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Viability and fertility of chilled and cryopreserved milt from wild African catfish (*Clarias gariepinus*) extended with coconut milk

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Abstract

Too many male catfish are sacrificed to obtain enough milt for fertilizing fish eggs. The wastage of these fish gametes during breeding due to seasonality and limitations of milt collection make it compelling to explore preservative measures for sperm storage. This study was carried out to evaluate milt characteristics and the effect of different extenders on the sperm of wild African catfish; *Clarias gariepinus* (*C. gariepinus*) in Maiduguri, Nigeria. The effects of preservation of *C. gariepinus* milt at 4℃ and at -196℃ was also investigated. Forty wild male *C. gariepinus* brood stock from Lake Alau, Maiduguri were used. The brood stocks were sacrificed, dissected and their reproductive tracts exposed and isolated. The testes were identified, stripped and milt samples collected, pooled and their pre-extension characteristics evaluated. Four different semen extenders; OviPlus® + egg yolk (E1), OviPlus® + coconut milk (E2), OviPlus® + coconut milk + egg yolk (E3) and coconut milk-based (E4) were used to extend and preserve the milt through chilling (4℃) and freezing (-196℃) for 4 and 2 days, respectively. The characteristics of extended, chilled and frozen milt were evaluated. The result of the study showed that E1, E2 and E3 maintained the sperm progressive motility up to 48 hours post extension (≥50%) and E4 maintained the progressive motility for up to 72 hours (>50%) at 4℃. All extenders used maintained progressive motility (>50%) and livability (>60%) post-thawed after freezing at -196℃. Conclusively, E4 showed better milt qualities preserved at 4℃ and -196℃. Thus, wild *C. gariepinus* milt can be collected and extended in coconut milk based extender and used to fertilize *C. gariepinus* eggs or preserved short-term at 4℃ or frozen at -196℃.

Keywords: Chilled storage, *Clarias gariepinus*, Coconut milk, Cryopreservation, Milt

Introduction

In Nigerian aquaculture, African catfish has a high commercial value, 2 to 3 times than that of other species, and can be artificially propagated to meet the demand for fingerlings (Omitogun *et al.,* 2010). However, catfish aquaculture faces a major hurdle of lack of dependable males for breeding (Omitogun *et al.,* 2010). This is because many fish brood stock are wasted during natural or induced spawning techniques. Fish farming is crucial for meeting the protein needs of a growing population, as fish offer a versatile and essential food source (Bamidele, 2007). To ensure a consistent supply of fish as a protein source, large-scale fish reproduction is therefore required throughout the year, independent of the breeding season (Ozigbo *et al.,* 2014). Artificial propagation methods constitute the major practicable means of providing enough high-quality seed for rearing in confined fish enclosure (Charo & Oirere, 2000). Identifying superior male catfish for sperm collection would significantly improve fish production. Additionally, preserving this sperm would allow females to be spawned even when males are unavailable (Nwankwo & Oroko, 2017).

Germplasm conservation of fish biodiversity being a practicable aid of preservation of genetic resources relies more on sperm cryopreservation only, as cryopreservation of oocyte and embryos is not possible in most species with telolecithal eggs (Labbé *et al.,* 2022; Ian & Martin, 2024). Even though fish sperm cryopreservation has achieved successes, it is challenging to standardize the technique across all fish species (Poh & Abd-Rashid, 2012). To date, successful cryopreservations of fish milt were reported in more than 200 freshwater species and 40 marine species globally (Gwo, 2000). However, cryopreservation protocols need to be individually established for each fish species, particularly regarding the medium used. Selecting a semen extender is crucial in artificial insemination, but many extenders vary in complexity and content (Ogbu *et al.,* 2014). Commercially available extenders require technical expertise for preparation, including choosing ingredients, precise measurements, and adjusting solution properties (Ogbu *et al.,* 2014). This makes them inaccessible to farmers in rural areas of Nigeria. Therefore, evaluating readily available natural extenders, like egg yolk and coconut milk, becomes necessary to maintain fish sperm quality under appropriate conditions for defined periods.

Studies show OviPlus®, a commercially available semen extender, is recommended for sperm recovery and preservation in various farm animals (Bukar *et al.,* 2017). Coconut milk and coconut water are promising natural extenders due to their energy compounds, which maintain sperm function (Iqbal & Solangi, 2011; Manisha & Shamapada, 2011; Ogbu *et al.,* 2014). Research suggests coconut water-based extenders are more effective at preserving sperm viability during cryopreservation. Egg yolk has also been explored as cryoprotectants in many studies, including those focused on African catfish sperm (Muchlisin, 2004; Muchlisin *et al.,* 2015), believing 10% egg yolk concentration is optimal for catfish sperm cryopreservation.

Wastage of fish genetic resources during breeding due to seasonality and limitations of current milt collection methods necessitates exploring cryopreservation for sperm storage (Omitogun *et al.,* 2010). Cryopreservation offers several advantages: it allows for year-round access to fish seed, facilitates genetic selection programs, and eliminates the need to sacrifice male catfish for each spawning event (Hecht & Britz, 1988; Nwankwo & Oroko, 2017).

The aim of this study was to obtain information on the milt characteristics and effect of some extenders on the milt quality of the wild male African catfish captured in Maiduguri, Nigeria with the intent of obtaining an optimal cryopreservation protocol that can serve as a means of preserving viable sperm for making fingerlings available to fish farmers all year round.

Materials and Methods

Ethical statement

All procedures performed in this experimental study were in accordance to the guidelines of the animal care and use community of the University of Maiduguri.

Study design

This study employed an experimental study design and a convenient sampling method.

Study location and timing

This study was conducted in the Artificial insemination (AI) Laboratory of the Department of Theriogenology, University of Maiduguri, Borno State, Nigeria. The annual rainfall in Borno state is averagely 320 mm, humidity of about 49% and evaporation of 203 mm per year. The rainfall is generally been heaviest in August. The annual temperature average is 35.4℃ (Bruton & Smith, 1988; Mayom & Mohammed, 2014). All fishes used were from Lake Alau, a natural water storage formed by River Ngada, situated off Maiduguri-Bama Road, some 14 km away from Maiduguri. This study was conducted in wet/spawning season (June to August).

Experimental fish

Forty adult males (≥ 250g body weight) of wild *C. gariepinus* were used for this study. All fish were bought live and transported from fish retailers; these fish were from Lake Alau in Maiduguri, Nigeria. Male *C. gariepinus* were identified according to the description by De-Graaf & Janssen (1996); Diyaware *et al*. (2010). Using a handheld digital electronic weighing scale (WeiHeng® 145.00), the body weight of each fish was measured and those that weighed ≥ 250 g were brought to the AI Laboratory and allowed to rest for at least 24 hours in a plastic tank before evaluations were carried out.

Preparation of extenders

Freshly laid eggs were collected, and egg yolks were extracted from them according to the method described by Olurode & Ajala (2016).

OviPlus® semen extender concentrate was prepared according to the manufacturers guide (composition: OviPlus®, citric acid, sugar, buffer, antibiotic, and ultra-purewater); 136 ml of bi-distilled water was added to the contents of the bottle (24 ml concentrate for the preparation of 200 ml ready to use extender), the stock solution was then stirred carefully into 40ml of egg yolk as extender one (OviPlus® + egg yolk) with pH of 8.0. In extender two (OviPlus® + coconut milk) with a pH of 7.6, egg yolk was substituted with coconut milk using the same procedure. While in extender three (OviPlus® + coconut milk + egg yolk) with a pH of 7.8, a combination of coconut milk + egg yolk 20 ml/20 ml (v/v) was substituted for egg yolk. The overall solutions were stored in the refrigerator at 4 °C until use and discarded after 3 days.

Tri-sodium citrate buffer was prepared by heating 100 ml of bi-distilled water to 60℃, and the preparation of 2.94 % weight/volume (w/v) of tri-sodium citrate was then made by using 2.94 g of tri-sodium citrate. This amount (2.94% Tri-sodium citrate) was dissolved in 250 ml flat-bottom flask. This solution with pH 7.4 was then shaken together and allowed to cool for a few minutes and kept refrigerated at 4℃ until use as described by Ogbu *et al*. (2014).

Coconut milk was obtained from the blended coconut flesh and water, and then centrifuged twice at 3000 revolutions per minute for 10 minutes, as described by Sule *et al.* (2007). The filtrate was used to prepare the extender. For extender four (coconut milk based semen extender), in preparation of 200 ml coconut milk semen extender (pH 7.3); 100 ml of 2.9% tri-sodium citrate - bi-distilled water solution containing 1g antibiotic in ratio 2:1 (1000 I.U crystalline penicillin and 1000 mg of streptomycin per ml, respectively) was mixed with 40ml coconut milk base, and the mixture was added to 10 ml egg yolk and stirred. This was refrigerated at 4℃ until use and discarded after 3 days. Further details on extender formulations and their compositions are highlighted on Table 1.

Milt collection and Pre-extension evaluation

Each fish was sacrificed and their testes were then excised, the milt samples was obtained by stripping and scraping the testes as described by Olumuji & Mustapha (2012) into a clean calibrated test tube for analyses.

The volume of pooled milt was measured and then divided into four portions. One portion was evaluated immediately for macroscopic characteristics (volume, viscosity, consistency, pH and color) and microscopic characteristics (concentration, motility, livability and morphologic abnormalities) as previously described by Hafez & Hafez (2000); Buyukhatipoglu & Holtz (2001); Rurangwa *et al.* (2004); Fauvel *et al.* (2010); Dominic & Abiodun (2014) and the other three portions were divided in to 5 aliquots, 4 aliquots was stored at 4℃ extended with four different extenders (OviPlus® + egg yolk, OviPlus® + coconut milk, OviPlus® + coconut milk + egg yolk extender and coconut milk-based extender), each of the four extended milts was further divided into four aliquots, and 1 aliquot was evaluated every day for four days. The remaining one aliquot was frozen using the two-step method described by Ofelia *et al.* (2012) and stored for at least 48 hours. All chilled and frozen milt was thawed rapidly at 37℃ for 10 seconds as described by Olaruwaju *et al.* (2015), and the post-thaw quality of the milt sample was evaluated immediately. Spermatozoa characteristics (such as motility, livability and morphology) were evaluated in fresh, chilled and post-thaw milt as described by Rurangwa *et al.* (2004); Fauvel *et al.* (2010).

Milt extension and storage

Sperm to diluents (extenders) ratios for milt storage were 1:10 (v/v) for both chilled and frozen semen as described by Azlina *et al.* (2012). Extenders used were OviPlus® + coconut milk (E1), OviPlus® + egg yolk (E2), OviPlus® + coconut milk + egg yolk (E3) and coconut milk-based extender (E4) as extender 1, 2, 3 and 4, respectively. The extended milt was then filled into sterile Ependorf tubes and chilled, while those that were to be frozen were fortified with 10% DMSO (dimethyl-sulphoxide) for cryoprotection as described by Basavaraja & Hedge (2004) then filled into 0.25 ml straws by capillary action and sealed, which were then subjected to freezing processes.

Cryopreservation

The two-step method of cryopreservation was used as described by Ofelia *et al.* (2012). After loading of milt into 0.25 ml straws of different colors for different extenders and completely sealed using an electric sealer (impulse sealer, PSF250A; AC 220V/50-60Hz, China), the straws were then immersed completely in cooled clean iced water to undergo cooling from 21℃ to 4℃ in a refrigerator and equilibrated for 2 hours for proper permeability of cryoprotectant (temperature measurement was observed using a digital electronic thermometer throughout the processes). After the equilibration procedure, milt samples were subjected to liquid nitrogen vapour exposure (temperature of -120℃) for 15 minutes in an insulated box filled with liquid nitrogen, with the samples placed at 4 cm above the liquid nitrogen, after which the samples were directly plunged into the liquid nitrogen tank at -196℃ for storage for at least 48 hours. Milt samples from each male broodstock were cryopreserved separately in order to maintain its inherent variability.

Thawing of chilled and cryopreserved milt

Chilled and cryopreserved milt were thawed rapidly using luke-warm water of 37℃ in a water bath; the chilled or frozen straws were removed from the refrigerator or liquid nitrogen tank respectively and thereafter, were completely immersed in to the water bath for not more than 10 seconds as described by Olaruwaju *et al.* (2015) and analyzed immediately.

Statistical analyses

All data generated from the study were analyzed using SPSS® software (version 20.0). Results were expressed as the Mean ± Standard Deviation. The differences in mean were tested using One-way Analysis of Variance (ANOVA), to determine significant difference in all parameters between groups. Repeated measures- ANOVA was used to determine over time related changes at storage durations while descriptive statistics was used to determine the mean and standard deviation in fewer than 2 groups. Differences with values of P<0.05 were considered to be statistically significant.

Results

The mean sperm concentration ranged from 1.9 × 10^9 to 3.8 × 10^9 with an average mean (± SD) of 2.75 ± 0.5 × 10^9 spermatozoa per ml. The average pH of the *C. gariepinus* milt in this study was 6.8 ± 0.25. The average mean semen volume was 3.45 ± 1.1ml. It was found that the avagrage progressive individual sperm motility was 83.5 ± 7.5% in all milt sample collected, 90.3 ± 6.9% live spermatozoa and 3.15 ± 2.2% morphologically abnormal spermatozoa (Table 2) were observed.

There was gradual decrease in sperm motility across all extenders with advancing storage time post-extension (Table 3). The result also revealed that individual progressive motility was still evident 96 hours post extension in all samples diluted with extension media stored at 4℃. However, the highest sperm motility was always observed in semen samples extended with coconut milk based extender than the other extension media used and differences

Table 1: Extenders and their components used in *Clarias gariepinus* milt extension

Extenders (E)	Components (in preparation of 200 ml extender)						
	OviPlus stock solution®	Egg yolk	Coconut milk	Tri-sodium citrate buffer (2.9%)	pH	Antibiotic	Bi-distilled water
E 1 (OE)	24 ml (12 %)	40 ml (20%)	-	Non added	8.0	-	136 ml (68%)
E 2 (OCM)	24 ml (12 %)	-	40 ml (20%)	Non added	7.6	-	136 ml (68%)
E 3 (OCME)	24 ml (12 %)	20 ml (10%)	20 ml (10%)	Non added	7.8	-	136 ml (68%)
E 4 (CMB)	-	20 ml (10%)	80 ml (40%)	Added	7.3	Added-1g	100 ml (50%)

Key: OE = OviPlus® + egg yolk, OCM = OviPlus® + coconut milk, OCME = OviPlus® + coconut milk + egg yolk, and CMB = coconut milk based

Table 2: Some pre-extension milt characteristics (mean ± SD) of wild *Clarias gariepinus* from Maiduguri, Nigeria

Milt characteristics	Mean ± Standard Deviation
Volume (ml)	3.45 ± 1.1
Progressive Motility (%)	83.5 ± 7.5
Livability (%)	90.3 ± 6.9
Morphologic Abnormalities (%)	3.15 ± 2.2
Concentration (x10^9/ml)	2.75 ± 0.5
pH	6.8 ± 0.25

between groups were statistically significant at $p < 0.05$ when evaluated at 24, 48, 72 and 96 hours post extension. More than 50% of *C. gariepinus* sperm were progressively motile post extension at 48 hours across all diluents used and up to 72 hours in milt extended in E4. The E4 maintained *C. gariepinus* sperm motility better than E2 and the difference was statistically significant ($p<0.05$) across the holding time. In addition, at 48 hours, 72 hours and up to 96 hours when compared to E3 and E1, E4 was significantly better in terms of the motility recorded.

Table 4 revealed that there was a gradual reduction in percentage livability of *C. gariepinus* sperm extended with E1, E2, E3 and E4 over storage time stored at 4°C and the differences were statistically significant at $p<0.05$.

The percentage morphologic abnormalities were higher in milt extended with E2 than in E1, E3 and E4, and changes were statistically significant between them ($p<0.05$) as presented in Table 5. It was also observed that the percentage morphologic abnormalities were higher in milt extended with E3 than in other extenders used (at 24 hrs, 72 hrs and 96 hrs) and was statistically significant ($p<0.05$) and were lower in milt extended with E4.

Table 6 Shows that 54.7 ± 17.9, 46.4 ± 19.5, 58.2 ± 21.4, and 69.7 ± 16.2% (extended with E1, E2, E3 and E4 respectively) of *C. gariepinus* sperm were observed to be progressively motile post thawed, difference in sperm motility was significant ($p<0.05$) comparing milt in E4 and E2. The post-thaw livability was significantly different ($p<0.05$) comparing E4 and E2. Milt extended with E4 had the highest number of live sperms (70.6 ± 12.7%). The sperm morphologic abnormalities also differ significantly ($p<0.05$) between E4 and E2, and between E4 and E3. Thepercentage morphologic abnormalities were lower ($p<0.05$) in milt extended with E4 (2.2 ± 2.2%).

Table 3: Progressive individual motility (%) of *Clarias gariepinus* sperm in extended milt chilled at 4°C from Maiduguri, Nigeria

Extension media	Post Extension Time (Hours)				
	0	24	48	72	96
E1	80.0 ± 6.7^{m}	76.4 ± 8.0^{n}	$64.5 \pm 10.3^{o,x,y,z}$	$47.8 \pm 14.5^{p,x,y}$	$13.9 \pm 7.6^{q,x,y}$
E2	84.9 ± 6.4^{m}	$71.1 \pm 5.8^{n,x}$	$51.2 \pm 8.1^{o,x,y}$	$28.2 \pm 10.9^{p,x,y}$	$7.70 \pm 7.7^{q,x,y}$
E3	87.9 ± 7.1^{m}	72.1 ± 7.9^{n}	$54.6 \pm 9.7^{o,z}$	$40.5 \pm 8.9^{p,y}$	$25.6 \pm 9.8^{q,y}$
E4	86.4 ± 6.8^{m}	$78.6 \pm 9.2^{n,x}$	$69.9 \pm 10.9^{o,x,z}$	$57.8 \pm 12.2^{p,x,y}$	$26.7 \pm 13.4^{q,x}$

*Values (Mean ± SD) on the same row with different superscripts $^{(m, n, o, p \text{ and } q)}$ differs significantly at $p<0.05$

*Values (Mean ± SD) on the same column with the same superscripts $^{(x,y,z)}$ differs significantly at $p<0.05$

Table 4: Livability (%) of *Clarias gariepinus* sperm in extended milt chilled at 4°C from Maiduguri, Nigeria

Extension media	Post Extension Time (Hours)				
	0	24	48	72	96
E1	89.6 ± 6.4^{m}	82.2 ± 7.6^{n}	78.7 ± 6.3^{o}	76.2 ± 6.7^{p}	66.6 ± 13.1^{q}
E2	89.4 ± 5.8^{m}	83.0 ± 5.2^{n}	78.6 ± 5.6^{o}	74.0 ± 6.0^{p}	69.3 ± 7.10^{q}
E3	90.9 ± 6.4^{m}	85.8 ± 7.1^{n}	80.2 ± 6.4^{o}	76.4 ± 7.1^{p}	71.5 ± 7.4^{q}
E4	89.7 ± 6.4^{m}	83.5 ± 6.4^{n}	81.0 ± 6.5^{o}	77.9 ± 6.8^{p}	72.2 ± 7.50^{q}

Values (Mean ± SD) on the same row with different superscripts $^{(m, n, o, p \text{ and } q)}$ differs significantly at $p<0.05$

Table 5: Morphologic abnormalities (%) of *Clarias gariepinus* sperm in extended milt chilled at 4°C from Maiduguri, Nigeria

Extension media	Post Extension Time (Hours)				
	0	24	48	72	96
E1	2.3 ± 2.0^{m}	2.2 ± 2.0	$1.8 \pm 1.7^{b,c}$	$1.9 \pm 1.6^{b,n}$	2.4 ± 1.7
E2	$3.3 \pm 1.3^{a,b,m}$	3.3 ± 1.5^{a}	$3.4 \pm 1.6^{a,b}$	$3.6 \pm 1.8^{a,b,c,n}$	3.4 ± 1.8^{a}
E3	$3.8 \pm 2.0^{b,m}$	2.9 ± 1.8^{b}	$3.6 \pm 2.2^{b,c}$	$2.7 \pm 1.0^{c,n}$	2.4 ± 1.3
E4	$1.7 \pm 1.7^{a,b,m}$	$1.3 \pm 1.3^{a,b}$	$1.2 \pm 1.2^{a,c}$	$1.5 \pm 1.0^{a,c,n}$	1.5 ± 1.5^{a}

*Values (Mean ± SD) on the row with different superscripts $^{(m,n)}$ differs significantly ($p<0.05$)

*Values (Mean ± SD) on the same column with the same superscripts $(^{a,b,c)}$ differs significantly at $p<0.05$

Table 6: Some Cryopreserved milt characteristics (Mean ± SD) of *Clarias gariepinus* from Maiduguri, Nigeria

Extension Media	Progressive Motility (%)		Livability (%)		Morphologic Abnormality (%)	
	Pre-freezing	Post-thaw	Pre-freezing	Post-thaw	Pre-freezing	Post-thaw
E1	80.0 ± 8.1	54.7 ± 17.9	89.9 ± 8.2	60.9 ± 15.2[a]	2.3 ± 2.0	3.0 ± 2.3
E2	84.9 ± 7.5	46.4 ± 19.5[a]	91.1 ± 6.9	58.7 ± 11.7[a]	3.3 ± 1.3	4.2 ± 2.4[a]
E3	86.9 ± 7.6	58.2 ± 21.4	90.9 ± 7.5	63.4 ± 14.6	3.8 ± 2.0	4.6 ± 2.5[b]
E4	87.6± 6.7	69.7 ± 16.2[a]	89.4 ± 7.8	70.6 ± 12.7[a]	1.7 ± 1.7	2.2 ± 2.2[a,b]

Values (Mean ± SD) on the same column with the same superscripts[a,b] differs significantly at $p < 0.05$

Discussion

The pre-extension milt quality assessment revealed that the mean value of sperm concentration was within standard range reference value (1.8 to 7.2 × 10^9/ml) similar to previous observation made by Viveiros *et al.* (2000) even with the fact that spermatocrit and viscosity of milt vary between specific males, species and across reproductive seasons (Ali *et al.,* 2022). All average characteristics of pre-diluted milt measured (progressive individual sperm motility, livability, and morphological abnormalities) aligned with the findings reported by Adeyemo *et al.* (2007); Ali *et al.* (2022) which indicated ≥85% progressive motility. This high motility might be due to collection and use of fresh semen from healthy broodstock which typically exhibits 90-100% motility, provided the sperm sample is not activated or contaminated by water, urine, or any other external medium during sampling, as noted by Saeed *et al.* (2010). The mean volume of milt and the slightly acidic mean pH observed were consistent with the observations made by Orlu & Ogbalu (2011); Yusuf *et al.* (2015); Ali *et al.* (2022) for wild African catfish species, which falls within the normal range (6.85 to 8.9). Regardless, a previous finding reported by Mansour *et al.* (2005) found that a milt pH between 7.0 to 9.0 has no effect on the sperm motility parameters of most teleost fish.

The present study showed that there was gradual reduction in percentage livability of *C. gariepinus* sperm across all diluents used over storage time and were ≥65% even at 96 hours post extension at chilled storage. This may be attributed to the high affinity of fish sperm in resistance to cold shock or a relative positive impact of semen extender medium used.

Some of the primary morphologic abnormalities seen during fresh milt evaluation were; coiled mid piece, small head and double head which might have resulted from either damages by xenobiotics or altered genetic mutations as reported by Fauvel *et al.* (2010). In addition, other morphologic abnormalities like detached heads and coiled tail were observed which may have resulted from handling and processing of the milt sample The sperm morphologic abnormalities in chilled milt, decreased with increase storage time (between 0 hour and 72 hours) which perhaps may have resulted from slow milt evaluation post-storage rather than the rapid milt analyses pre-storage time and or optimized storage temperature for preservation, this agrees with what was reported by Albiaty *et al.* (2016) who reported a decrease in morphologic abnormalities of sperm with increase in storage time. Sperm morphologic abnormalities were lower in milt extended with E4. The high percentage morphologic abnormalities of sperms in extenders containing egg yolk could be due to presence of microbial contamination (Ugwu & Igboeli, 2009) and or alteration in pH and lactic acid accumulation (Rehman *et al.,* 2013) which are detrimental to sperm.

The progressive sperm motility should be > 30% to achieve acceptable fertility according to the society of Theriogenology (Barth, 2007). The current study found that frozen-thawed milt evaluation revealed > 50% sperm fraction were progressively motile across all diluents used. The study confirmed that E4 provided the best cryoprotection to the sperm for all post-thawed sperm quality measurements. High viability maintenance observed in Milt preserved with E4 might be due to high sugar content and antioxidant effect of coconut milk and coconut water (Evans & Halliwell, 2001). However, milt samples diluted in E2 showed the highest morphologic abnormalities, lower viability and lowest sperm motility. Moreover, studies by Viveiros *et al*. (2000); Oetome *et al*. (1996) found that fresh and cryopreserved semen results in no significant difference in hatching rate with 82.25% and 78.9% sperm motility of fresh and cryopreserved respectively, besides, all extenders used in this study (E1, E2, E3 and E4) was able to preserve good milt qualities. It was observed that 10% DMSO showed increased total motility of sperm and relatively gave a good results post-thawed, this could be attributed to the fast penetration into sperm and by its interaction with the phospholipids of the sperm membrane. Furthermore, it could be inferred that cryopreserved sperm still needs to be completely activated after thawing before being evaluated or used for egg hatching by addition of aqueous solution.

In conclusion, our observation suggest that progressive motility of wild *C. gariepinus* sperm can be maintained for 72 hours at 4℃ when preserved in coconut milk based extender. However, addition of either egg yolk or coconut milk or a combination of both to OviPlus® extender preserved motility for 48 hours at chilled liquid storage. It was deduced that coconut milk based extenders maintained a good post-thaw qualities better than egg yolk based extender in both chilled (4℃) and cryopreserved (-196℃) *C. gariepinus* milt. This study has shed more light on achieving an optimal cryopreservation protocol for sperm storage and utilization of germ cells from wild male *C. gariepinus*.

Based on our findings, we recommend the use of coconut milk-based extenders for short and long-term preservation of wild *C. gariepinus* sperm due to their superior performance in maintaining motility and good post-thaw quality.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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