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EFFECTS OF EXTENDER TYPE, DILUTION LEVEL, AND STORAGE DURATION ON SPERM QUALITY AND REPRODUCTIVE PERFORMANCE OF PRESERVED *Clarias gariepinus* MILT

***[1]SALIFU C., [1]GALADIMA M.A., [2]OBAJE C.O., [1]DAMBO A., [3]UMAR R., [4]OGABIDU A.O.**
[1]Department of Biology, Ahmadu Bello University, Zaria
[2]Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Lokoja
[3]National Agricultural Extension Research and Liaison Services, ABU, Zaria
[4]Scientific and Industrial Research Department, National Research Institute for Chemical Technology, Zaria
**Correspondence Email: salifucomfort23@gmail.com +234 8162688231,*
https://orcid.org/0009-0002-6126-1709

Abstract
Success in the production of Clarias gariepinus through artificial propagation via hatchery culture is influenced by the quality of sperm used for fertilization. This study investigated the impacts of extender type, diluent level, and preservation periods on sperm quality and the reproductive efficiency of the preserved milt. Fresh milt was diluted with goat milk, chicken egg yolk, and glycerol at 10%, 20%, and 30% concentrations using trisodium citrate buffer and preserved for up to 144 hours at 4°C. Sperm quality parameters (motility, viability, concentration, morphology, and lipid peroxidation) were measured at 24-hour intervals while reproductive performance indices (fertilization, hatchability, and larvae survival rate) were determined at 0, 72, and 144 hours.The motility of sperm gradually reduced as the storage period increased. Sperm quality declined progressively with increasing storage duration across all treatments, with differences becoming more pronounced at higher dilutions and longer storage periods. Goat milk consistently maintained superior sperm quality, with higher motility, viability, and normal morphology, alongside moderate lipid peroxidation levels. Chicken egg yolk provided moderate short-term preservation but deteriorated with extended storage, while glycerol showed the poorest performance. Reproductive performance followed similar trends, with goat milk maintaining higher fertilization (75-80%), hatchability (>80%), and larval survival up to 72 hours compared to other extenders. Fresh milt showed optimal performance at 0 hours but declined rapidly without preservation. At 144 hours, reproductive performance was significantly reduced across all treatments, indicating the biological limits of chilled storage. The study concludes that goat milk is an effective, low-cost natural extender for short-term preservation of C. gariepinus milt, with optimal results at 30% dilution within 72 hours at 4°C.

Keywords: Clarias gariepinus, semen extenders, goat milk, chilled storage, sperm quality, fertilization, hatchability, larval survival

INTRODUCTION

Aquaculture is one of the most rapidly developing sectors of food production worldwide, with developing nations making a considerable contribution to its development (Mair *et al*., 2023). In Nigeria, there is an estimated aquaculture potential of 2.5 million metric tonnes of fish, while current production is still far from that mark, resulting in a significant shortage of fish in relation to its demand (Ogunji *et al*., 2023). The development of aquaculture is largely determined by effective hatchery operation, including the use of quality gametes for fish propagation. However, the insufficient number of quality broodstock and low effectiveness of current fish breeding have made it necessary to develop measures aimed at preserving valuable genetic material and enhancing fish seed production (Siddique *et al*., 2025) The African catfish (*Clarias gariepinus*) can be considered one of the most promising candidates for further development in aquaculture throughout Africa owing to such advantageous characteristics as fast growth rates, adaptability, and commercial attractiveness (Srikulnath *et al*., 2025). However, the lack of ability to produce sufficient numbers of quality fingerlings remains one of the critical problems related to their inefficient reproduction.

One of the most significant constraints facing the artificial propagation of *C. gariepinus* is the difficulty of acquiring good-quality sperm, since acquiring male sperm involves the death of the male stock. This leads to reduced genetic stock and increased cost in production (Tilahun and Yalew, 2024). Moreover, poor fertilization, hatching, and survival of larvae are some of the factors limiting hatchery performance (Siddiqu *et al*., 2022). This necessitates the development of advanced sperm handling techniques in order to improve the viability and longevity of the sperms without any wastage of stock animals. One of the methods through which this can be achieved is the use of sperm extenders to preserve the sperms temporarily. While cryopreservation can provide for long storage periods, this is not feasible in many developing countries due to associated high costs (Valdebenito and Gallego, 2015; Martínez-Páramo *et al*., 2017). Hence, chilled sperm storage at 4°C emerges as a better alternative for preserving fish sperm.

The success of preserving chilled sperm is determined to a large extent by the extenders used due to the fact that extenders ensure membrane stabilization, regulation of pH, and decreased oxidative stress, thus preventing the death of sperm cells (Hameed *et al*., 2023).Chemical extenders like glycerol and Ringer's solution have been

extensively employed in semen preservation; however, they are associated with drawbacks, including toxicity, expensive cost, and absence of inherent antioxidants (Onyia et *al., 2017*). On the other hand, natural semen extenders, such as goat milk and chicken egg yolk, contain lipid components, proteins, and vitamin antioxidants that can improve sperm quality due to their ability to neutralize oxidative stress (Valdebenito and Gallego, 2015). Their efficacy depends on the degree of dilution and period of storage, but there is insufficient comparative data concerning their efficacy compared to artificial semen extenders in *Clarias gariepinus* (Mustapha and Alade, 2022). In addition, oxidative stress, especially lipid peroxidation due to the presence of reactive oxygen species, is still one of the crucial elements that play an important role in sperm degeneration (Egger *et al*., 2021). Unfortunately, little research has been conducted to identify these connections.

In addition, most studies on sperm preservation have focused primarily on pre-fertilization quality indicators such as motility and viability, without adequately linking these parameters to functional reproductive performance, including fertilization rate, hatchability, and larval survival. This disconnect limits the development of effective preservation protocols, as the ultimate measure of success in hatchery operations is the ability of preserved sperm to achieve successful fertilization and support early larval development (Beirão *et al.,* 2019). For this reason, there is a need to conduct integrative evaluations where the sperm quality tests are combined with the reproductive success performances using different types of extenders. These methods are important in enhancing conservation techniques that are biologically efficient and practical within aquaculture production systems.

Due to the growing need for cost-effective, environmentally friendly, and practical aquaculture practices in Nigeria, it has become necessary to investigate natural extender substances as potential alternatives to synthetic chemical extenders. Improving sperm preservation techniques could reduce the need for broodstock sacrifice, enhance hatchery efficiency, and promote better genetic resource management in catfish production systems (Omitogun *et al.,* 2012; Kwikiriz *et al.,* 2025). In view of these considerations, the present study investigated the effects of extender type, dilution level, and storage duration on sperm quality characteristics and reproductive performance of preserved *Clarias gariepinus* milt. Specifically, the study evaluated key sperm quality parameters, including motility, viability, morphology, concentration, and lipid peroxidation, and related these to reproductive outcomes such as fertilization rate, hatchability, and larval survival.

MATERIALS AND METHODS

Study Location

The study was conducted at the National Animal Production Research Institute (NAPRI) Fertility Laboratory Ahmadu Bello University, Zaria, and the fisheries hatchery of the Division of Agricultural Colleges (DAC), Ahmadu Bello University, Zaria, Nigeria.

Experimental Fish and Sample Collection

A total of (12) mature male and (5) female *Clarias gariepinus* broodstocks, weighing between 1.5 and 2.5 kg, were procured from a commercial fish farm in Zaria, Kaduna State, Nigeria. The fish were transported in oxygenated tanks to the hatchery facility and acclimatized for two weeks. During acclimatization, broodstocks were fed Coppens feed (42% crude protein) at 5% body weight twice daily, while water quality and fish health were closely monitored.

Natural extenders, including chicken egg yolk and goat milk, were obtained from the National Animal Production Research Institute (NAPRI), Zaria, while glycerol was sourced from the Chemistry Multipurpose Laboratory, Ahmadu Bello University, Zaria.

Experimental Design

The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment. Fresh milt was diluted using three extender types (goat milk, chicken egg yolk, and glycerol) at concentrations of 10%, 20%, and 30%. Glycerol served as the synthetic control, while undiluted milt served as the negative control. The diluted samples were stored at 4°C for 0-144 hours. Samples withdrawn at 72 and 144 hours were used for fertilization trials, and hatched fry were monitored for survival over a 14-day period.

Preparation of Extenders and Milt Collection

Freshly laid chicken eggs were disinfected with 70% alcohol, and the yolk was separated under sterile conditions. Goat milk obtained from West African Dwarf goats was centrifuged to remove the fat layer, and the skim milk fraction was used to improve homogeneity. Glycerol was used directly without further preparation.

Male broodstocks were identified by genital papilla characteristics and euthanized following cold shock. The testes were dissected, rinsed in saline solution, and milt was extracted by gentle laceration and collected into sterile Petri dishes.

Preparation of Semen-Extender Mixtures and Storage

A 2.9% (w/v) trisodium citrate buffer was prepared and used as the base solution. Extenders were prepared at different concentrations by mixing appropriate volumes of extender and buffer to obtain 10%, 20%, and 30% dilutions. Fresh milt was diluted at a ratio of 1:10 (sperm: extender).

Aliquots of 2 mL were dispensed into labeled tubes and stored at 4°C for up to 144 hours. Samples were withdrawn at 24-hour intervals for sperm quality assessment. Prior to analysis, samples were equilibrated to room temperature.

Sperm Quality Assessment

Sperm Motility

Motility was evaluated microscopically after activation with distilled water and expressed as the percentage of motile sperm cells (Adgnihotri *et al.,* 2016):

$$\% \text{ motile sperm} = \frac{\text{Total number of motile sperm}}{\text{Total number of sperm}} \times 100$$

Sperm Viability

Viability was determined using 0.2% eosin staining. Live sperm cells appeared transparent, while dead cells were stained pink. At least 200 cells were counted per sample (Kumar *et al*., 2017):

$$\% \text{ viability} = \frac{\text{Number of live sperm}}{\text{Total number of sperm}} \times 100 \text{ (Kumar } et\ al., 2017)$$

Sperm Concentration

Sperm concentration was determined using a hemocytometer after proper dilution and expressed as $\times 10^6$ cells mL^{-1}.

Sperm Morphology

Morphological abnormalities (e.g., bent tails, detached heads) were assessed using eosin-nigrosin stained slides, and the percentage of normal sperm cells was recorded.

Lipid Peroxidation (MDA Determination)

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) using the thiobarbituric acid (TBA) method. Absorbance was measured at 532 nm, and MDA concentration was calculated using the Beer-Lambert equation:

$$\text{MDA } (\frac{\text{nmol}}{\text{mL}}) \frac{A532}{\epsilon \times l}$$

where A_{532} is absorbance, $\mathcal{E}$= 1.56 × 10^5 M^{-1} cm^{-1}, and l = 1cm

Reproductive Performance Evaluation

Induced Breeding and Egg Collection

Female broodstocks were injected with Ovaprim at 0.5 mL kg^{-1} body weight. After an 8-hour latency period, eggs were stripped by gentle abdominal pressure.

Fertilization and Hatchability

Fertilization was carried out using preserved milt samples. Unfertilized eggs were identified and removed after 12 hours. Fertilization and hatchability were calculated as (Muchlisin *et al.,* 2010; Ubah *et al*., 2023):

$$\text{Percentage (\%)fertilization} = \frac{\text{Total number of eggs fertilized}}{\text{Total number of eggs incubated}} \times 100$$

$$\text{Percentage (\%)hatchability} = \frac{\text{Total number of hatched larvae}}{\text{Total number of fertilized eggs}} \times 100$$

Larval Survival Rate

Larvae were maintained on yolk reserves for 3-4 days and subsequently fed Artemia. Survival was assessed 14 days post hatch as:

$$\text{Percentage (\%)survival} = \frac{\text{Total number of surviving larvae}}{\text{Total number of hatched larvae}} \times 100$$

Statistical Analysis

Data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. One-way ANOVA was used to evaluate changes across storage time, while two-way ANOVA was used to assess the effects of extender type and dilution level, including their interaction. Significant differences among means were separated using Duncan's Multiple Range Test at $p \leq 0.05$. All results were expressed in charts. Bars and line points represent means and error bars represent SEM.

RESULTS AND DISCUSSION

Quality of Preserved *Clarias gariepinus* Milt

As shown in figures 1A and 1B, sperm motility was significantly influenced ($P < 0.05$) by extender type, dilution level, and storage duration. At lower dilutions (10-20%), differences in motility among extenders were minimal. However, at 30% dilution, clear variation was observed, with goat milk maintaining the highest motility values, followed by chicken egg yolk and glycerol. Motility declined progressively across all treatments over the 144-hour storage period, with fresh milt showing rapid loss of motility within 72 hours.

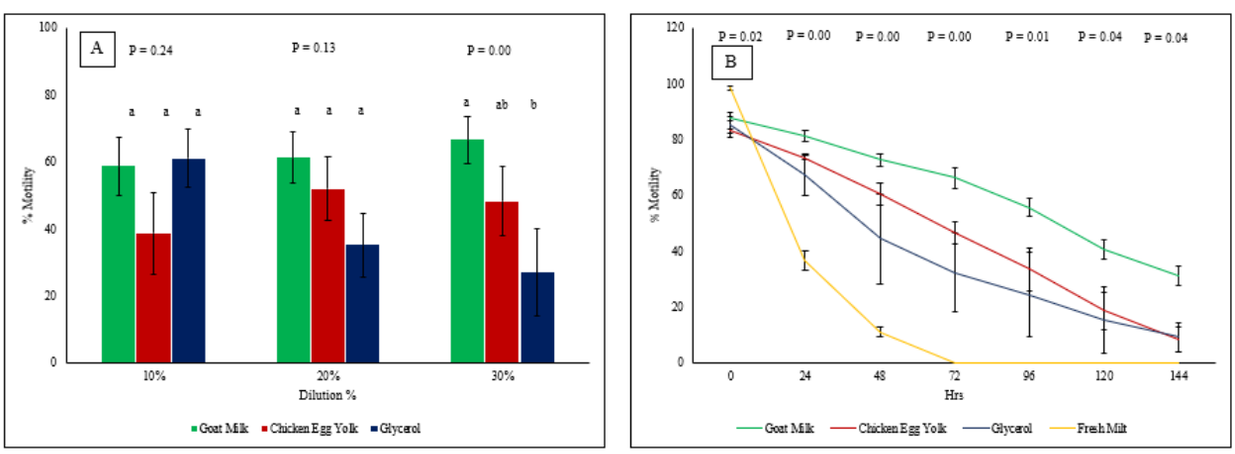

Figure 1: A - Effect of extender type and dilution level on sperm motility of *Clarias gariepinus* milt
B - Changes in sperm motility of *Clarias gariepinus* milt during preservation

Sperm viability followed a similar trend to motility as shown in figures 2A and 2B. No significant differences were observed among extenders at lower dilution levels (10-20%), but at 30% dilution, goat milk maintained higher viability compared to egg yolk and glycerol. Viability decreased with increasing storage duration across all treatments, with glycerol showing the most rapid decline

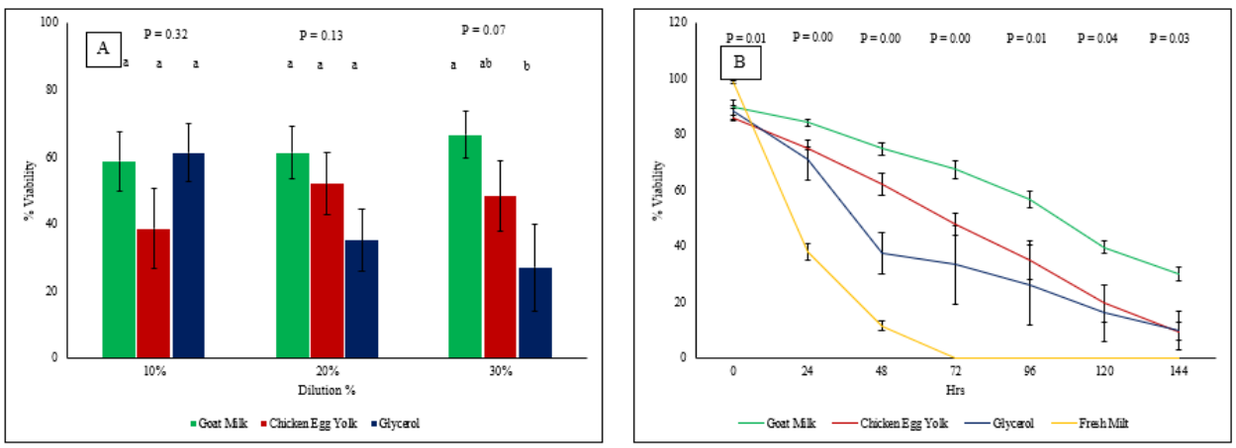

Figure 2: A - Effect of extender type and dilution level on sperm viability of *Clarias gariepinus* milt
B - Changes in sperm viability of *Clarias gariepinus* milt during preservation

As shown in figures 3A and 3B, sperm concentration was primarily influenced by dilution and storage duration. Fresh milt recorded the highest initial concentration (425.62 ± 7.35 × 10^6 cells mL^{-1}) but declined rapidly during storage. Among extenders, goat milk maintained higher residual sperm concentrations over time, while glycerol showed a more pronounced decline.

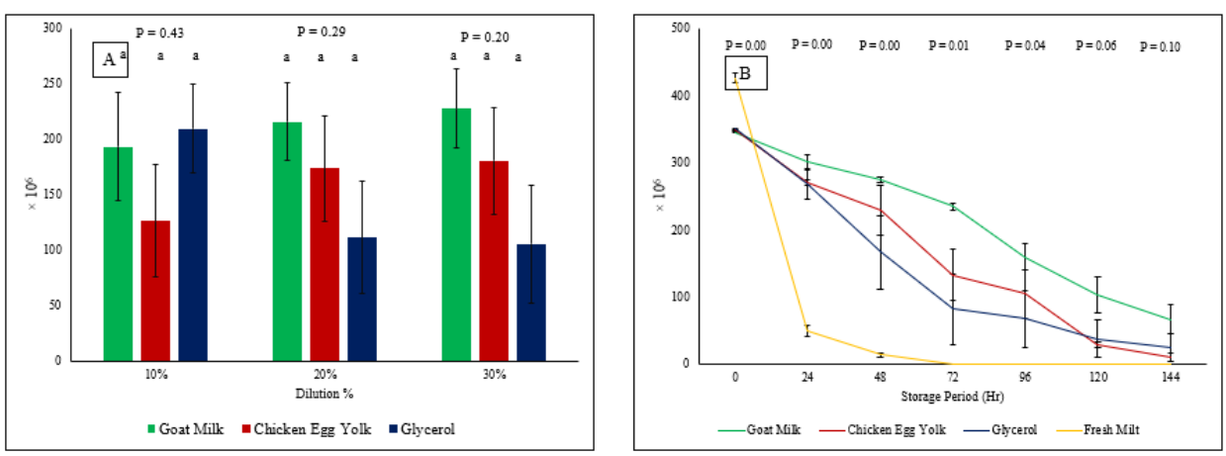

Figure 3: A - Effect of extender type and dilution level on sperm concentration of *Clarias gariepinus* milt
B - Changes in sperm viability of *Clarias gariepinus* milt during preservation

As shown in figures 4A and 4B, sperm morphology was significantly affected by extender type and dilution level. At 30% dilution, goat milk recorded the highest percentage of normal sperm (84-85%), followed by egg yolk (80-81%), while glycerol showed markedly lower values (56-63%). Morphological integrity declined with increasing storage duration across all treatments

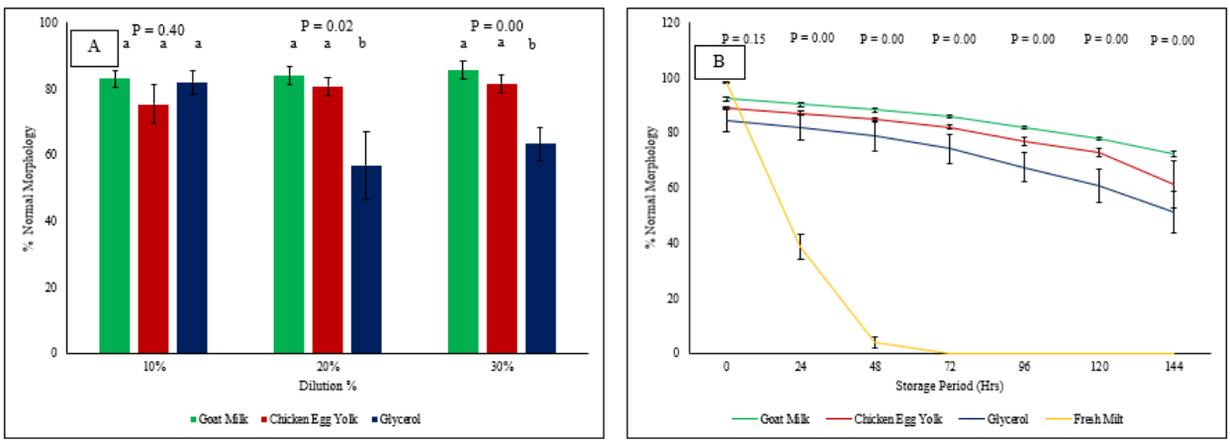

Figure 4: A - Effect of extender type and dilution level on normal sperm morphology of *Clarias gariepinus* milt
B - Changes in sperm normal sperm morphology of *Clarias gariepinus* milt during preservation

Lipid peroxidation, measured as malondialdehyde (MDA), varied significantly among extenders and increased with storage duration as shown in figures 5A and 5B. Glycerol consistently recorded the highest MDA levels, while egg yolk showed the lowest values. Goat milk exhibited intermediate MDA levels. Slight reductions in MDA were observed at higher dilutions.

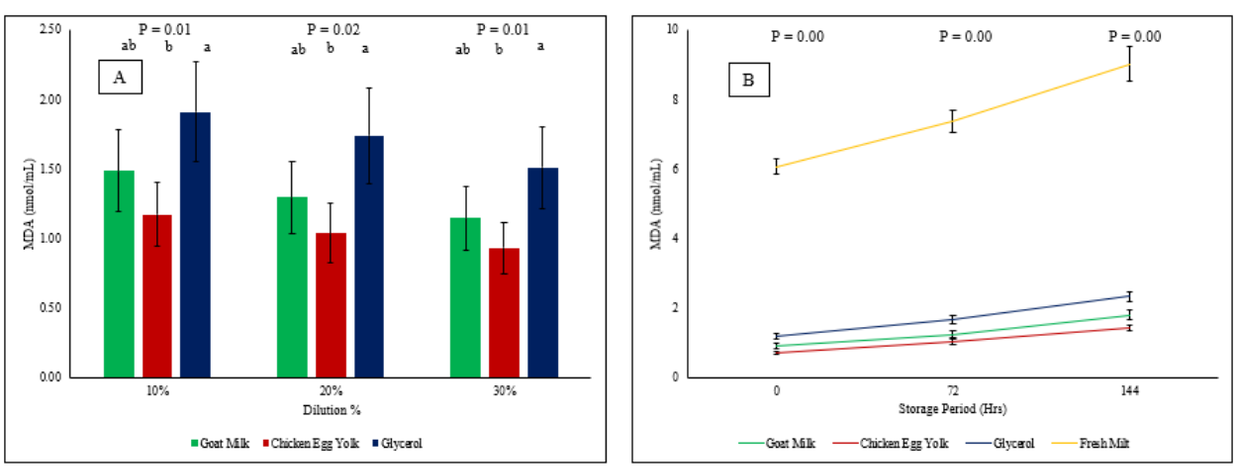

Figure 5: A - Effect of extender type and dilution level on lipid peroxidation (MDA) levels of *Clarias gariepinus* milt
B - Changes in sperm lipid peroxidation (MDA) levels of *Clarias gariepinus* milt during preservation
Reproductive Performance of Preserved *Clarias gariepinus* Milt

Fertilization rate, hatchability, and larval survival were influenced by extender type, dilution level, and storage duration.

Fertilization rate did not differ significantly ($p > 0.05$) among goat milk, chicken egg yolk, and glycerol extenders across 10-30% dilution levels as shown in figure 6A. However, numerical variations were observed, with goat milk showing slightly higher values at increased dilutions, while glycerol showed a declining trend. Across storage duration as shown in figure 6B, fertilization rate varied significantly ($p < 0.05$). Fresh milt at 0 h recorded the highest fertilization rate (99.33 ± 0.17%), significantly higher than preserved samples. Among extenders at 0 h, goat milk (81.17 ± 2.96%), chicken egg yolk (72.83 ± 1.92%), and glycerol (70.00 ± 10.21%) showed no significant differences. At 72 h, fertilization rates declined, with goat milk (76.33 ± 4.17%) maintaining higher values than chicken egg yolk (56.33 ± 2.86%) and glycerol (49.17 ± 12.69%). At 144 h, fertilization rates were markedly reduced across all treatments, with no fertilization observed in chicken egg yolk and low values in goat milk and glycerol.

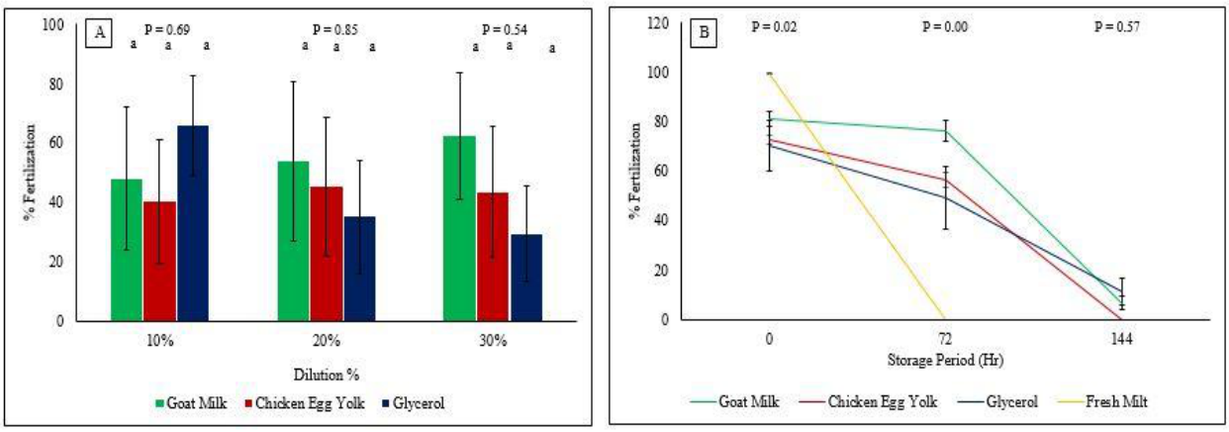

Figure 6: A - Effect of extender type and dilution level on fertilization rate of *Clarias gariepinus*
B - Changes in fertilization rate of *Clarias gariepinus* using preserved milt

Hatchability showed no significant differences ($p > 0.05$) among extenders across dilution levels as shown in figure 7A, although goat milk recorded higher mean values at 30% dilution compared to other treatments. Storage duration significantly affected hatchability ($p < 0.05$) as shown in figure 7B. Fresh milt recorded the highest hatchability at 0 h (99.13 ± 1.86%). At 72 h, goat milk maintained the highest hatchability (85.07 ± 0.38%), followed by chicken egg Yolk (78.90 ± 4.51%) and glycerol (77.10 ± 2.60%). At 144 h, hatchability declined significantly across all treatments, with no hatchability observed in chicken egg yolk or fresh milt, while goat milk and glycerol recorded low values.

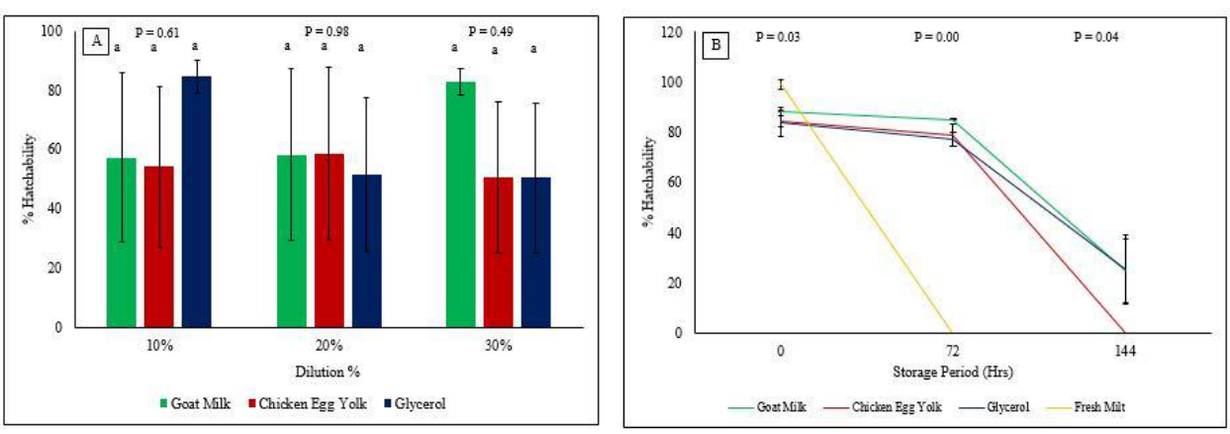

Figure 7: A -Effect of extender type and dilution level on hatchability of *Clarias gariepinus* eggs fertilized with preserved milt
B - Changes in hatchability of *Clarias gariepinus* eggs fertilized with preserved milt

Larval survival at 14 days post-hatch did not differ significantly ($p > 0.05$) among extenders across dilution levels as shown in figure 8A, although goat milk and glycerol recorded relatively higher survival rates at 30% dilution. Storage duration significantly influenced larval survival ($p \leq 0.05$) as shown in figure 8B. At 0 h, larvae produced from fresh milt recorded the highest survival (87.97 ± 0.98%), followed by goat milk (73.73 ± 1.83%), glycerol (70.17 ± 9.12%), and chicken egg yolk (65.50 ± 3.85%). At 72 h, survival declined across all treatments, with goat milk maintaining higher values than other extenders, while no survival was recorded in fresh milt. At 144 h, survival became quite low in moat milk and glycerol, while there was no survival at all in chicken egg yolk and fresh milt.

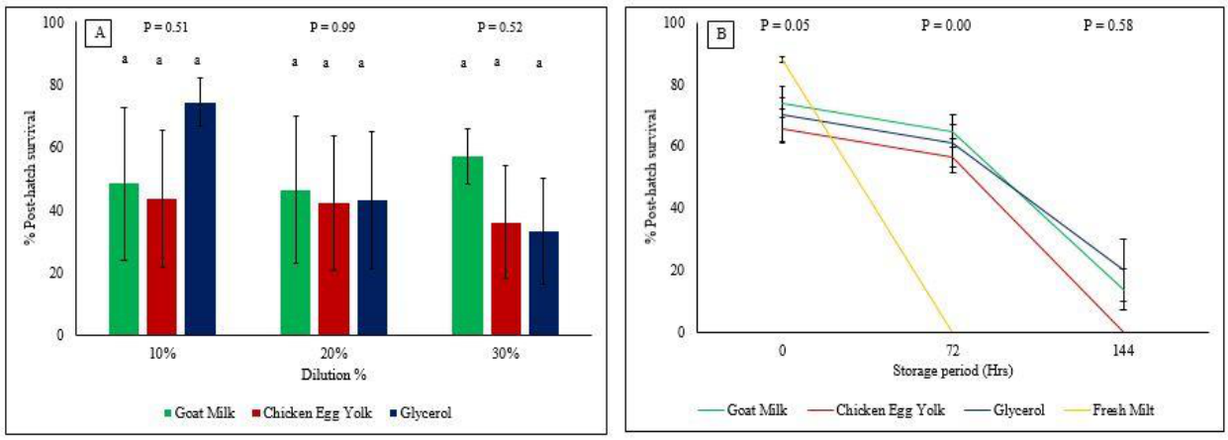

Figure 8: A -Effect of extender type and dilution level on larval survival of *Clarias gariepinus* at 14 days post-hatch
B - Changes in larval survival of *Clarias gariepinus* larvae (14 days post-hatch) produced using preserved milt

DISCUSSION

The changes in the motility of the sperm point to the effects of the extender type, concentration of dilution, and storage duration on the activity of the sperm. The slight differences seen at lower concentrations might be attributed to the effect of the seminal plasma that provides osmotic support and biochemical protection. At high concentrations of dilution, however, (30%), the extender becomes very important for the maintenance of the viability of the sperm. This superior ability is due to antioxidants present in the extenders, such as vitamin C that help maintain ATP levels essential for the motility (Akhter *et al*., 2023). The decrease in sperm motility with increasing duration indicates the metabolic exhaustion, increase in ROS production, and cell membrane instability, while quick deterioration of fresh milt stresses the need for an extender (Bugau *et al*., 2022; Fuentes-Albero *et al*., 2021).

Viability trends closely mirrored motility, reinforcing the strong relationship between membrane integrity and sperm functionality. The superior viability observed in goat milk, particularly at higher dilution, indicates effective antioxidant protection against oxidative stress. In contrast, the rapid decline in glycerol treated samples may be attributed to cumulative osmotic imbalance and lack of intrinsic antioxidant defense (Akhter *et al*., 2023). These findings agree with previous studies demonstrating that antioxidant-rich extenders prolong sperm lifespan and enhance reproductive potential (Rurangwa *et al.,* 2011; Bugau *et al.,* 2022.,Bello *et al* 2022).

The decline in sperm concentration over time reflects cell degradation and structural breakdown during storage. Goat milk's ability to maintain higher residual concentrations suggests improved membrane stabilization and reduced cellular damage. While egg yolk contains lipid-soluble antioxidants, its lower efficiency compared to goat milk indicates that aqueous-phase antioxidants play a more critical role in preserving sperm cell integrity (Fuentes-Albero *et al.,* 2021).

The morphological integrity underscored the relevance of the extender composition. The greater proportion of normal sperm in goat milk and egg yolk extender showed the efficiency of their use in alleviating damage from aging. In contrast, the pronounced morphological defects in sperm samples in glycerol extender indicated high sensitivity to osmotic shock and oxidation that caused head and tail malformations (Akhter *et al.*, 2023; Fuentes-Albero *et al*., 2021).

Lipid peroxidation analysis supported the oxidative hypothesis on sperm damage in chilled conditions. The higher level of MDA was explained by the lack of antioxidants in glycerol extender that made sperm susceptible to oxidative damage initiated by ROS. Among the extenders tested, egg yolk exhibited the highest protective effect thanks to vitamin E, whereas goat milk provided a moderate level of protection due to vitamin C and protein content. The slightly lower MDA concentration in samples stored at a higher dilution ratio was likely to be associated with low sperm density that reduced the amount of available substrates (Larsson *et al.*, 2016; Shaliutina-Kolešová & Nian, 2022).

The process of fertilization is influenced by various parameters of sperm function such as motility, viability, integrity of the membranes, and acrosome reaction. There were no statistical differences between different extenders with varying degrees of dilutions, which implies that all the extenders ensured an appropriate physiochemical milieu for sperm and egg interaction. Similar findings have been obtained in other studies involving *Clarias gariepinus* and other freshwater fish species wherein moderate dilutions ensure the balance of ionic strength and fertilization (Amachree *et al*., 2018; Nenova *et al*., 2026). The numerically better results with goat milk at high levels of dilution imply the better integrity of sperm, whereas the decreasing values in glycerol may indicate osmotic or membrane stresses (Rurangwa et al., 2011; Mustapha & Alade, 2022).

The sharp fall in the fertilization rate as the storage time

increases demonstrates the deteriorating status of the sperm during its storage under refrigerated conditions. Perfect fertilization rate in fresh milt is due to intact membranes and lack of oxidative stress, whereas the higher success rate of goat milk at 72 h is attributed to protection from oxidative stress as well as integrity of the acrosome. Sharp decline in the fertilization rate after 144 h among all groups shows damage to the sperm membrane and enzyme deactivation, as stated by Rurangwa *et al*. (2011) and Behera *et al*. (2023).

The hatchability trend followed the same pattern as the fertilization trend, thereby establishing that embryogenesis is highly reliant on the quality of the sperm cells used during fertilization. The fact that the hatchability trend was relatively consistent among the various extenders at low dilutions is a clear indication that all the necessary ingredients for embryo development were present. On the other hand, the better results recorded by goat milk at 72 hours could indicate increased protection of sperm DNA as well as other elements needed for embryo development. This is probably because Egg Yolk lost its efficiency due to lipid peroxidation (Lein *et al*., 2023).

The survival rate of the larvae was an indicator that showed the compounded effect of sperm quality, fertilization, and the embryo's ability to develop properly. The lack of any differences between the dilutions shows that the extenders offered an adequate biochemical medium for the early developmental stages of the larvae. Nevertheless, the increased survival rate among the goat milk group shows its effectiveness in maintaining the viability of sperm cells and reducing oxidative damage caused by the chilling process. The decreasing rate of survival among the different periods of time, especially after 144 hours, proves the biological limitations of chilled sperm preservation. The effectiveness of the goat milk extenders lies in its composition, which consists of proteins, sugar, and antioxidant properties (Bello *et al* 2022).

CONCLUSION AND RECOMMENDATIONS

This study showed that there was an interactive effect of the extender used, the dilution rate and the period of storage on the quality of the sperm and reproductive success of *C. gariepinus* semen stored under cold condition. The quality parameters assessed such as sperm motility, viability, concentration, morphology and lipid peroxidation level decreased progressively with increased storage time irrespective of the extender used. However, at higher dilution rates of 30% and longer storage time of between 72-144 h, the nature of extender became very important in ensuring optimal sperm storage.

In terms of performance, goat milk showed good results by providing better motility, viability and better sperm morphology together with minimal lipid peroxidation during the storage period. Egg yolk from chicken showed good performance in the initial stages of storage but lost its ability to protect the milt from oxidation and maintain morphologically normal sperm cells over prolonged storage times. Glycerol which is well known as a cryoprotectant showed poor performance in terms of high lipid peroxidation levels and poor sperm quality.

Parameters related to reproductive performance such as fertilization rate, hatchability, and larval survival were consistent with the trend seen in semen quality. Fresh milt showed optimum reproductive performance after collection, which quickly declined without extenders within the first few hours after collection. For stored sperm, the goat milk extender provided the best reproductive performance in terms of fertilization rates (75-80%), hatchability (over 80%), and larval survival (up to 72 hours). The other extenders, namely, chicken egg yolk and glycerol, provided poorer results. However, at 144 hours, all sperm samples, irrespective of extender used, showed low reproductive performance.

The following recommendations is made based on the results of this study:

i. Goat milk extender diluted 30% is recommended for the short-term chilled preservation of the milt of *Clarias gariepinus*. Its high physical and chemical stability, buffer capacity, and natural antioxidant effects resulted in lower levels of lipid peroxidation and better quality spermatozoa compared to artificial extenders, providing a cheap alternative in the hatchery industry.
ii. The storage period of diluted milt should not exceed 72 hours at 4ºC for the highest spermatozoa quality and fertilization rate. Beyond that time period, the quality of spermatozoa and their fertility drop in all the examined groups of extender. If goat milk is unavailable, the egg yolk of chicken could be applied as an extender but for the shortest possible period not exceeding 72 hours.
iii. To increase the rates of fertilization, hatchability, and larval survival, the utilization of 30% diluted goat milk milt is highly recommended.

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