling, semen samples were divided and allocated into the different extender treatment groups. Semen was diluted with the respective extenders at a ratio of 0.2 ml semen to 2.0 ml extender preparation.

The diluted semen samples were stored under chilled conditions at approximately 4–5°C. Semen quality evaluation was performed after 24, 48, and 72 h of liquid storage to assess extender performance over time.

2.9 Evaluation of semen quality parameters

Semen quality assessment was performed at each storage duration by evaluating sperm motility and viability characteristics. The major outcome parameters assessed were sperm motility percentage, live sperm percentage, and dead sperm percentage.

2.9.1 Sperm motility assessment

Sperm motility was assessed using light microscopy. A representative semen sample from each treatment group was examined under ×40 magnification, and the percentage of motile spermatozoa was estimated based on observed sperm movement.

Motility values were expressed as percentages, representing the proportion of spermatozoa exhibiting observable movement.

2.9.2 Sperm viability assessment

Sperm viability was assessed using eosin–nigrosin staining. Prepared semen smears were stained and examined microscopically to differentiate viable and non-viable sperm cells based on membrane integrity.

The numbers of live and dead sperm cells were recorded, and the percentages of live and dead spermatozoa were calculated for each sample.

220 2.10 Experimental design

221 The experimental design consisted of 11 extender formulations evaluated across three liquid storage durations (24,
222 48, and 72 h). The study included two independent pooled semen experimental batches, with three technical
223 replicate measurements performed within extender–storage duration combinations.

224 Extender formulation and storage duration represented the main experimental factors. Individual buck identity was
225 not included as an experimental factor because ejaculates from the five bucks were pooled before allocation into
226 extender treatments. Therefore, statistical comparisons focused on extender-level effects on pooled semen
227 preservation performance.

228 2.11 Statistical analysis

229 2.11.1 Data preparation and validation

230 Raw sperm motility and live/dead viability datasets were merged using unique experimental identifiers comprising
231 extender formulation, experimental batch, storage duration, and laboratory replicate. Before analysis, dataset
232 integrity was assessed by evaluating variable structure, coding consistency, missing data, duplicate records, and
233 biological plausibility of semen quality measurements.

234 Duplicate records generated during dataset merging were identified using Extender × Experiment × Storage duration
235 × Replicate combinations and removed by retaining only the first valid occurrence. Sperm motility, live sperm
236 percentage, and dead sperm percentage values were validated to ensure biologically acceptable ranges (0–100%).
237 Zero sperm motility values were retained as valid observations representing complete loss of motility during
238 preservation.

239 The final dataset structure, variable definitions, completeness assessment, and data-cleaning procedures are provided
240 in the Online Resource 1 (Supplementary Tables S1 and S2). The final experimental structure, treatment allocation,
241 storage duration design, and analytical classification of variables used for modelling are summarised in the Online
242 Resource 1 (Supplementary Table S3).

243 2.11.2 Model assumption assessment

244 Before inferential analyses, semen quality variables were evaluated for distributional characteristics, variance
245 homogeneity, and potential influential observations. Normality was assessed using the Shapiro–Wilk test together
246 with visual inspection of diagnostic plots. Homogeneity of variance was evaluated using Levene's test, while

potential outliers were assessed using standardized residuals and the interquartile range method. No observations were removed unless considered biologically implausible.

2.11.3 Descriptive analyses

Descriptive analyses were performed to summarize semen quality characteristics across extender formulations and storage durations. Continuous semen quality outcomes, including sperm motility (%), live sperm (%), and dead sperm (%), were summarized using means and standard deviations (SD). Mean estimates with corresponding 95% confidence intervals (CI) were calculated to describe the precision of semen quality measurements within each extender–storage duration combination. Graphical visualization was performed using boxplots, line plots, and heatmaps to evaluate treatment-related differences and storage-dependent changes in semen quality parameters.

2.11.4 Mixed-effects modelling

Linear mixed-effects models were used to evaluate the effects of extender formulation and storage duration on semen quality outcomes while accounting for variability among independent semen processing batches. Separate models were fitted for sperm motility (%), live sperm (%), and dead sperm (%). For each outcome, extender formulation, storage duration, and the extender × storage duration interaction were included as fixed effects, while experimental batch was included as a random intercept.

The general model structure was:

$$Y_{ijkl} = \mu + E_i + (ET)_{ij} + b_k + \varepsilon_{ijkl}$$

$$b_k \sim N(0, \sigma_b^2), \qquad \varepsilon_{ijkl} \sim N(0, \sigma_e^2)$$

where Y_{ijkl} represents the observed semen quality outcome (sperm motility percentage, live sperm percentage, or dead sperm percentage) for the i^{th} extender formulation, j^{th} storage duration, k^{th} experimental batch, and l^{th} laboratory replicate; μ represents the overall mean response; E_i represents the fixed effect of extender formulation

$i = (1, \ldots, 11)$; T_i represents the fixed effect of storage duration $j = 1,2,3$, corresponding to 24, 48, and 72 h); $(ET)_{ij}$ represents the fixed interaction effect between extender formulation and storage duration; b_k represents the random intercept effect of the independent pooled semen experimental batch $(k = 1,2)$, assumed to be normally distributed with mean zero and variance σ_b^2; and ε_{ijkl} represents the residual error term associated with replicate-level observations, assumed to be normally distributed with mean zero and variance σ_e^2. The random batch effects and residual errors were assumed to be independent.

Storage duration was modelled as a categorical variable to allow comparison among preservation periods without assuming a linear decline in semen quality. Buck identity was not included as a random effect because ejaculates collected from individual bucks were pooled before allocation into extender treatments; therefore, treatment responses represented pooled semen characteristics rather than individual buck variation.

Overall significance of fixed effects was assessed using Type III analysis of variance with Satterthwaite's approximation for denominator degrees of freedom. Model outputs included fixed-effect significance tests, variance components, residual variance, and model fit statistics. Mixed-effects analyses were conducted using the lme4 and lmerTest packages in R (Bates *et al*., 2015; Kuznetsova *et al*., 2017).

2.11.5 Post-hoc comparisons

Where significant extender, storage duration, or interaction effects were detected, post-hoc comparisons were performed using estimated marginal means derived from the fitted mixed-effects models. Pairwise comparisons among extender formulations within storage durations were conducted with Tukey adjustment to control for multiple testing. Compact letter displays were generated to identify statistically different treatment groups, with significance declared at $p < 0.05$. Estimated marginal means and multiple comparisons were obtained using the emmeans package in R (Lenth, 2024).

2.11.6 Extender preservation efficiency assessment

To evaluate practical semen preservation performance, additional descriptive preservation indices were calculated for each extender formulation. Motility retention was calculated as the percentage of sperm motility maintained at 72 h relative to 24 h, while viability retention represented the percentage of live sperm maintained over the same period. Motility loss was calculated as the difference in mean sperm motility between 24 and 72 h, and sperm mortality increase was calculated as the increase in dead sperm percentage between 24 and 72 h of storage.

Extender preservation performance was interpreted based on combined assessment of sperm motility retention, viability maintenance, reduction in sperm mortality, and consistency of semen quality during storage. These preservation indices were considered descriptive measures of practical extender performance and were interpreted together with results from mixed-effects models and post-hoc comparisons.

3. Results

3.1 Experimental dataset characteristics

Details of the experimental dataset structure, variable completeness, and analytical framework are provided in the Online Resource 1 (Supplementary Tables S1–S3).

After dataset validation and removal of merge-generated duplicate entries, the final analytical dataset consisted of 108 unique observations from 11 extender formulations evaluated at three storage durations (24, 48, and 72 h). The dataset included two independent pooled semen experimental batches and three technical replicate measurements. Complete viability measurements were available for all observations, whereas sperm motility data were available for 99 observations.

3.2 Descriptive semen quality changes during storage

The effects of extender formulation and storage duration on sperm motility, viability, and mortality are presented in Table 2. Progressive reductions in sperm motility and live sperm percentage were observed across all extender formulations as storage duration increased from 24 to 72 h. However, the magnitude of deterioration differed among extender formulations. The EYC extender maintained the highest sperm motility after 72 h of preservation (45.00 ± 8.66%), followed by TJSCEY (35.00 ± 0.00%). Similarly, TJSCEY maintained the highest sperm viability after 72 h (81.89 ± 3.55%) and the lowest sperm mortality (18.11 ± 3.55%), indicating superior preservation of sperm viability during extended storage.

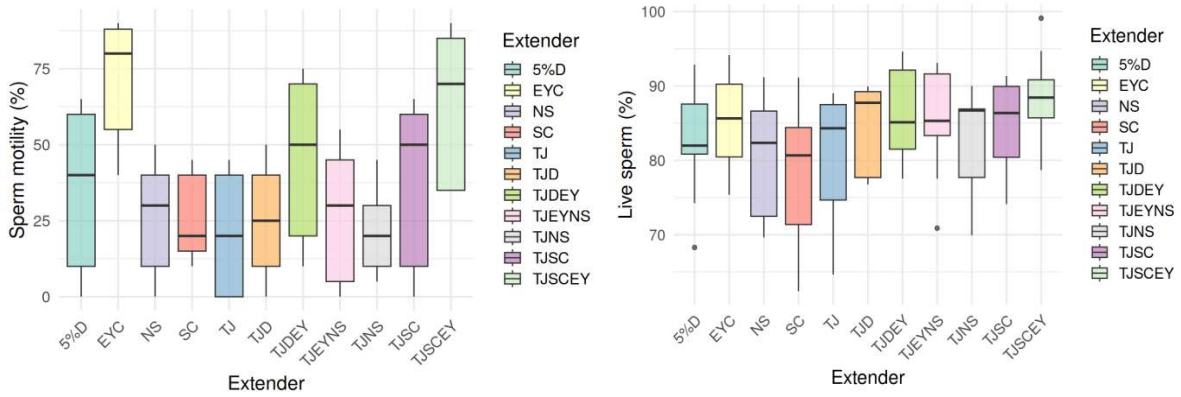
Extenders without egg yolk supplementation generally demonstrated greater reductions in motility during storage, with TJ showing complete loss of observable motility by 72 h despite the persistence of viable sperm cells. This highlights that sperm viability and motility represent related but distinct indicators of semen preservation quality.

Table 2. Effect of extender formulation and storage duration on motility and viability characteristics of pooled Red Sokoto buck semen during liquid preservation

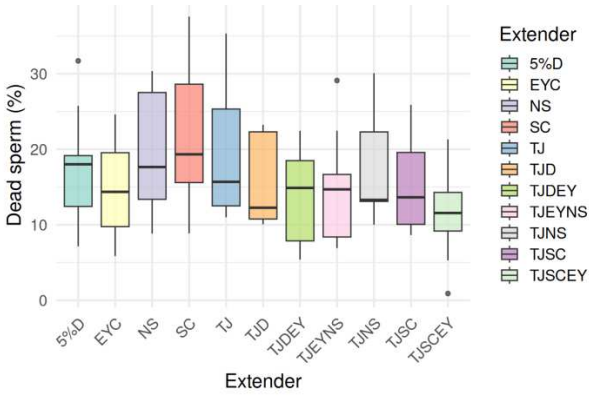
Extender	Storage duration (h)	n	Sperm motility (%)	Live sperm (%)	Dead sperm (%)
5%D	24	3	61.67 ± 2.89	91.00 ± 2.97	9.00 ± 2.97
	48	3	41.67 ± 2.89	82.70 ± 2.91	17.30 ± 2.91
	72	3	3.33 ± 5.77	74.84 ± 6.87	25.16 ± 6.87
EYC	24	3	88.33 ± 2.89	92.02 ± 1.97	7.98 ± 1.97
	48	3	82.67 ± 4.62	85.14 ± 1.61	14.86 ± 1.61
	72	3	45.00 ± 8.66	77.72 ± 2.56	22.28 ± 2.56
NS	24	3	45.00 ± 5.00	89.17 ± 2.32	10.83 ± 2.32
	48	3	30.00 ± 5.00	82.17 ± 3.09	17.83 ± 3.09
	72	3	5.00 ± 5.00	71.46 ± 1.58	28.54 ± 1.58
SC	24	3–6	41.67 ± 2.89	87.14 ± 3.82	12.86 ± 3.82
	48	3–6	18.33 ± 2.89	80.43 ± 3.59	19.57 ± 3.59
	72	3–6	11.67 ± 2.89	68.26 ± 3.36	31.74 ± 3.36
TJ	24	3	41.67 ± 2.89	88.10 ± 0.81	11.90 ± 0.81
	48	3	21.67 ± 2.89	83.95 ± 1.59	16.05 ± 1.59
	72	3	0.00 ± 0.00	70.57 ± 5.24	29.43 ± 5.24
TJD	24	3	43.33 ± 5.77	89.04 ± 1.02	10.96 ± 1.02
	48	3	26.67 ± 2.89	87.85 ± 1.41	12.15 ± 1.41
	72	3	3.33 ± 5.77	77.11 ± 0.51	22.89 ± 0.51
TJDEY	24	3	73.33 ± 2.89	93.65 ± 1.33	6.35 ± 1.33
	48	3	51.67 ± 2.89	85.94 ± 1.53	14.06 ± 1.53
	72	3	13.33 ± 5.77	79.48 ± 1.98	20.52 ± 1.98

TJEYNS	24	3	50.00 ± 5.00	92.34 ± 0.74	7.66 ± 0.74
	48	3	28.33 ± 7.64	85.48 ± 1.94	14.52 ± 1.94
	72	3	3.33 ± 2.89	77.25 ± 6.23	22.75 ± 6.23
TJNS	24	3	38.33 ± 7.64	88.53 ± 1.55	11.47 ± 1.55
	48	3	21.67 ± 2.89	86.25 ± 0.95	13.75 ± 0.95
	72	3	8.33 ± 2.89	74.59 ± 4.10	25.41 ± 4.10
TJSC	24	3	61.67 ± 2.89	90.51 ± 0.75	9.49 ± 0.75
	48	3	48.33 ± 2.89	85.99 ± 1.36	14.01 ± 1.36
	72	3	3.33 ± 5.77	77.01 ± 3.18	22.99 ± 3.18
TJSCEY	24	3	88.33 ± 2.89	94.88 ± 4.14	5.12 ± 4.14
	48	3	71.67 ± 2.89	88.46 ± 1.03	11.54 ± 1.03
	72	3	35.00 ± 0.00	81.89 ± 3.55	18.11 ± 3.55

Footnote: *Values are presented as mean ± standard deviation. EYC = egg yolk citrate extender (control). Ejaculates collected from five Red Sokoto bucks were pooled before allocation into extender treatments; therefore, values represent pooled semen responses. Zero sperm motility values indicate complete loss of observable sperm movement after preservation and were retained as valid biological observations*.



A. Sperm motility (%) **B.** Live sperm (%)



C. Dead sperm (%)

Figure 1. Distribution of sperm motility and viability characteristics of pooled Red Sokoto buck semen preserved using different extender formulations over 72 h of liquid storage. Boxplots illustrate variation in semen quality parameters among extender formulations at 24, 48, and 72 h of storage. Panels represent: **(A)** sperm motility (%), **(B)** live sperm (%), and **(C)** dead sperm (%). Boxes indicate the interquartile range, horizontal lines represent median values, and whiskers show data variability across replicate measurements. EYC = egg yolk citrate extender.

Descriptive changes in semen quality parameters across extender formulations and storage durations are presented in Table 2 and visually summarized in Fig. 1. A progressive decline in sperm quality was observed during liquid preservation, with sperm motility showing the greatest storage-associated reduction (Fig. 1A). Across all extender formulations, mean sperm motility decreased from 57.58 ± 18.38% at 24 h to 40.15 ± 20.96% at 48 h and further declined to 12.02 ± 14.71% at 72 h, demonstrating substantial loss of sperm functional capacity with prolonged storage.

Marked extender-dependent variation was observed, particularly after extended preservation. At 72 h, EYC maintained the highest sperm motility (45.00 ± 8.66%), followed by TJSCEY (35.00 ± 0.00%), whereas TJ showed complete loss of observable motility (0.00 ± 0.00%). Other tomato-based combinations showed variable preservation capacity, indicating formulation-dependent differences in maintaining sperm movement during storage.

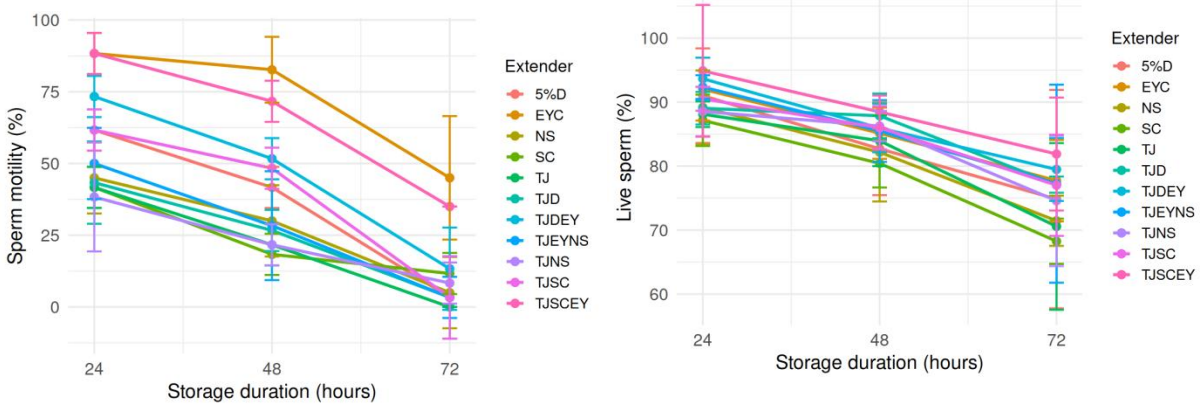
Live sperm percentage showed a more gradual decline compared with motility (Fig. 1B). Overall viability decreased from 90.29 ± 3.25% at 24 h to 84.61 ± 3.24% at 48 h and 74.93 ± 5.41% at 72 h. At the end of storage, TJSCEY maintained the highest sperm viability (81.89 ± 3.55%), followed by TJDEY (79.48 ± 1.98%) and EYC (77.72 ± 2.56%).

Correspondingly, sperm mortality increased progressively during preservation (Fig. 1C), rising from 9.71 ± 3.25% at 24 h to 15.39 ± 3.24% at 48 h and 25.07 ± 5.41% at 72 h. The lowest sperm mortality after 72 h was observed with TJSCEY (18.11 ± 3.55%), followed by TJDEY (20.52 ± 1.98%) and EYC (22.28 ± 2.56%), suggesting improved membrane preservation in egg-yolk-containing extender combinations.

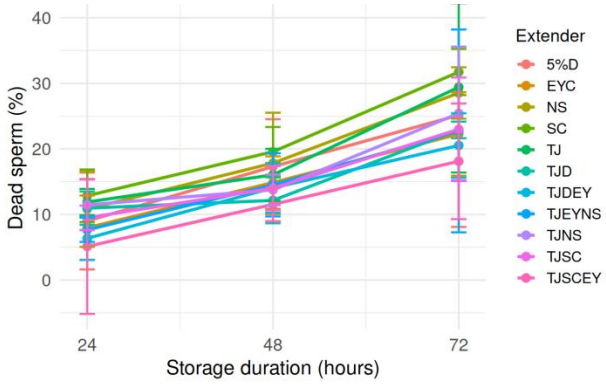
Overall, the exploratory visualization demonstrated a clear time-dependent decline in semen quality, with EYC providing superior motility preservation, whereas TJSCEY demonstrated the most favourable overall viability and mortality profile after prolonged storage.

3.3 Storage-dependent preservation patterns among extender formulations

Semen quality showed progressive deterioration during liquid storage, although the magnitude of decline varied considerably among extender formulations (Fig. 2).



A. Sperm motility (%) **B.** Live sperm (%)



C. Dead sperm (%)

Figure 2. Storage-dependent changes in sperm motility and viability characteristics of pooled Red Sokoto buck semen preserved using different extender formulations over 72 h of liquid storage. Line plots illustrate temporal changes in semen quality parameters among extender formulations at 24, 48, and 72 h of preservation. Panels represent: **(A)** sperm motility (%), **(B)** live sperm (%), and **(C)** dead sperm (%). Lines represent extender-specific preservation trends, points indicate mean values at each storage duration, and error bars show 95% confidence intervals across replicate measurements. EYC = egg yolk citrate extender.

Sperm motility showed the greatest storage-associated reduction among the evaluated semen quality parameters (Fig. 2A). The conventional egg yolk citrate extender (EYC) maintained the highest motility throughout storage, declining from 88.33 ± 2.89% at 24 h to 82.67 ± 4.62% at 48 h and 45.00 ± 8.66% after 72 h. Among experimental formulations, TJSCEY demonstrated the closest preservation performance, maintaining motility values of 88.33 ± 2.89%, 71.67 ± 2.89%, and 35.00 ± 0.00% at 24, 48, and 72 h, respectively.

Live sperm percentage decreased more gradually compared with motility (Fig. 2B). After 72 h of storage, the highest sperm viability was maintained by TJSCEY (81.89 ± 3.55%), followed by TJDEY (79.48 ± 1.98%) and EYC (77.72 ± 2.56%), suggesting improved maintenance of sperm membrane integrity in extender combinations containing egg yolk.

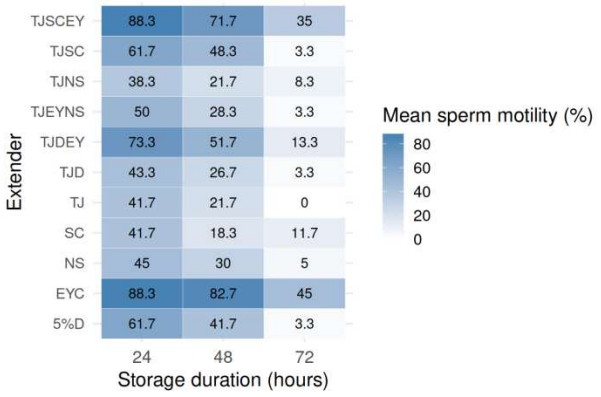
A corresponding increase in dead sperm percentage was observed with prolonged preservation (Fig. 2C). After 72 h, TJSCEY resulted in the lowest sperm mortality (18.11 ± 3.55%), followed by TJDEY (20.52 ± 1.98%) and EYC (22.28 ± 2.56%). In contrast, higher mortality was observed in formulations such as SC (31.74 ± 3.36%) and TJ (29.43 ± 5.24%) after prolonged storage.

Overall, the storage trend analysis demonstrated that EYC provided superior preservation of sperm motility, whereas TJSCEY showed the most favourable viability maintenance and lowest sperm mortality after extended storage.

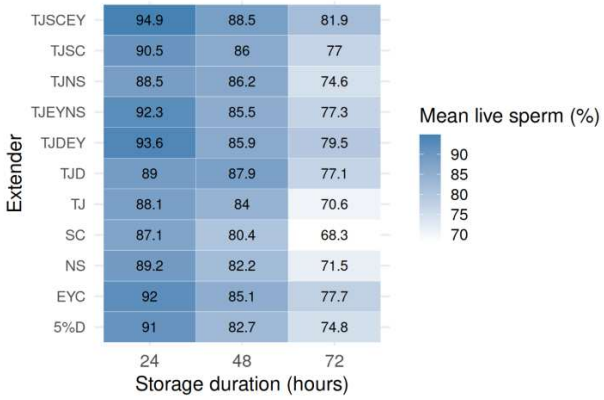
Detailed numerical summaries of extender-specific semen quality responses across storage durations, including mean values, standard deviations, and corresponding 95% confidence intervals are provided in the Online Resource 1 (Supplementary Table S4).

3.4 Comparative visualization of extender preservation performance

Heatmap visualization provided a comparative overview of extender-dependent differences in semen preservation capacity throughout storage (Fig. 3).



A. Sperm motility (%)



B. Live sperm (%)



C. Dead sperm (%)

Figure 3. Heatmap visualization of sperm motility and viability preservation patterns in pooled Red Sokoto buck semen stored with different extender formulations over 72 h of liquid preservation. Heatmaps illustrate mean semen quality values for each extender formulation at 24, 48, and 72 h of storage. Panels represent: **(A)** sperm motility (%), **(B)** live sperm (%), and **(C)** dead sperm (%). Cell values represent mean percentages, with colour gradients indicating relative differences in semen preservation performance across extender formulations and storage durations. EYC = egg yolk citrate extender.

Distinct preservation patterns were observed among extender formulations, particularly after prolonged storage. For sperm motility (Fig. 3A), EYC maintained the highest values throughout preservation, decreasing from 88.3% at 24 h to 82.7% at 48 h and 45.0% at 72 h. Among experimental formulations, TJSCEY demonstrated the strongest motility preservation profile, maintaining 88.3%, 71.7%, and 35.0% motility at 24, 48, and 72 h, respectively.

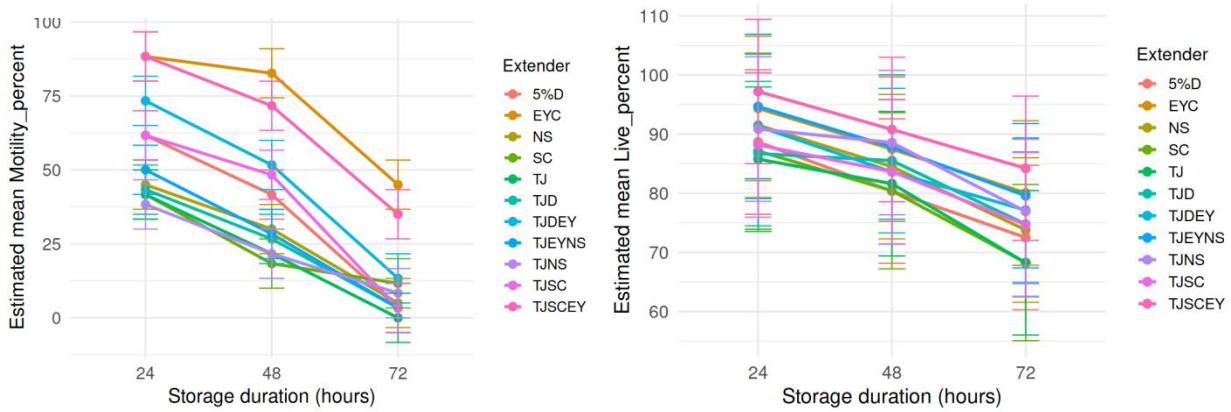
For sperm viability (Fig. 3B), egg-yolk-containing combinations showed favourable preservation patterns. After 72 h, TJSCEY maintained the highest live sperm percentage (81.9%), followed by TJDEY (79.5%), EYC (77.7%), and TJEYNS (77.3%). Conversely, SC showed the lowest viability after prolonged storage (68.3%).

The inverse pattern was observed for sperm mortality (Fig. 3C), where lower values indicated better preservation. At 72 h, TJSCEY showed the lowest percentage of dead sperm cells (18.1%), followed by TJDEY (20.5%), EYC (22.3%), and TJEYNS (22.7%). The highest mortality after 72 h occurred in SC (31.7%).

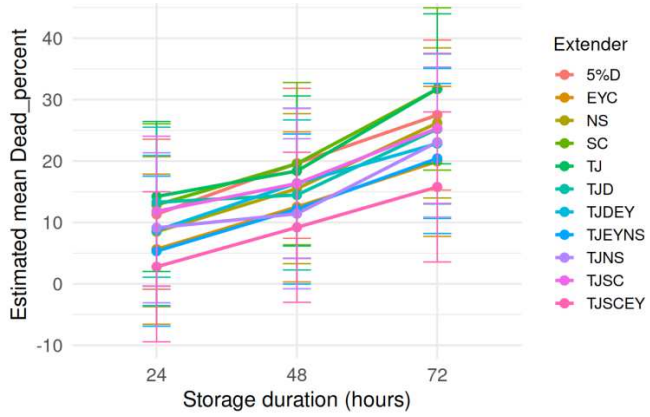
Overall, heatmap visualization confirmed that EYC remained superior for maintaining sperm motility, whereas TJSCEY provided the most favourable overall preservation profile by maintaining higher viability and reducing sperm mortality during extended storage.

3.5 Extender × storage duration interaction effects on semen preservation performance

The interaction patterns between extender formulation and storage duration demonstrated clear differences in the ability of extender formulations to maintain semen quality during liquid preservation (Fig. 4).



A. Sperm motility (%) **B.** Live sperm (%)



C. Dead sperm (%)

Figure 4. Interaction effects of extender formulation and storage duration on sperm motility and viability characteristics of pooled Red Sokoto buck semen during 72 h of liquid preservation.

Interaction plots illustrate estimated marginal mean changes in semen quality parameters among extender formulations across storage durations. Panels represent: **(A)** sperm motility (%), **(B)** live sperm (%), and **(C)** dead sperm (%). Points represent estimated marginal means, and error bars indicate model-derived standard errors. Non-parallel response patterns indicate differences among extender formulations in their ability to maintain semen quality during storage. EYC = egg yolk citrate extender.

Sperm motility showed a progressive decline across all extender formulations; however, the magnitude of decline varied substantially among treatments (Fig. 4A). At 24 h, EYC and TJSCEY showed the highest estimated motility values (88.33 ± 2.58%) and belonged to the highest statistical grouping. TJDEY also demonstrated relatively high early motility preservation (73.33 ± 2.58%).

After 48 h storage, EYC maintained the greatest sperm motility (82.67 ± 2.58%), followed by TJSCEY (71.67 ± 2.58%). These treatments remained within the upper Tukey groupings, demonstrating better resistance to storage-associated motility decline compared with other extender formulations.

At the end of the 72 h preservation period, motility differences became more pronounced. EYC retained the highest motility (45.00 ± 2.58%), followed by TJSCEY (35.00 ± 2.58%). In contrast, TJ showed complete loss of observable motility (0.00 ± 2.58%), indicating poor preservation capacity when tomato juice was used without additional protective components.

Live sperm percentage showed a slower decline compared with motility (Fig. 4B). TJSCEY consistently maintained the highest estimated viability throughout preservation, with values of 97.22 ± 3.04%, 90.79 ± 3.04%, and 84.22 ± 3.04% at 24, 48, and 72 h, respectively. At 72 h, TJSCEY remained among the highest statistical groups, followed by EYC (80.06 ± 3.04%), TJEYNS (79.58 ± 3.04%), and TJDEY (77.14 ± 3.04%).

Correspondingly, the interaction pattern for sperm mortality (Fig. 4C) demonstrated an inverse trend to viability, with mortality increasing during storage. Egg-yolk-containing combinations, particularly TJSCEY, showed lower increases in sperm mortality, suggesting improved protection of sperm membrane integrity during prolonged preservation.

Overall, interaction analysis confirmed that extender performance was storage-duration dependent. EYC provided the strongest preservation of sperm motility, whereas TJSCEY demonstrated the most balanced preservation profile by maintaining high viability and relatively strong motility after 72 h of storage.

3.6 Effects of extender formulation and storage duration on semen quality

Prior to inferential modelling, diagnostic evaluation showed evidence of departure from normality for sperm motility, live sperm percentage, and dead sperm percentage (Shapiro–Wilk $p \leq 0.0042$); however, variance homogeneity assumptions were satisfied (Levene's test $p \geq 0.764$), and no influential outliers were detected using standardized residual or interquartile range approaches, as shown in the Online Resource 1 (Supplementary Table S5). Therefore, linear mixed-effects models were fitted to determine the effects of extender formulation, storage duration, and their interaction on semen quality traits.

Linear mixed-effects modelling demonstrated significant effects of extender formulation, storage duration, and their interaction on semen preservation outcomes (Table 3).

Table 3. Effects of extender formulation, storage duration, and their interaction on semen quality traits of pooled Red Sokoto buck semen evaluated using linear mixed-effects models

A. Fixed-effect analysis

Outcome	**Fixed effect**	**Num df**	**Den df**	**F-value**	**p-value**	**Interpretation**
Sperm motility (%)	Extender	10	99	217.88	<0.001	Significant extender effect
	Storage duration	2	99	1374.81	<0.001	Significant storage effect
	Extender × storage duration	20	99	11.12	<0.001	Significant interaction
Live sperm (%)	Extender	10	105.42	18.98	<0.001	Significant extender effect
	Storage duration	2	105.62	380.1	<0.001	Significant storage

						effect
	Extender × storage duration	20	105.62	1.68	0.049	Significant interaction
Dead sperm (%)	Extender	10	105.42	18.98	<0.001	Significant extender effect
	Storage duration	2	105.62	380.1	<0.001	Significant storage effect
	Extender × storage duration	20	105.62	1.68	0.049	Significant interaction

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507 B. Variance components and model fit statistics

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Outcome	**Experimental batch random-effect SD**	**Residual SD**	**AIC**	**BIC**	**Model interpretation**
Sperm motility (%)	0	3.57	603	694	Minimal between-experiment variability
Live sperm (%)	2.35	2.3	565	659	Experimental batch variability accounted for
Dead sperm (%)	2.35	2.3	565	659	Experimental batch variability accounted for

509 **Footnote:** *Linear mixed-effects models included extender formulation, storage duration, and their interaction as*
510 *fixed effects, with independent pooled semen experimental batch fitted as a random intercept. Storage duration was*
511 *modelled as a categorical variable. Fixed effects were evaluated using Type III analysis of variance with*
512 *Satterthwaite's approximation for denominator degrees of freedom. A random-effect standard deviation of zero*
513 *indicates negligible additional variability attributable to experimental batch after accounting for fixed effects*.

514

For sperm motility, extender formulation had a significant effect ($F_{10,99} = 217.88$, $p < 0.001$), indicating substantial differences in the ability of extender formulations to maintain sperm movement during storage. Storage duration exerted the strongest influence on motility ($F_{2,99} = 1374.81$, $p < 0.001$), confirming progressive deterioration during liquid preservation. The significant extender × storage duration interaction ($F_{20,99} = 11.12$, $p < 0.001$) demonstrated that the rate of motility decline differed among extender formulations.

Similar effects were observed for sperm viability. Live sperm percentage was significantly affected by extender formulation ($F_{10,105.42} = 18.98$, $p < 0.001$) and storage duration ($F_{2,105.62} = 380.10$, $p < 0.001$). The significant interaction between extender and storage duration ($F_{20,105.62} = 1.68$, $p = 0.049$) indicated formulation-dependent differences in maintaining sperm membrane integrity over time.

Because dead sperm percentage represents the inverse relationship of sperm viability, comparable effects were observed, with significant extender, storage duration, and interaction effects (Table 3). These findings confirm that extender composition influenced both functional sperm preservation and cellular survival during storage.

Model variance estimates showed negligible experimental batch variability for sperm motility, whereas live and dead sperm models retained measurable between-experiment variation (random-effect SD = 2.35). This indicates that most variation in sperm motility was explained by extender formulation and storage duration rather than differences between pooled semen processing batches.

Because significant extender × storage duration interactions were detected for semen quality traits, Tukey-adjusted post-hoc comparisons were performed to identify extender-specific differences at each storage duration.

3.7 Post-hoc comparison of extender-specific semen preservation performance

The significant interaction effects observed in the mixed-effects models were further explored using Tukey-adjusted post-hoc comparisons (Table 4).

Table 4. Estimated marginal means and Tukey-adjusted comparisons of sperm motility and viability among extender formulations during 72 h liquid preservation of pooled Red Sokoto buck semen

Extender	Motility 24 h	Motility 48 h	Motility 72 h	Live sperm 24 h	Live sperm 48 h	Live sperm 72 h
5%D	61.67^{c}	41.67^{cd}	3.33^{ab}	88.7^{abcd}	80.4^{ab}	72.5^{abc}
EYC	88.33^{e}	82.67^{e}	45.00^{c}	94.4^{bcd}	87.5^{bc}	80.1^{cd}
NS	45.00^{ab}	30.00^{bc}	5.00^{ab}	91.5^{abcd}	84.5^{abc}	73.8^{abc}
SC	41.67^{ab}	18.33^{a}	11.67^{ab}	87.1^{a}	80.4^{a}	68.3^{a}
TJ	41.67^{ab}	21.67^{ab}	0.00^{a}	85.8^{ab}	81.6^{ab}	68.2^{ab}
TJD	43.33^{ab}	26.67^{ab}	3.33^{ab}	86.7^{abc}	85.5^{abc}	74.8^{abc}
TJDEY	73.33^{d}	51.67^{d}	13.33^{b}	91.3^{abcd}	83.6^{abc}	77.1^{cd}
TJEYNS	50.00^{bc}	28.33^{ab}	3.33^{ab}	94.7^{cd}	87.8^{bc}	79.6^{cd}
TJNS	38.33^{a}	21.67^{ab}	8.33^{ab}	90.9^{abcd}	88.6^{bc}	76.9^{bcd}
TJSC	61.67^{c}	48.33^{d}	3.33^{ab}	88.2^{abc}	83.7^{abc}	74.7^{abc}
TJSCEY	88.33^{e}	71.67^{e}	35.00^{c}	97.2^{d}	90.8^{c}	84.2^{d}

Footnote: *Values represent estimated marginal means (%) obtained from linear mixed-effects models containing extender formulation, storage duration, and their interaction as fixed effects, with experimental batch fitted as a random intercept. Within each column, means sharing at least one superscript letter are not significantly different, whereas means without a common superscript differ significantly based on Tukey-adjusted pairwise comparisons ($p < 0.05$). EYC = egg yolk citrate extender.*

During early preservation (24 h), EYC and TJSCEY produced the highest sperm motility values (88.33%) and shared the highest statistical grouping, indicating superior initial preservation of sperm movement. TJDEY also maintained relatively high motility (73.33%) but was significantly lower than EYC and TJSCEY.

After 48 h of storage, EYC maintained the highest motility (82.67%) and remained statistically comparable with TJSCEY (71.67%), whereas several tomato-based formulations without egg yolk showed greater reductions in sperm movement.

At 72 h, extender differences became more pronounced. EYC maintained the highest sperm motility (45.00%), followed by TJSCEY (35.00%), with both belonging to the highest statistical grouping. In contrast, TJ resulted in complete loss of observable sperm movement (0.00%), confirming poor long-term preservation capacity when tomato juice was used alone.

For sperm viability, TJSCEY consistently demonstrated superior preservation. It maintained the highest live sperm percentage at 24 h (97.2%), 48 h (90.8%), and 72 h (84.2%). At the final storage period, TJSCEY showed significantly greater viability preservation compared with poorer-performing formulations such as SC (68.3%) and TJ (**68.2%).

Overall, post-hoc comparisons confirmed that EYC provided the strongest protection of sperm motility, whereas TJSCEY offered the best combined preservation of viability and sustained motility, supporting its potential as an alternative extender formulation for short-term liquid storage of Red Sokoto buck semen.

3.8 Overall preservation efficiency ranking of extender formulations

Preservation efficiency indices demonstrated clear differences among extender formulations in their ability to maintain semen quality between 24 and 72 h of liquid storage (Table 5).

Table 5. Preservation efficiency ranking of extender formulations based on sperm motility retention, viability maintenance, and mortality changes in pooled Red Sokoto buck semen after 72 h of liquid storage

Extender	**Motility retention**	**Viability retention**	**Motility loss**	**Mortality**	**Overall descriptive note**

	(%)	(%)	(%)	increase (%)	
EYC	50.9	84.5	43.3	14.3	Highest motility retention
TJSCEY	39.6	86.3	53.3	12.99	Strong combined motility and viability retention
SC	28	78.3	30	18.88	Lower viability retention despite moderate motility retention
TJNS	21.7	84.2	30	13.94	Moderate preservation profile
TJDEY	18.2	84.9	60	14.17	Good viability retention but marked motility loss
NS	11.1	80.1	40	17.71	Limited motility retention
TJD	7.69	86.6	40	11.93	Highest viability retention and lowest mortality increase
TJEYNS	6.67	83.7	46.7	15.09	Low motility retention but reasonable viability retention
5%D	5.41	82.2	58.3	16.16	Poor motility retention
TJSC	5.41	85.1	58.3	13.5	Low motility retention but good viability retention
TJ	0	80.1	41.7	17.53	Complete loss of motility by 72 h

Footnote: *Preservation indices were calculated from mean semen quality values observed at 24 and 72 h of liquid storage. Motility and viability retention indicate the percentage of initial values maintained after storage, whereas motility loss and mortality increase represent absolute percentage-point changes. These descriptive indices were used to summarize biological preservation efficiency and should be interpreted alongside mixed-effects models and Tukey-adjusted comparisons*.

EYC demonstrated the highest preservation of sperm functional activity, retaining 50.9% of its initial motility after 72 h of storage. This was followed by TJSCEY, which retained 39.6% of initial motility. Although EYC showed superior motility preservation, it was associated with a slightly lower viability retention (84.5%) compared with some tomato-based extender combinations.

TJSCEY demonstrated the most balanced preservation profile, combining relatively high motility retention (39.6%) with excellent viability maintenance (86.3%) and a comparatively low increase in sperm mortality (12.99%). This indicated that the combination of tomato juice, sodium citrate, and egg yolk provided effective protection of sperm functional activity and membrane integrity during prolonged liquid storage.

Among the tomato-based combinations, TJD showed the highest viability retention (86.6%) and the lowest increase in sperm mortality (11.93%), suggesting effective maintenance of sperm membrane integrity. However, this was not accompanied by sustained motility preservation, as motility retention was only 7.69% after 72 h.

Similarly, TJSC and TJDEY maintained good viability retention (85.1% and 84.9%, respectively) but showed considerable motility losses (58.3% and 60.0%, respectively), suggesting that preservation of sperm viability did not necessarily correspond with maintenance of progressive movement.

The poorest preservation of sperm motility was observed in TJ, which showed complete loss of observable motility by 72 h (0% retention) despite retaining approximately 80.1% viability, indicating that some sperm cells remained structurally viable but had lost functional movement capacity.

Overall, preservation efficiency ranking confirmed the findings from the mixed-effects models and post-hoc comparisons, demonstrating clear differences in extender performance during liquid storage. EYC remained the most effective formulation for maintaining sperm motility, whereas TJSCEY provided the most balanced preservation profile by combining relatively high motility retention with the greatest overall sperm viability and lowest mortality. In contrast, extenders without effective protective combinations, particularly TJ, showed reduced preservation capacity, mainly due to substantial loss of sperm motility during prolonged storage.

A composite ranking approach incorporating overall sperm motility, viability, mortality, and storage stability, which further supported the preservation efficiency findings are provided in the Online Resource 1 (Supplementary Table S6; Supplementary Fig. S1). TJSCEY achieved the highest overall ranking due to its combination of high mean sperm viability (88.4%), lowest sperm mortality (11.6%), and balanced motility preservation (65.0%). Although

EYC demonstrated the highest mean motility (72.0%), TJSCEY provided a more balanced preservation profile across multiple semen quality indicators. Conversely, TJ ranked lowest overall due to reduced motility preservation despite moderate maintenance of sperm viability.

Correlation analysis was performed to further describe relationships among semen quality indicators during liquid preservation shown in the Online Resource 1 (Supplementary Table S7; Supplementary Fig. S2). Sperm motility showed a strong positive correlation with live sperm percentage (Pearson $r = 0.756$, 95% CI: 0.656–0.829; $p < 0.001$; Spearman $\rho = 0.787$), indicating that samples maintaining higher viability generally exhibited greater functional sperm movement, as shown in the Online Resource 1 (Supplementary Fig. S2A).

Conversely, sperm motility was strongly negatively correlated with dead sperm percentage (Pearson $r = -0.756$, 95% CI: −0.829 to −0.656; $p < 0.001$; Spearman $\rho = -0.787$), demonstrating that increased sperm mortality was associated with reduced motility during preservation, as shown in the Online Resource 1 (Supplementary Fig. S2B). Live and dead sperm percentages showed a perfect inverse relationship (Pearson $r = -1.000$; Spearman $\rho = -1.000$; $p < 0.001$), reflecting their complementary calculation from the same viability assessment rather than an independent biological association, as shown in the Online Resource 1 (Supplementary Fig. S2C).

4. Discussion

4.1 General effects of storage duration on semen deterioration

This study evaluated the influence of different extender formulations on the preservation of pooled Red Sokoto buck semen during liquid storage. The results demonstrated a progressive deterioration in semen quality with increasing storage duration, characterized by declining sperm motility and viability and increasing sperm mortality. The significant effect of storage duration observed across semen quality parameters indicates that preservation time remains a critical factor affecting sperm functional competence, even when protective extender formulations are used.

The marked reduction in sperm motility during storage agrees with previous studies showing that sperm movement characteristics are among the earliest indicators affected by preservation-induced cellular stress (Salamon and Maxwell, 2000; Gundogan, 2009; Rizkallah *et al*., 2022). Sperm motility depends on the maintenance of plasma membrane integrity, mitochondrial function, ATP production, and flagellar activity, all of which may become progressively impaired during storage (Vahedi Raad *et al*., 2024; Xu *et al*., 2025). In the present study, motility declined more rapidly than sperm viability, suggesting that functional impairment of sperm movement occurred before complete loss of membrane integrity.

During liquid storage, spermatozoa are exposed to multiple stressors, including temperature changes, accumulation of metabolic by-products, membrane destabilization, and oxidative damage (Pintus & Ros-Santaella, 2021; Wang *et al*., 2025). Cooling-induced stress can alter membrane lipid organization, resulting in reduced membrane fluidity and impaired sperm function (Castro *et al.,* 2025). These changes are particularly important in buck semen because sperm plasma membranes contain high concentrations of polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation (Van Tran *et al*., 2017; Simsek *et al*., 2025).

Oxidative stress has been identified as one of the major causes of sperm deterioration during semen preservation. Although physiological concentrations of reactive oxygen species (ROS) are necessary for sperm capacitation and fertilization processes, excessive ROS production during storage overwhelms endogenous antioxidant defence systems, causing lipid peroxidation, mitochondrial dysfunction, DNA damage, and reduced sperm survival (Kaltsas, 2023; Fleming & Thomson, 2025; Aitken *et al.,* 2026). Therefore, incorporation of protective compounds capable of reducing oxidative injury represents an important strategy for improving semen extender efficiency.

4.2 Effectiveness of conventional egg yolk citrate extender

Egg yolk citrate demonstrated superior preservation of sperm motility during storage, confirming its effectiveness as a conventional semen extender. The ability of EYC to maintain higher sperm motility agrees with previous reports demonstrating the protective role of egg-yolk-based extenders during preservation of mammalian semen (Alcay *et al.,* 2016; Bustani & Baiee, 2021).

The protective effects of egg yolk are mainly attributed to low-density lipoproteins (LDL), which interact with the sperm plasma membrane and reduce damage associated with cooling and storage (Akhter *et al*., 2011; Dong *et al*., 2011; Chang *et al.,* 2025). LDL molecules help stabilize membrane phospholipids, prevent excessive lipid loss, and reduce cold shock injury, thereby supporting sperm motility and viability during preservation (Quinn *et al.,* 1980; Ondřej *et al*., 2019; Castro *et al.,* 2025).

The superior motility observed with EYC in this study suggests effective maintenance of sperm functional capacity. However, semen preservation success depends on multiple characteristics, including motility, viability, membrane integrity, and resistance to oxidative damage (Tarig *et al.,* 2017; Bustani & Baiee, 2021). Therefore, although EYC maintained the highest sperm motility, the combined evaluation of semen quality traits indicated that tomato juice-containing formulations with egg yolk components may provide additional protective benefits.

4.3 Protective effects of tomato juice-based extender combinations

Among tomato juice-containing formulations, TJSCEY demonstrated the most balanced preservation performance, maintaining favourable sperm motility, the highest viability, and reduced sperm mortality during storage. TJDEY also demonstrated improved preservation compared with several other tomato-containing formulations. These findings suggest that tomato-derived compounds may provide beneficial effects when combined with extender components that provide adequate membrane stabilization.

The improved performance of tomato–egg yolk combinations may be associated with complementary protective mechanisms. While egg yolk components protect sperm membranes against cold shock, tomato-derived antioxidants may reduce oxidative damage occurring during storage. Tomato is particularly rich in lycopene, vitamin C, carotenoids, flavonoids, and phenolic compounds, all of which contribute to antioxidant activity (Silvestre *et al*., 2021).

Lycopene is one of the most powerful naturally occurring carotenoid antioxidants and has been reported to neutralize singlet oxygen and reduce lipid peroxidation in biological membranes (Rao *et al.,* 2006; Imran *et al*., 2020; Shafe *et al.,* 2024). Previous studies have shown that lycopene supplementation can improve semen quality by enhancing antioxidant status, reducing oxidative stress markers, and supporting sperm motility and membrane integrity (Durairajanayagam *et al*., 2014; Tvrdá *et al*., 2016).

The beneficial response observed with TJSCEY therefore suggests that antioxidant supplementation may be most effective when combined with extender systems that simultaneously provide nutritional support, buffering capacity, and membrane protection. Similar observations have been reported with other plant-derived antioxidant additives, where improvements in semen quality depended on antioxidant concentration, extender composition, and storage conditions (Cammisotto *et al*., 2021; Meulmeester *et al*., 2022).

4.4 Limited preservation ability of tomato juice alone

Despite the presence of antioxidant compounds, tomato juice alone demonstrated poor preservation efficiency, with complete loss of observable sperm motility after prolonged storage. This finding highlights that antioxidant capacity alone is insufficient for maintaining sperm function during liquid preservation.

Effective semen extenders must perform multiple roles, including maintaining osmotic balance, supplying energy substrates, regulating pH, protecting sperm membranes, and reducing oxidative injury (Bustani & Baiee, 2021; Gacem *et al*., 2025). Although tomato juice contains antioxidants, it may lack sufficient membrane-stabilizing molecules required to protect spermatozoa against cold shock and structural damage.

Furthermore, antioxidant supplementation follows a dose-dependent response, and excessive or unbalanced antioxidant concentrations may disrupt normal oxidation–reduction processes required for sperm function (Kaltsas, 2023; Bouhadana *et al.,* 2025; Moustakli *et al.,* 2025). Therefore, natural antioxidants require appropriate formulation and concentration optimization before use in semen preservation systems.

The contrasting performance between TJ alone and TJSCEY demonstrates that tomato juice may be more valuable as a complementary antioxidant additive rather than a complete substitute for established extender components such as egg yolk citrate.

4.5 Practical implications for goat artificial insemination

Improvement of semen preservation methods is essential for wider adoption of artificial insemination and genetic improvement programmes in goats. Artificial insemination facilitates dissemination of superior genetics, conservation of valuable breeds, and improved reproductive management; however, its application in smallholder systems is often constrained by semen handling and preservation limitations (Cseh *et al*., 2012; Foote, 2022; Dhara *et al.,* 2023).

The promising performance of TJSCEY suggests that locally available antioxidant sources may contribute to developing more accessible extender strategies, particularly in resource-limited livestock production systems. The use of affordable plant-derived additives could reduce dependence on expensive commercial extender supplements while improving reproductive biotechnology adoption.

However, laboratory semen quality does not always directly predict fertility outcomes. Therefore, further studies evaluating conception rates, fertility performance, microbial safety, extender optimization, and long-term preservation potential are required before practical field recommendations can be made.

4.6 Strengths and limitations

This study has several strengths, including the controlled comparison of multiple extender formulations, repeated assessment across different storage durations, and simultaneous evaluation of sperm motility, viability, and mortality. Additionally, the use of linear mixed-effects modelling allowed experimental batch variability to be incorporated, providing more robust estimates of extender and storage effects compared with simple fixed-effect analyses.

However, some limitations should be considered. First, semen from individual bucks was pooled before allocation into extender treatments. Although pooling minimized individual variation and enabled direct comparison of extender effects, it prevented assessment of buck-specific differences in semen preservation response. Individual male variation is known to influence semen characteristics and preservation tolerance (Holt, 2000; Benson *et al.,* 2012).

Second, this study evaluated laboratory semen quality indicators only. Although motility and viability are important predictors of sperm function, fertility trials remain necessary because successful reproduction depends on additional factors including sperm transport, capacitation, fertilization ability, and embryo development (Rodríguez-Martínez, 2006; Tanga *et al.,* 2021).

Finally, the evaluation was limited to short-term liquid storage. Future studies should investigate the effects of tomato-based extender combinations during longer preservation periods, cryopreservation, and artificial insemination trials under field conditions.

5. Conclusion

Short-term liquid storage resulted in progressive deterioration of Red Sokoto buck semen quality, characterised by declining sperm motility and viability and increasing sperm mortality. Preservation efficiency differed significantly among extender formulations, demonstrating that extender composition is a major determinant of semen quality during storage. While the conventional egg yolk citrate extender provided the greatest protection of sperm motility, tomato juice supplementation enhanced semen preservation when incorporated into appropriate extender combinations rather than when used alone. Among the formulations evaluated, the tomato juice-sodium citrate-egg yolk (TJSCEY) extender demonstrated the most favourable overall preservation profile by providing the best balance between motility retention, viability maintenance, and reduced sperm mortality during 72 h of liquid storage. These findings suggest that tomato-derived antioxidants can serve as cost-effective complementary

additives for improving buck semen extenders and support further investigation of TJSCEY under field conditions, including fertility trials, optimization of formulation, and assessment of oxidative stress biomarkers.

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Author contributions

S.S. conceived and designed the study. S.S., AT.S. and H.L.J. conducted the laboratory experiments. J.Y. and A.A.S. assisted with semen collection and sample storage. A.S. performed the statistical analyses, interpreted the data, and prepared the first draft of the manuscript. S.S., A.S. and E.I.O. contributed to the study design, critically revised the manuscript for important intellectual content, and approved the final version for publication. All authors read and approved the final manuscript.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

All experimental procedures involving animals were approved by the Animal Research Ethics Committee of Usmanu Danfodiyo University, Sokoto (Approval No. UDUS/IACUC/2026/R09) and were conducted in accordance with the institutional guidelines for the care and use of animals in research.

Competing interests

The authors declare that they have no competing interests.

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