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Effects of different extenders and storage temperatures on longevity of small East African goat (*Capra hircus*) semen

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ABSTRACT

Goat production is central to resource-poor rural livelihoods in the tropics. However, it is constrained by high inbreeding levels and poor access to good quality breeding bucks. In this study, semen extenders differing in egg yolk and energy level were compared in their ability to maintain liquid semen from a tropical goat breed, when stored at physiological (37 °C) or refrigerator (4 °C) temperature. Semen was collected weekly for nine weeks from seven bucks of the Small East African goat breed, diluted in three extenders and incubated at 37 °C or 4 °C for 24 h. The semen parameters: viability, motility and morphology were better preserved when semen was incubated at 4 °C compared to 37 °C across all extenders. Sperm motility after 24 h at 4 °C was 50.4 ± 5.5%, 50.4 ± 6.2% and 54.3 ± 5.4% for Extender 1 (high glucose, 18% egg yolk), Extender 2 (low glucose, 2.5% egg yolk) and Extender 3 (low fructose, no egg yolk), respectively. Extender 2 maintained significantly higher viability values (67.7 ± 2.5%) at 24 h compared to Extenders 3 (59.3 ± 2.1%) and 1 (46.7 ± 4.2%). Normal morphology after 24 h (88.1 ± 3.1% at 4 °C and 85.6 ± 3.1% at 37 °C) fell within the acceptable range for good quality semen. Acceptable quality was maintained within 8 h and up to 24 h from collection for non-frozen goat semen stored at 37 °C and 4 °C temperature, respectively. These results imply that Small East African goat semen diluted in low or non-egg yolk based extenders and stored at refrigerator temperature can be valuable for artificial reproduction of native goats on low resource capacity farms in developing countries.

1. Introduction

The Small East African (SEA) goat is a group of unimproved and heterogeneous type of goat indigenous to eastern and southern parts of Africa. These goats form an integral component of smallholder multi-enterprise farming systems, through their contribution to food production, household dietary and income diversity, meeting socio-cultural obligations and as a livelihood safety net (Swanepoel et al., 2010). Prolificacy coupled with hardiness and adaptation to the local environment, make the SEA goat an important and unique genetic resource for smallholder farmers in resource-poor communities. However, goat production is constrained by high inbreeding levels and shortage of quality breeding stock. Reproductive biotechnologies such as semen preservation and artificial insemination could be used to address these challenges and improve the performance of goats in poor communities (Rege et al., 2011).

Semen collection, preservation and transportation facilitate exchange of genetic material between goat flocks and breeding animals across spatial and temporal scales (Morrell, 2011; van Arendonk, 2011). The ability to preserve integrity and functionality of semen for a certain time is critical for artificial livestock breeding programs (Lehloenya, 2008). For long term storage, semen is cryopreserved at ultra-low temperature in liquid nitrogen (−196 °C). However, cryogenic storage is expensive and inaccessible for most smallholder goat keepers in remote environments (Rege et al., 2011; van Arendonk, 2011). Under these circumstances, storage of non-frozen goat semen under optimal extender and temperature conditions for short term use are possible solutions. Extended semen has been incubated at physiological (36–37 °C), room (15–20 °C) or refrigerator (4–5 °C) temperature (Leboeuf et al., 2000; Lemma, 2011). Traditionally, non-frozen semen is used within 6 h of collection, which gives little room for transportation of semen from a semen station to inseminate females in heat on the farms. If the longevity of diluted, non-frozen goat semen could be improved, use of liquid semen under farm conditions would be facilitated.

Semen maintained *in vitro* is subject to post-ejaculatory handling and storage conditions that quickly diminish its quality and fertilising potential. These conditions include generation of reactive oxygen species, depletion of energy and other nutrients, changes in osmotic conditions, and temperature related shocks (Lieberman et al., 2016). To protect against these challenges, semen is generally diluted in extender

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media. Semen extenders contain buffering systems in the form of ionic or non-ionic substances (such as Tris, sodium phosphate, sodium citrate, citric acid) to reduce osmotic pressure variations and maintain pH of the medium; cryoprotectants (such as glycerol, propylene glycol and ethylene glycol), a source of lipoprotein or high-molecular-weight material (such as egg yolk, milk, or soybean lecithin) to prevent cold shock, sources of nutrients like carbohydrates (fructose or glucose), lipids, and proteins (egg yolk, skim milk) to provide nourishment for the sperm cells, antibiotics (streptomycin, penicillin) to ensure a microbial free environment, and other additives such as enzymes and antioxidants (Rehman et al., 2013; Sieme et al., 2016). Semen is known to naturally contain fructose as energy source and during extension, glucose and other sugars can also be utilized by spermatozoa.

Most extenders for both fresh and frozen ruminant semen, including goats, are composed of Tris, egg yolk and glycerol. Low density lipoproteins in egg yolk are known to protect the spermatozoa from cold shock as semen is cooled from physiological to 4–5 °C (Sieme et al., 2016). However, negative interactions have been reported between egg yolk phospholipids and a bulbourethral gland secretion present in goat seminal plasma named egg yolk coagulating enzyme (EYCE). The EYCE is known to catalyse the hydrolysis of egg yolk lecithin into fatty acids and lysolecithin, which are cytotoxic (Sen et al., 2015; Ngoma et al., 2016). In addition, susceptibility to EYCE has been observed to vary between breeds of goat (Purdy, 2006; Ramukhithi et al., 2011). For these reasons, goat semen extenders are made with a safe margin of egg yolk to avoid coagulation and yet provide sperm cells with required nutrients. The effect of extending goat semen has been determined by many researchers (Leboeuf et al., 2000; Ashmawy et al., 2010; Medrano et al., 2010; Ramukhithi et al., 2011). To our knowledge, the effect of different extenders or storage temperature on the longevity of non-frozen buck semen has not been determined in Small East African goats. The present study sought to evaluate the longevity of non-frozen semen from a tropical goat breed when diluted in three extenders differing in egg yolk and energy concentration and stored at either 37 °C or 4 °C.

2. Materials and methods

2.1. Location and animal management

The Small East African (SEA) goat flock maintained at the research farm of Chinhoyi University of Technology was used for the experiment. The research farm is located outside Chinhoyi town, about 120 km northwest of Harare, Zimbabwe, between 17° 21′ 10.8″ S latitude and 30° 12′ 21.6″ E longitude. The farm is located in a warm (20–30 °C), high rainfall (750–1000 mm/year, unimodal) area (Vincent et al., 1960). Vegetation is dominantly wooded shrub land with *Terminalia sericea, Brachystegia boehmii, Combretum molle* and *Acacia* spp., in association with various *Hyparrhenia* and *Hyperthelia* grass species. Soils are rich, deep red clays. The goat flock is reared under semi-intensive management system consisting of daily grazing for about 7 h with some supplementary concentrate feed at the end of the day. Seven experimental bucks (mean body weight 36.7 ± 6.3 kg; aged 2–3 years) were separated from the rest of the flock a month before the start of the experiment. They were accommodated in a group pen with an open air run and given concentrate feed (200 g/day, 12% CP and 13 MJ/kg ME) twice a day and *ad libitum* access to water and good quality native hay (6% CP, *Hyperrhenia* spp.). In addition, the bucks received routine health inspection, anti-helminthic drenching, dipping and vaccination against endemic diseases.

2.2. Extenders used in the experiment

Three Tris-sodium citrate extenders differing in the level of egg yolk (0%, 2.5% and 18%) and type type of sugar (glucose or fructose) were prepared for the experiment (Table 1). Extender 1 was a high energy (1.38% w/v glucose), high egg yolk (18% v/v) extender. Extender 2 was a low energy (0.30% w/v glucose), low egg yolk (2.5% v/v) extender. Extender 3 was a low energy (0.20% w/v fructose), non-egg yolk (0% egg yolk) extender.

Table 1
Composition of the goat semen extenders used in the study.

Ingredients	Extender 1	Extender 2	Extender 3
Fructose (g)	0.00	0.00	0.20
Glucose (g)	1.38	0.30	0.00
Fresh egg yolk (ml)	18.00	2.50	0.00
Tris (g)	3.025	3.025	4.000
Sodium Citrate (g)	1.70	1.99	2.00
Glycerol (ml)	0.00	2.00	1.40
Distilled water (ml)	94.0	91.0	93.0

2.3. Semen collection and processing

Semen collection was done between February and April 2016, under the guidance of a qualified veterinarian and according to the 'Guidelines: Animal Handling and Sample Collection' of Chinhoyi University of Technology. Semen was collected in the morning (0600 − 0700 h) using an electro-ejaculator standardized for small ruminants (Minitube, Germany). A lubricated probe was inserted into the rectum and an electrical stimulation was applied for 4–8 s. When the electro-stimulation was stopped briefly, further massage was applied with the probe. This cycle was repeated for 3–4 electro-stimulations gradually increasing the current until an ejaculatory response was obtained. Semen was collected into pre-warmed graduated falcon tubes, divided into three fractions and diluted (1:1) in pre-warmed (37 °C) extender (Extender 1, 2, and 3). Each fraction of the extended semen was further divided into two and immediately incubated at 4 °C and 37 °C for up to 24 h.

2.4. Semen evaluation

Each semen fraction was evaluated for sperm motility, viability and morphology at 0, 2, 4, 8 and 24 h intervals after collection following Baracaldo et al. (2006). Eosin-nigrosin (eosin-Y 1.67 g, nigrosin 10 g, and sodium citrate 2.9 g, dissolved in 100 ml distilled water) dry-mount smears were made for the evaluation of viability and morphology at the lapse of each time interval. A semen smear was made on a pre-warmed microscope slide after mixing sperm suspension (˜10 µl) with stain (1:1 v/v). The slide was immediately air dried and stored for viewing later. Viability was assessed by counting 200 cells under bright-field microscopy at 400× magnification. Sperm displaying partial or complete purple staining were considered non-viable and those showing strict exclusion of stain were counted as viable. The same slides for viability were used for sperm morphological evaluation under bright-field microscopy at 400× magnification. Proportions of sperm with normal shape and size were determined at each time period. Occurrence of sperm morphological defects was determined at 0 h and 24 h after collection by counting 200 cells per slide. Each normal sperm was counted once, and each defect was counted separately even if multiple defects occurred on the same sperm cell.

For sperm evaluation, a bright-field microscope (Amscope, USA) with a pre-heated stage (37 °C) was used. An aliquot (10 µl) of semen was placed on a pre-warmed (37 °C) slide, covered with a cover slip and subjectively evaluated at 400× magnification. Multiple groups of ˜10 sperm per field were counted, estimating the proportion of motile *versus* immotile spermatozoa. Ten fields of view per slide were evaluated this way and averaged.

2.5. Statistical analyses

The study was carried out and analysed as a 3 × 2 factorial

experiment with repeated measures. Semen from each of the seven bucks was subjected to three extender and two storage temperature (4 °C and 37 °C) treatments and evaluated for sperm motility, viability and morphology at five time periods (0, 2, 4, 8 and 24 h). Five to nine semen samples per buck were collected and analysed. Statistical analysis was performed in IBM SPSS Statistics Version 20 for Windows (IBM, 2011) using repeated measures analyses of variance (RM-ANOVA) to evaluate within-subjects effects of incubation time and between-subjects effects of storage temperature and extender on the response variables. Effect sizes of the model factors and their interactions for each variable were determined using the Partial Eta Squared (η_P^2) parameter. Mauchly's W test indicated that all response variables violated the sphericity assumption ($P < 0.01$). Therefore, Greenhouse-Geisser ($\varepsilon < 0.75$) corrected results were reported (Field, 2013). The assumption of normality, required for ANOVA, was not violated (Box's test, $P > 0.05$). Where significant interaction between storage temperature and extender across time were observed ($p < 0.05$), analyses of main effects were ignored. Individual variability among the bucks in the quality of freshly ejaculated semen was investigated by one-way analysis of variance, and effects of model factors on sperm quality after 24 h of incubation were evaluated using the General Linear Model procedure of Minitab 17 (Minitab, 2014). *Post hoc* treatment of data was done using Tukey's HSD, at the 5% level of significance.

3. Results

Fresh semen characteristics from the seven SEA goat bucks used in this study are summarized in Table 2. Significant individual variability in fresh semen parameters was observed for ejaculate volume, normal morphology, viability and the sperm defects: decapitated heads, bent tails and stump tails. Other sperm motility morphological abnormalities observed but that did not differ ($P > 0.05$) between individual bucks include the occurrence of coiled tails, double tails, double heads and cytoplasmic droplets. The large standard deviations indicated significant variability in semen parameters from one ejaculate to another for individual bucks.

The interaction of extender and temperature across time reached significance but with very small effect sizes for the parameters sperm motility, $F(8,1268) = 2.627$, $P = 0.022$, $\eta_P^2 = 0.016$, and viability, $F(4,1268) = 3.63$, $P < 0.01$, $\eta_P^2 = 0.019$; thus making little contribution to the model. Effect size on the observed rate of decline in sperm motility over time was very small but significant for extender, $F(8,1268) = 3.29$, $P < 0.01$, $\eta_P^2 = 0.020$, and large for storage temperature, $F(4,1268) = 1667.06$, $P < 0.001$, $\eta_P^2 = 0.840$. Thus the two storage temperatures and three extenders did not produce similar effects for the sperm motility variable across time. Sperm motility after 24 h at 4 °C was 50.4 ± 5.5%, 50.4 ± 6.2% and 54.3 ± 5.4% for Extender 1 (18% egg yolk), Extender 2 (2.5% egg yolk) and Extender 3 (0% egg yolk), respectively (Fig. 1). Extender 3 maintained significantly higher sperm motility ($P < 0.01$) compared to Extenders 1 and 2, which had similar figures. At 37 °C, sperm motility at the end of the experimental period was significantly higher ($P < 0.01$) for Extender 3 (11.2 ± 6.1%) compared to Extender 1 (4.6 ± 2.8%) and Extender 2 (4.2 ± 5.2) which produced similar effects. Thus extender 3 performed marginally better than the other two extenders at each time period and in the maintenance of sperm motility in non-frozen Small East African goat semen.

The effect of the different extenders on viability at each storage temperature over time is given in Fig. 2. When viewed separately, storage temperature had a moderate effect size on the decline of sperm viability over time, $F(4,1268) = 217.04$, $P < 0.001$ $\eta_P^2 = 0.269$. Extender 2 maintained significantly higher viability values (67.7 ± 2.5%) after 24 h at 4 °C compared to Extender 3 (59.3 ± 2.1%) and Extender 1 (46.7 ± 4.2%). At 37 °C, there was no consistent performance of the different extenders in terms of maintenance of sperm viability. However, after 24 h Extender 3 maintained significantly higher ($P < 0.05$) viability values as compared with Extender 1 and Extender 2.

Interaction effects of the model factors for the normal sperm morphology parameter were significant only for time by temperature, $F(4,1268) = 118.11$, $p < 0.001$, $\eta_P^2 = 0.205$, with a moderate effect size. Thus, time had a large effect size while storage temperature across time had moderate effect size on the observed decline in the proportion of morphologically normal sperm. Over time, the three extenders did not differ in their effects on normal sperm morphology. At physiological temperature, the decline in the proportion of normal sperm over the 24 h of incubation period was linear and constant, while at refrigerator temperature it was curvilinear. Overall, the proportion of morphologically normal sperm differed for the two incubation temperatures only at 2 h (37 °C > 4 °C) and at 24 h (4 °C > 37 °C) of incubation (Fig. 3). For samples incubated at 4 °C, Extender 3 (89.4 ± 0.3%) maintained significantly higher proportion of normal sperm after the 24 h incubation period compared to Extender 1 (88.6 ± 0.2%) and Extender 2 (87.8 ± 0.4%), which had a similar effect.

An incubation time of up to 24 h had significant effects of increasing the proportion of sperm with decapitated heads, simple bent tails, coiled tails ($P < 0.001$) and tail stumps ($P < 0.01$). During the same period, the quality variables: motility, viability and normal morphology, decreased significantly compared to fresh semen. Statistically significant interaction effects of extender and temperature were observed for decapitated heads ($P < 0.001$) and tail stumps ($P < 0.01$) but not for normal sperm morphology, sperm motility, coiled tails and bent tails ($P > 0.05$), after storage for 24 h post ejaculation (Fig. 4). After 24 h, Extender 3 (32.7 ± 2.2%) had better overall sperm motility

Table 2
Fresh semen ejaculate characteristics (Mean ± SD) of seven goat bucks of the Small East African breed.

Semen variable	Buck (ejaculates)								Overall
	Buck 1	Buck 2	Buck 3	Buck 4	Buck 5	Buck 6	Buck 7	P-value	
Number of ejaculates	9	7	8	8	8	8	5		53
Ejaculate volume ml	0.49 ± 0.18^{bcd}	0.44 ± 0.16^{cd}	0.45 ± 0.19^{cd}	0.52 ± 0.18^{bc}	0.76 ± 0.28^{a}	0.57 ± 0.22^{b}	0.38 ± 0.08^{d}	0.000	0.52 ± 0.22
Gross motility %	94.1 ± 3.57	92.5 ± 4.70	93.0 ± 4.30	94.1 ± 4.17	93.5 ± 4.27	93.5 ± 4.12	95.0 ± 2.52	0.148	93.6 ± 4.09
Normal sperm %	97.1 ± 1.31^{a}	96.0 ± 2.21^{b}	96.5 ± 1.56^{ab}	97.1 ± 1.43^{a}	96.7 ± 1.53^{ab}	96.5 ± 1.44^{ab}	96.6 ± 1.33^{ab}	0.004	96.7 ± 1.61
Viability %	80.0 ± 2.14^{b}	81.1 ± 8.71^{b}	84.9 ± 3.89^{a}	83.1 ± 4.35^{a}	80.1 ± 3.85^{b}	80.3 ± 4.22^{b}	85.0 ± 7.50^{a}	0.023	82.8 ± 4.03
Decapitated head %	0.24 ± 0.35^{a}	0.46 ± 0.60^{b}	0.31 ± 0.35^{ab}	0.23 ± 0.33^{a}	0.25 ± 0.36^{a}	0.23 ± 0.33^{a}	0.30 ± 0.36^{ab}	0.007	0.30 ± 0.45
Bent tail %	1.81 ± 0.77^{bc}	1.64 ± 0.82^{a}	1.63 ± 0.77^{a}	1.63 ± 0.53^{a}	1.85 ± 0.81^{c}	1.88 ± 0.73^{c}	1.67 ± 0.76^{b}	0.000	1.78 ± 0.74
Coiled tail %	0.44 ± 0.57	0.80 ± 0.71	0.63 ± 0.64	0.38 ± 0.39	0.52 ± 0.59	0.63 ± 0.61	0.80 ± 0.71	0.063	0.57 ± 0.59
Double tail %	0.00 ± 0.00	0.10 ± 0.21	0.08 ± 0.19	0.00 ± 0.00	0.04 ± 0.14	0.04 ± 0.14	0.07 ± 0.14	0.052	0.04 ± 0.14
Stump tail %	0.24 ± 0.32^{a}	0.55 ± 0.51^{c}	0.50 ± 0.41^{bc}	0.35 ± 0.37^{ab}	0.44 ± 0.39^{abc}	0.46 ± 0.38^{abc}	0.27 ± 0.37^{ab}	0.019	0.36 ± 0.40
Double head %	0.15 ± 0.23	0.10 ± 0.21	0.08 ± 0.19	0.06 ± 0.17	0.08 ± 0.18	0.08 ± 0.19	0.20 ± 0.25	0.074	0.10 ± 0.20
Cytoplasmic droplet %	0.26 ± 0.40	0.30 ± 0.41	0.42 ± 0.50	0.35 ± 0.52	0.44 ± 0.49	0.46 ± 0.48	0.20 ± 0.25	0.129	0.35 ± 0.44

^{abc}Different superscripts within the same row indicate significant difference ($P \leq 0.05$) between bucks for that parameter.

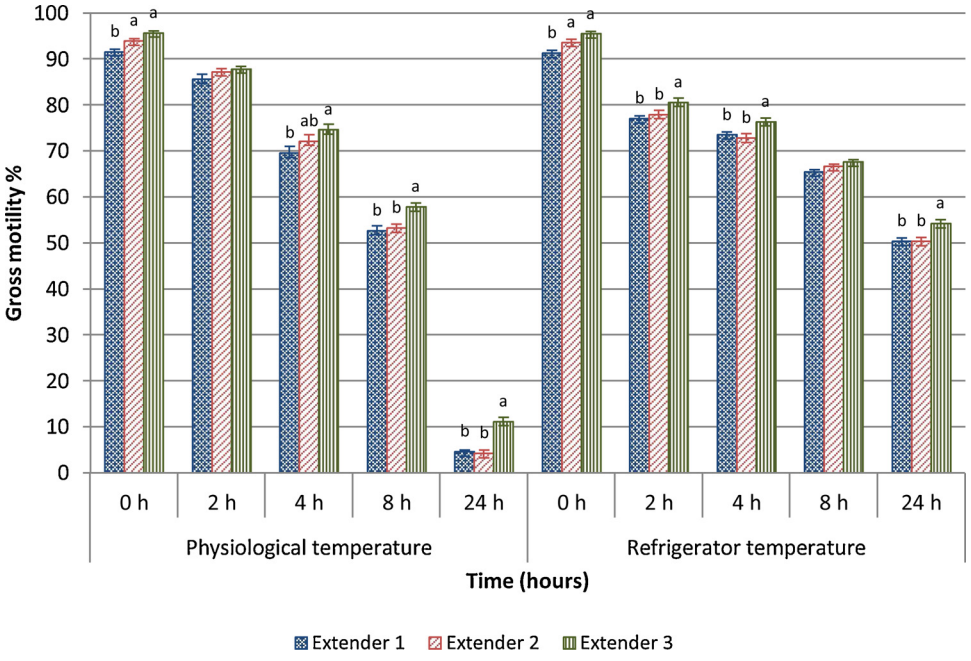

Fig. 1. Percent sperm gross motility (Mean ± SEM %) of non-frozen Small East African goat semen diluted in three extenders and evaluated after 0, 2, 4, 8 and 24 h storage at physiological (37 °C) and refrigerator (4 °C) temperature. Different letters (a, b) indicate differences between extenders at each sampling time within storage temperature regime.

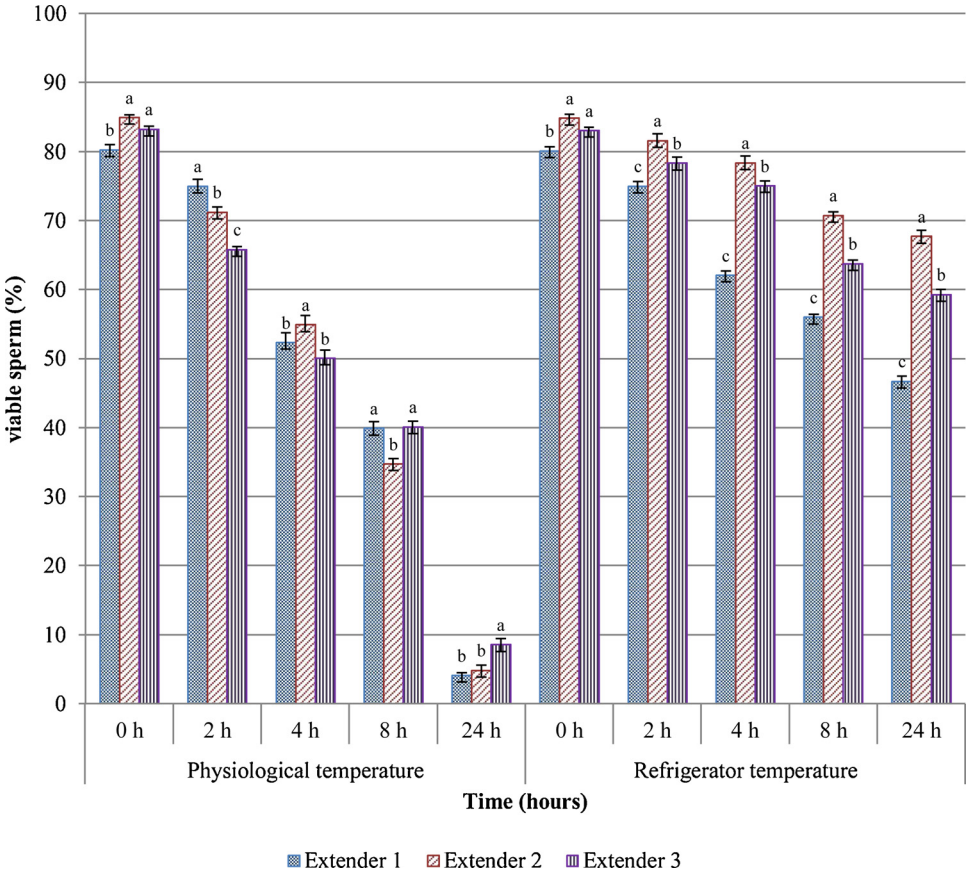

Fig. 2. Viability (Mean ± SEM %) of non-frozen Small East African goat semen diluted in three extenders and evaluated after 0, 2, 4, 8 and 24 h storage at physiological (37 °C) and refrigerator (4 °C) temperature. Different letters (a–c) indicate differences between extenders at each sampling time within storage temperature regime.

($P < 0.001$) compared to Extender 1 (27.7 ± 2.3%) and Extender 2 (27.3 ± 2.3%), which performed similarly. Semen incubated at 37 °C had significantly lower sperm motility values (6.7 ± 0.5%) compared to semen stored at 4 °C (51.7 ± 0.5%) after 24 h. At the end of the experimental period, samples incubated at 4 °C had higher proportions of morphologically normal sperm (88.6 ± 0.2%) compared to those incubated at 37 °C (86.2 ± 0.2%). Compared to other extenders, Extender 1 resulted in significantly higher decapitated heads at 37 °C, and significantly lower decapitated heads at 4 °C. Extenders 2 and 3 performed similarly at each temperature treatment. In terms of tail stump defects, the three extenders did not perform similarly across storage temperature. At the higher temperature, Extenders 2 and 3 had higher tail stumps compared to Extender 1. However, at the lower storage temperature, all three extenders differed from each other ($P < 0.05$), with Extender 3 giving the least and Extender 2 giving the highest proportion of stump tails. Temperature effects were observed in the

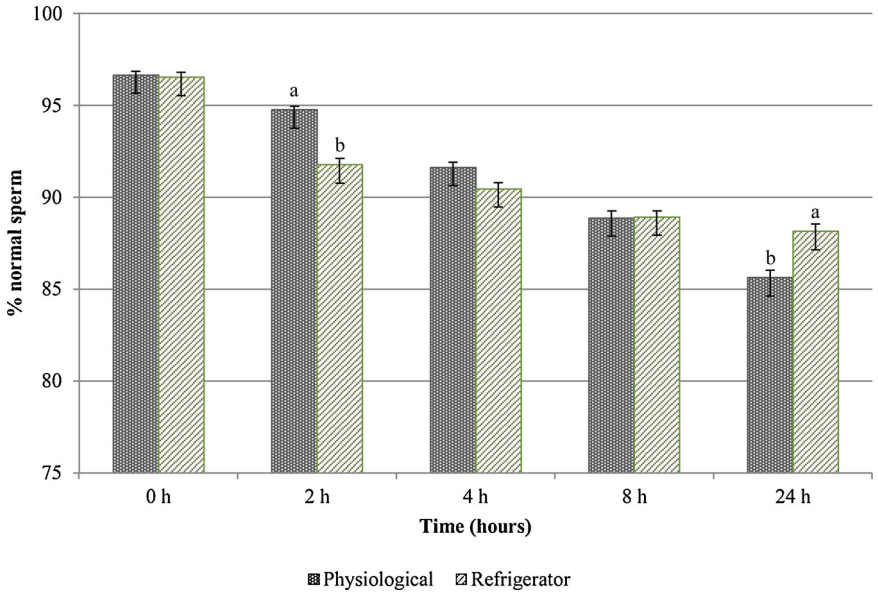

Fig. 3. Effect of storage temperature (4 °C and 37 °C) on the proportion of morphologically normal spermatozoa (Mean ± SEM %) of non-frozen Small East African goat semen following 0, 2, 4, 8 and 24 h incubation. Different letters (a, b) indicate significant differences between storage temperatures at each sampling time.

proportion of bent tails after 24 h of storage. Lower temperature storage resulted in lower proportions of bent tails compared to physiological temperature storage. No treatment effects were observed for the coiled tail parameter, except the effect of time. However, these sperm defects occurred in very small proportions to be of any biological consequence.

4. Discussion

In this study, three Tris-sodium citrate extenders were evaluated in their ability to maintain non-frozen Small East African goat semen quality over the course of 24 h under two storage temperature regimes: physiological (37 °C) and refrigerator (4 °C) temperature. Extender 1 was a high-glucose high-egg yolk extender, Extender 2 was a low-glucose low-egg yolk extender, and Extender 3 was a low-fructose non-egg yolk extender. Significant variability in fresh semen quality and longevity among the bucks was observed. This variability was higher with storage time, indicating that semen from individual males respond differently to storage conditions. These differences are thought to have both a genetic and biochemical basis (Seshoka et al., 2016). The ejaculate volume of 0.52 ± 0.224 ml observed in this study for the Small East African goat breed was lower compared to 0.70 ± 0.08 ml (Ramukhithi et al., 2011) and 1.77 ± 0.30 ml (Webb et al., 2004) obtained for South African indigenous goats using the same collection method. However, the yield is still within the range of 0.50–2.00 ml expected for goats (Almeida et al., 2007). The low semen yield may be related to the relatively small size of the breed compared to other goats in the region. It may also be due to lack of mineral supplementation in the experimental bucks prior to and during the study. Feed and mineral supplementation of breeding animals is known to have beneficial effects on testicular development and semen yield and quality (Almeida et al., 2007; Ramukhithi et al., 2011).

A progressive decline in all sperm quality variables studied, save for some defects whose proportion increased, was observed. Time had a significant and large effect size on the loss of sperm quality. Loss of quality over time is inevitable for spermatozoa as they are, by nature, catabolic cells with little or no healing capacity. The quality of sperm cells maintained *in vitro* is quickly diminished by energy and nutrient depletion, generation of reactive oxygen species, and changes in osmotic conditions over time. Thus, the aim of semen extension is to provide conditions that reduce catabolism and oxidative stress (Rehman et al., 2013; Sieme et al., 2016). One such condition investigated in this study is temperature. The hypothesis was that storing extended semen at reduced temperature as compared to physiological temperature helps to extend sperm life by slowing their metabolism. Incubation at 4 °C resulted in greater longevity of the semen compared to when the semen was incubated at 37 °C. Acceptable quality in non-frozen goat semen was maintained within 8 h and up to 24 h from collection when semen was incubated at 37 °C and 4 °C respectively, confirming findings by Morrell (2011). Similarly, Bayemi et al. (2010) observed greater longevity for non-frozen cattle semen incubated at refrigerator temperature compared to that at physiological temperature.

The second storage condition investigated was extender type. The three extenders used in this study had compositional differences in terms of the level of egg yolk and the type and concentration of sugar. This confers to the extenders differences in the ability to supply required nutrients, provide the right in-vitro environment and protect sperm cells from cold shock during the chilling process. Generally, it was observed in this study that any alteration in egg yolk concentration leads to a drastic decline in sperm motility and viability, while the absence of fructose was related to the loss of sperm viability and increase in the occurrence of sperm abnormalities. Sugars such as glucose and fructose are usually added to semen extenders since spermatozoa readily utilise these sugars for respiration. In addition, sugars provide osmotic balance and cold shock protection. Fructose is known to have the greatest molar concentration in goat seminal plasma (Qureshi et al., 2013). The three extenders all had sugar added in the form of either glucose or fructose. However they differed in the concentration of the sugar, with Extender 1 being a high energy diluent, probably above the normal concentration in goat seminal plasma. Although the molar concentration may have had an effect on the performance of Extender 1, it is unlikely that differences in the type of sugar contributed to differential performance of Extender 2 and Extender 3.

The extenders also differed in the level of egg yolk. Most extenders for goat semen use egg yolk as the basic non-permeating cryo-protective agent. Egg yolk protects against thermal shock due to the presence of phosphatidyl choline (lecithins) and lipoproteins (Aguiar et al., 2013). However, the dilution of goat semen in extenders containing egg yolk has been found to be toxic for goat semen due to the presence in goat seminal plasma of the enzyme, phospholipase A (also called egg yolk coagulating enzyme or EYCE), secreted by the bulbourethral glands. The EYCE is known to catalyse the hydrolysis of lecithin in egg yolk to

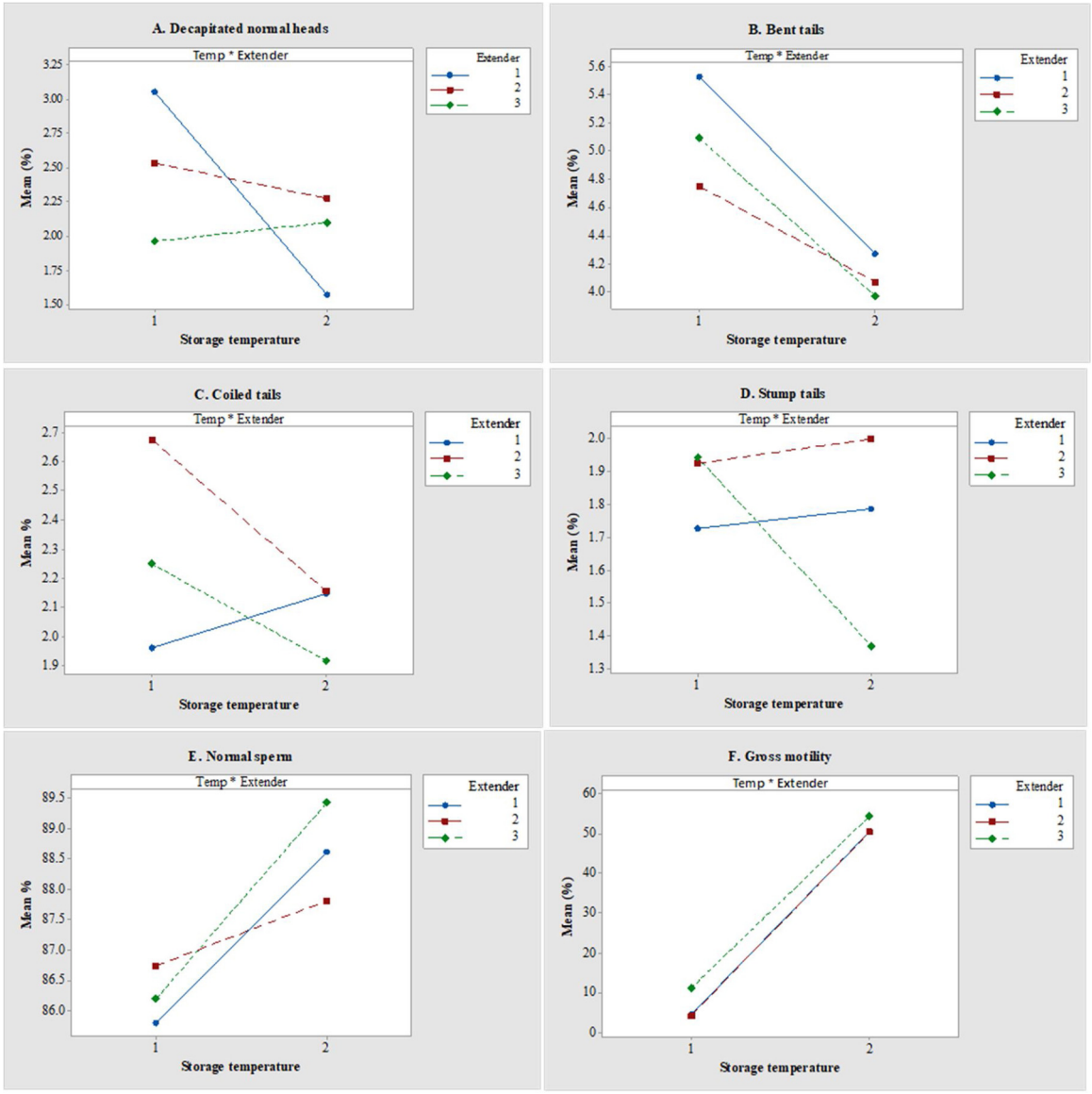

Fig. 4. Factorial plots for various sperm quality attributes of non-frozen Small East African goat semen 24 h post-ejaculation. Storage temperature: 1 = 37°C, 2 = 4°C; Extenders: 1 - glucose, no glycerol, 18% egg yolk; 2 - glucose-glycerol, 2.5% egg yolk; 3 - fructose-glycerol, no egg yolk.

fatty acids and lysolecithins, which are toxic and cause coagulation of the storage medium (Sen et al., 2015; Ngoma et al., 2016). However, research results from literature are somewhat contradictory in respect to the use of egg yolk in goat semen diluents. For instance, Medrano et al. (2010) reported limited tolerance by goat semen to extenders containing high levels of egg yolk or skim milk, while other researchers (Cabrera et al., 2005; Memon et al., 2013) reported better sperm quality preservation with increase in egg yolk concentration. In a number of papers (Purdy, 2006; Ramukhithi et al., 2011; Memon et al., 2013), breed variation in the susceptibility of goat semen to EYCE is reported, suggesting a genetic basis for these differences. Memon et al. (2013) proposed that egg yolk could be more detrimental to the survival of semen from temperate compared to tropical goat breeds. The present study used three extenders differing in egg yolk concentration (0%, 2.5% and 18%) for preserving non-frozen semen from a tropical goat breed. Extender 3 (no egg yolk) maintained better motility and viability compared to Extender 2 (2.5% egg yolk) and Extender 1 (18% egg yolk). Thus, the present study confirmed that egg yolk concentration has negative effects on the quality of non-frozen semen of a tropical goat breed. Future research in this area could compare a number of goat breeds in the susceptibility of their semen to EYCE.

Sperm morphology was an important semen trait in this study as low proportion of normal sperm and some sperm defects are associated with reduced rates of fertilization and embryonic development (Freneau et al., 2010; Chatiza et al., 2011; Memon et al., 2013). A number of sperm defects such as decapitated and double head forms, bent tails, coiled tails, cytoplasmic droplets, and tail stumps were observed in the study. Double head forms and tail stumps are classified as major sperm defects and are correlated to impaired fertility (Blom, 1983; Freneau et al., 2010). All other observed defects were minor and compensable (Saacke, 2008; Memon et al., 2013). This means that their effects could be compensated for by increasing the insemination dose. Semen storage resulted in an increase in the occurrence of detached heads, coiled tails and bent tails. Such increases may be attributed to sperm aging, temporary hyper-osmotic stress associated with addition of extenders (Guthrie et al., 2002), and temperature shock effects during handling (Lieberman et al., 2016). Such processes lead to ultra-structural, biochemical, and functional damage to the spermatozoa, resulting in an increase in sperm defects. Normal sperm morphology after 24 h of incubation (> 85% in this study) was well within the

minimum standards of > 70% acceptable for use of the semen in artificial reproduction practice (Chatiza et al., 2011).

5. Conclusions

In this study, the longevity of non-frozen semen from a tropical goat breed was evaluated when diluted in three extenders differing in the level of egg yolk and energy level and stored at either 37 °C or 4 °C. Acceptable sperm quality was maintained within 8 h and up to 24 h of incubation for samples stored at 37 °C and 4 °C, respectively. Semen was better preserved in low or non-egg yolk extenders. Across all storage temperatures and extenders, normal morphology after 24 h fell within the acceptable range required for good quality semen. These results show that for the best longevity, liquid preserved goat semen should be diluted in a low or non-egg yolk based extender, and incubated at 4 °C. This facilitates artificial reproduction of native goats on low resource capacity farms in developing countries. Whether such longevity differences would result in better fertility following artificial insemination remains mere speculation and needs to be investigated in a field trial with real female goats.

Conflict of interest statement

The authors declare that they have no affiliations with or involvement in any organization or entity with any interest (financial or non-financial) in the subject matter or materials discussed in this manuscript. In addition, there has been no significant financial support for this work that could have influenced its outcome.

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