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Full Length Research

A novel approach to non-lethal milt collection in *Clarias gariepinus* to improve broodstock management

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ABSTRACT: This study evaluated the feasibility of orchiotomy as an alternative to partial orchiectomy for milt collection in *Clarias gariepinus* (African catfish) to maintain fertilisation potential. In the partial orchiectomy group, 12 male fish underwent initial surgery where one-third of the testis was removed to extract milt, which was collected in a sterile bottle. Follow-up surgeries were conducted on six males after 30 days and on the remaining six after 40 days to collect additional milt from the remaining two-thirds of the testis. In the orchiotomy group, milt was obtained from 12 males via a small incision at the proximal testicular pole, allowing the milt to be gently expressed. Recollection followed the same 30- and 40-day schedule as the partial orchiectomy group. Milt quality and fertilisation capacity were assessed by using 1 g of eggs per milt sample. At the initial collection (day 0), there were no significant differences in milt volume, sperm concentration, or fertilisation success between the two methods. However, at 30 and 40 days post-surgery, the partial orchiectomy group showed significantly lower (P<0.05) milt volume, sperm concentration, and fertilisation rate compared to the orchiotomy group, except for fertilisation rate at 40 days, which was not significantly different. Within the partial orchiectomy group, a significant decline ($p<0.05$) was observed in milt volume, concentration, and fertilisation potential at 30 and 40 days compared to day 0, with no significant difference between the 30- and 40-day marks. In contrast, the orchiotomy group maintained consistent milt quality across all time points. Overall, orchiotomy proved superior, offering higher milt volume, concentration, and fertilisation success, along with minimal tissue adhesion, no visible scarring, faster healing, and quicker regeneration.

Keywords: *Clarias gariepinus*, non-lethal milt collection, orchiotomy, partial orchiectomy.

INTRODUCTION

To guarantee seed production, clariid farming mostly depends on induced breeding methods. These methods involve giving female fish hormones to cause superovulation, followed by stripping them after a 9 to 12 hours latency period. Mature male fish are sacrificed for fertilisation, and the testes are then taken out and manually squeezed to release milt (Steyn and van Vuren, 1987). This approach hampers efforts to preserve superior genetic features for selective breeding and other genetic research, even though it works well for immediate breeding.

The testes of African catfish (Clariidae) have a yellowish, lobular appearance and are situated dorsally within the abdominal cavity along the body's longitudinal axis

(Zakariah *et al*., 2016). According to Diyaware *et al*. (2010b), the intestines are located above the testes, and large projections of the seminal vesicles further restrict milt release, even under applied pressure. These anatomical constraints have made non-lethal milt collection particularly challenging. To address genetic conservation, several milt preservation techniques have been developed to maintain the genetic quality of valuable fish species (Nguenga *et al*., 1996; Viveiros *et al*., 2001; Woelders and Hiemstra, 2005; Pavlov, 2006; Ali et al., 2025). However, attempts to increase milt volume through hormonal induction followed by stripping have achieved limited success (Nguenga *et al*., 1996; Melo and Godinho, 2006). This limitation is largely attributed to the presence of accessory glands and seminal vesicles in the male reproductive system, which secrete a viscous fluid rich in mucopolysaccharides and proteins that impedes effective milt stripping (Eduardo *et al*., 2001). Further attempts to obtain milt from male *Clarias gariepinus* using gonadotropin-releasing hormone analogue combined with pituitary suspension (nGnRHa + PS) were unsuccessful (Viveiros *et al*., 2001). Internal examination revealed that the testes remained underdeveloped despite the fish being nine months old with a mean body weight of 1.5 ± 0.2 kg, explaining the failure of stripping (Viveiros *et al*., 2002). Although stripping was later achieved following treatment with Clarias pituitary suspension (1 ml/kg) and Ovaprim (0.5 ml/kg), the resulting milt was watery and blood-stained, with no motile sperm cells upon activation with water (Viveiros *et al*., 2002). Consequently, fertilisation outcomes were poor, with a hatching rate of only 4% after a 24-hour latency period.

Currently, milt collection in African catfish is most commonly achieved through testicular ablation (Diyaware *et al.,* 2010b; Adebayo *et al.,* 2012; Pronina and Petrushin, 2019). Although syringe-assisted milt harvesting from live males has been reported, its success rate remains extremely low (3.3%) (Idahor, 2014). Surgical procedures like orchiectomy by removing one or both testicles or a portion (partial orchiectomy), as well as orchiotomy, which involves incising into the testis, could be a better alternative for milt collection in catfish aquaculture. Abdominal massage is largely ineffective due to the anatomical positioning of the intestines and seminal vesicles, as well as the absence of a luteinizing hormone (LH) surge required for sperm release (Viveiros *et al*., 2001). Consequently, sacrificing male broodstock remains the most reliable method of milt collection, a practice that severely limits the reuse of high-quality males and reduces broodstock availability. Although testicular ablation allows milt recovery without immediate sacrifice, the prolonged regeneration period presents an additional constraint (Diyaware *et al.,* 2010b; Pronina and Petrushin, 2019). Overall, the routine sacrifice of male African catfish represents a significant loss of valuable genetic resources and contributes to the scarcity of quality male broodstock. Consequently, this study focuses on the critical need to develop alternative, non-lethal methods of milt collection that support sustainable breeding practices and the preservation of superior genetic stocks.

MATERIAL AND METHODS

Study area

The study was conducted between the months of June and August 2020 at the Andrology and Artificial Insemination Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Sokoto lies between 5º and 6ºE longitude and between 13 and 14ºN latitude with an average annual temperature of 40ºC and mean annual rainfall of 300 mm – 1200 mm (Abdulrahim *et al*., 2013).

Fish source and management

A total of twenty-eight sexually mature *C. gariepinus,* twenty-four (24) males weighing between 1.8–2.5 kg and four (4) females weighing between 1.5–2.0 kg were sourced from the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State. The fish were acclimatised in a 3.5 m x 3.5 m x 1.5 m outdoor tarpaulin tank for 2 weeks with mean temperature, pH and dissolved oxygen of 29.20 ± 1.89°C, 7.00 ± 0.79 and 4.95 ± 0.29 mg/l, respectively. They were fed a commercial diet containing 40% CP (Blue Crown®).

Water quality parameters

Water quality parameters such as dissolved oxygen (DO), temperature (ºC) and pH in the tank were recorded twice daily in the morning (08.00 h) and evening (18.00 h). With digital DO/temperature analyser meter (AMTAST® with Model No: AMT07) and pH meter with (Model No: PH-220W).

Study design

The 24 males of *C*. *gariepinus* were randomly divided into two groups (A and B, 12 males/group), each group was further divided into 2 sub-groups; 6 per group (A1, A2, B1 and B2, 6 males/subgroup) as shown in Table 1. The fish were tagged with different colours of beads by passing thread and needle through the hole of the bead, and a knot was made on the dorsal fin for ease of identification. Partial orchiectomy method of milt collection was carried out on all fish in group A (which were re-opened after 30 and 40 days in group A1 and A2, respectively), while all fish in group B (which were re-opened after 30 and 40 days in group B1 and B2, respectively), were subjected to orchiotomy method of milt collection.

Table 1. Grouping of male C*. gariepinus*for the experiment.

Group	No.	sub-group	No.	Milt collection method	Re-opening period (days)
A	12	A1	6	Partial Orchiectomy	30
		A2	6	Partial Orchiectomy	40
B	12	B1	6	Orchiotomy	30
		B2	6	Orchiotomy	40

Milt collection by partial orchiectomy

Milt collection by partial orchiectomy was performed by a surgical operation; the fish was placed on dorsal recumbency on a wet, draped cloth on a surgical table, and its head was covered with a wet, draped cloth for proper restraint (appropriate confinement in order to collect milt by partial orchiectomy). The abdominal surface was scrubbed with 70% isopropyl alcohol and was anaesthetised subcutaneously by linear infiltration using 0.5 ml/kg of 2% lidocaine. An incision was made on the mid ventral aspect of the abdomen on the skin down to the linea alba using a (size 15) scalpel blade. Using sterile Metzenbaum scissors, the cranial incision was expanded 3–5 cm to reveal the internal organs. A sterile gauze bandage was then used to clean the blood. The testes were exposed by lifting out and pushing aside the digestive tract. A 5 ml syringe was used to measure the milt after a partial orchiectomy, in which one-third of the testicles on the proximal side were removed during the original procedure and two-thirds at the reopening phases. The milt was then squeezed into a sterile bottle. The skin, subcutaneous tissue, and muscle were all sutured together during surgical incision closure using a straightforward interrupted suture pattern with chromic catgut size 2/0. Each procedure took fifteen minutes to complete. Immediately after the surgery, they were put in a treatment tank with 500 L of water containing Oxytetracycline antibiotic at a dosage of 50 mg/L for 5 days as described by Diyaware *et al*. (2010b). These were carried out in A group of fish at day 0 and were reopened after 30 and 40 days in A1 and A2, respectively.

Milt collection by the orchiotomy method

Milt collection by orchiotomy was a surgical operation in which the fish was placed on dorsal recumbency on a wet, draped cloth on a surgical table. The head of the fish was covered with a wet, draped cloth for proper restraint. The abdominal surface was scrubbed with 70% isopropyl alcohol and was anaesthetised subcutaneously by linear infiltration using 0.5 ml/kg of 2% lidocaine. Incision was made on the mid ventral aspect of the abdomen on the skin down to the linea alba using a (size 15) scalpel blade. The incision was extended cranially 3-5 cm long with a sterile Metzenbaum scissors to expose the internal organs, and the blood was cleaned using a sterile gauze bandage. The digestive tract was lifted out and pushed aside to reveal the testes. The technique of milt collection in this method was by making a nick incision on the proximal part of the testes, and the suspensory ligament was loose at the proximal part to facilitate milt collection. The milt was measured in a 5 ml syringe and poured into a sterile bottle that was positioned close to the incision site. Following the procedure, they were put in a treatment tank with 500 L of water that contained 50 mg/L of the antibiotic oxytetracycline for five days. These were carried out in the B group of fish at day 0 and were reopened after 30 and 40 days in B1 and B2, respectively.

Milt quality assessment

Milt quality was evaluated at initial collection (day 0) and after reopening at day 30, and 40post-surgery for the groups. Milt volume was measured using a 5 ml syringe. Sperm concentration of the milt was determined using the Neubauer impure haemocytometer. A cover slip was placed on the hemocytometer after supporting the rails with water. This helps to hold down the cover slip while loading the sperm. Using a microtitre pipette,10-15 µl of the diluted sperm (1:20) was dropped under the cover slip on each side of the haemocytometer. The haemocytometer was carefully placed in the pre-wetted chamber. The bottom of the haemocytometer was dried and was placed under a microscope without tilting it. The haemocytometer was viewed at × 40 Objective, making sure to count 5 squares on each side of the haemocytometer, and then the average was calculated. Each slide of the haemacytometer has an identical grid system consisting of 25 large squares in which each large square is divided into 16 smaller squares. The sperm heads were counted in the 5 large squares (top right, top left, bottom right, bottom left and the centre) of which contains 16 smaller squares. The formula for sperm count is given as:

$$\text{Sperm count} = \frac{\text{Total no of sperm counted from the 5 squares} \times \text{ dilution factor}}{\text{volume} \times 1000}$$

Sperm per ml = the number of sperm counted $\times 10^9$

Table 2. Tank water quality parameters between morning and evening period.

Parameters		Groups			
		Partial Orchiectomy 30 days (A1)	Partial Orchiectomy 40 days (A2)	Orchiotomy 30 days (B1)	Orchiotomy 40 days (B2)
T ºC	Morning	29.53± 3.02	27.45± 2.90	29.56 ± 3.55	28.20± 1.99
	Evening	29.55± 5.10	27.86± 4.60	29.56 ± 3.99	28.25± 2.50
DO_2 mg/l	Morning	4.50± 0.56	4.32± 0.25	4.42± 0.34	4.92± 0.19
	Evening	5.25± 0.32	4.81 ± 0.13	4.95± 0.29	4.95± 0.29
pH	Morning	6.71±0.97	6.84± 0.83	7.00± 0.77	7.00± 0.77
	Evening	6.74± 0.87	6.90± 1.00	7.00± 0.69	6.70± 0.92

Values are expressed as mean ± SD.

Determination of fertilizability

The females were injected intramuscularly at a dose rate of 0.5 ml/kg to induce ovulation with ovaprim® manufactured by Western Chemical Inc. After 10 hrs latency period, the females were stripped by abdominal massage, and the eggs were collected from the genital opening of the female into a bowl. Potency of milt obtained from the experiment was tested by fertilising 1 g of eggs and mixing with milt and aqueous solution in a plastic container, and was rocked for 15 seconds, then incubated in conventional sieves (locally known as rariya) for 12 hours in a plastic tank under a flow-through system as described by Diyaware *et al*. (2010b). The Fertilization success was determined based on the appearance of the eggs, in which the fertilised eggs appeared greenish in colour, while the unfertilized eggs appeared whitish in colour, according to Oyebola and Awodiran (2015), by counting. This was confirmed by counting the unfertilized eggs under the microscope by observing the development of the animal pole via formation of perivitelline space and cell division, while the unfertilized eggs remained unchanged; the estimate was then subtracted from the initial counted number of eggs from the subset (1 gram). This was estimated 5-7 hours after fertilisation procedures and was calculated using the formula below as described by Esa *et al.* (2023).

$$\text{Fertilisation rate} = \frac{\text{Number of fertilised eggs}}{\text{Number of estimated eggs in 1g}} x100$$

Statistical analysis

Data on water quality parameters (pH, DO, and temperature) were analysed using analysis of variance (ANOVA). The data obtained for comparison between partial orchiectomy and orchiotomy method of milt collection were analysed using an unpaired t-test and were presented in tables. While the data obtained for comparison between the days of milt collection of the two methods was analysed using a paired t-test and was presented in graphs by using InVivoStat version 3.7.0.0, with $p<0.05$ as significant.

Ethical approval

Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, with reference number UDUS/IACUC/2020/AUP-R0-20. All experiments have been examined and approved by the committee.

RESULTS

The mean water quality parameters for the tanks where *C. gariepinus* groups were kept showed no significant differences observed in all parameters measured between the morning and evening time readings among all the tanks (Table 2). Quality and fertilizability of milt collected from C. *gariepinus* using partial orchiectomy and orchiotomy methods were presented in Tables 3a, 3b and 3c. At initial surgery (Table 3a), the sperm concentration, fertilizability and milt volume showed no significant difference between the two groups. At 30 days (table 3b) the partial orchiectomy method had a mean sperm concentration of 16.67 ± 20.66 × 10^9 /ml-1, mean fertilizability of 22.50 ± 25.25% and mean milt volume of 0.13 ± 0.16 ml which was significantly lower than the fertility indices for milt collected using orchiotomy method (P<0.05) with a mean sperm concentration of 68.00 ± 39.15×10^9 ml^{-1}, mean fertilizability of 62.50 ± 33.13% and mean milt volume of 0.89 ± 0.72 ml. At 40 days (table 3c), there was a significant decrease (P<0.05) in the mean sperm concentration, 26.50 ± 27.19 × 109 /ml-1, and mean milt volume 0.37±0.39 ml for partial orchiectomy compared to orchiotomy method with mean sperm concentration 72.00 ± 33.13 × 109 /ml-1 and mean milt volume 1.12 ± 0.66 ml. There was also a decrease in mean fertilizability, 40.00 ± 35.64% of milt collected using the partial orchiectomy method at 40 days, but not statistically significant ($p<0.05$) compared to the orchiotomy method

Table 3a. Characteristics of milt collected using Partial Orchiectomy and Orchiotomy Methods at 0 day.

Milt characteristics	Initial collection (Day 0)	
	Partial Orchiectomy (n=12)	**Orchiotomy (n=12)**
Sperm concentration ($\times 10^9$ /ml^{-1})	47.42 ± 30.30	66.92± 39.31
Fertilizability (%)	59.17 ± 31.39	63.75 ± 33.45
Milt volume (ml)	0.76 ± 0.52	1.13 ± 0.70

Values are expressed as mean ± SD.

Table 3b. Characteristics of milt collected using Partial Orchiectomy and Orchiotomy Methods at 30 days.

Milt characteristics	Post-surgery (day 30)	
	Partial Orchiectomy **(n=6)**	**Orchiotomy (n-6)**
Sperm concentration (x 10^9/ ml^{-1})	16.67 ± 20.66^a	68.00 ± 39.15^a
Fertilizability (%)	22.50 ± 25.25^a	62.50 ± 33.13^a
Milt volume (ml)	0.13 ± 0.16^a	0.89 ± 0.72^a

Values on the same rows with the same superscript$^{(a)}$ showed statistically significant difference at ($p<0.05$). Values are expressed as mean ± SD.

Table 3c. Characteristics of milt collected using Partial Orchiectomy and Orchiotomy Methods at 40 days.

Milt characteristics	Post-surgery (day 40)	
	Partial Orchiectomy **(n=6)**	**Orchiotomy (n=6)**
Sperm concentration (x 10^9/ml^{-1})	26.50 ± 27.19^b	72.00 ± 33.13^b
Fertilizability (%)	40.00 ± 35.64	66.67 ± 33.57
Milt volume (ml)	0.37 ± 0.39^b	1.12 ± 0.66^b

Values on the same rows with the same superscript$^{(a)}$ showed statistically significant difference at ($P < 0.05$). Values are expressed as mean ± SD.

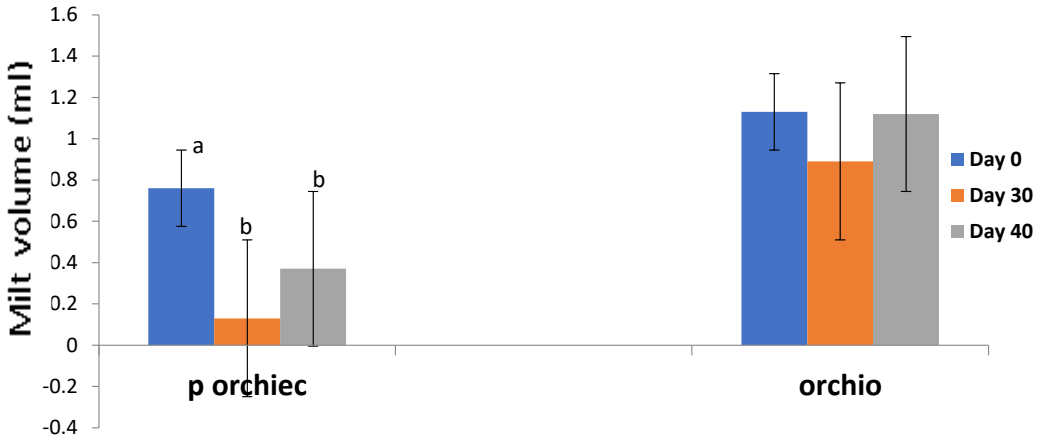

Figure 1. Milt Volume (ml) at 0, 30 and 40 days for Partial Orchiectomy and Orchiotomy Methods. Bars with different superscript $^{(a,b)}$ showed statistically significant difference at ($p<0.05$).

with mean fertilizability 66.67 ± 33.57%.

Milt volume, concentration and fertilizability at 0, 30 and 40 days of partial orchiectomy, and orchiotomy method are presented in Figures 1, 2 and 3, respectively. In the partial orchiectomy, there was a significant decrease ($p<0.05$) in the sperm concentration, fertilizability and milt volume at

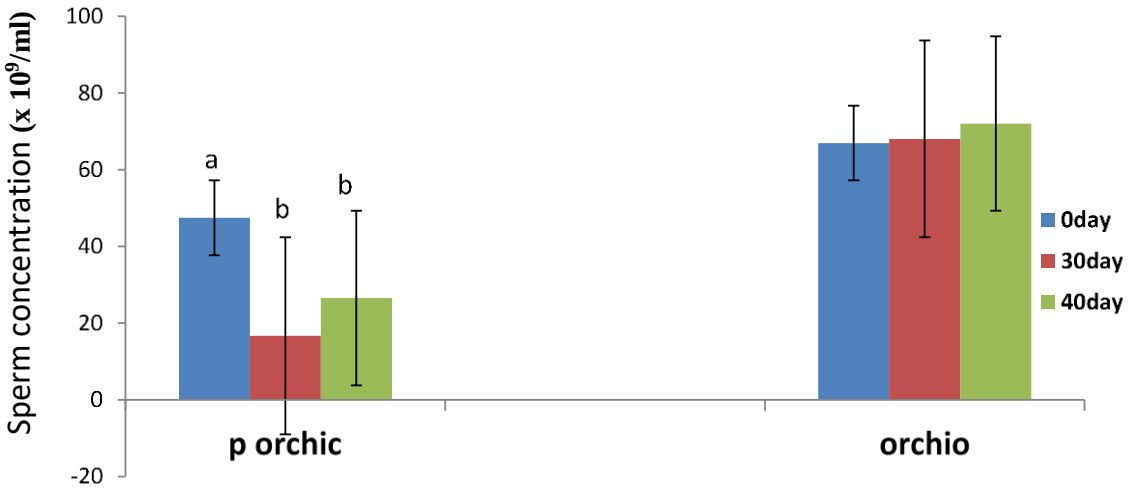

Figure 2. Sperm concentration (× 10^9 /ml^{-1}) at 0, 30 and 40 days for Partial Orchiectomy and Orchiotomy Methods. Bars with different superscript $^{(a,b)}$ showed statistically significant difference at ($p<0.05$).

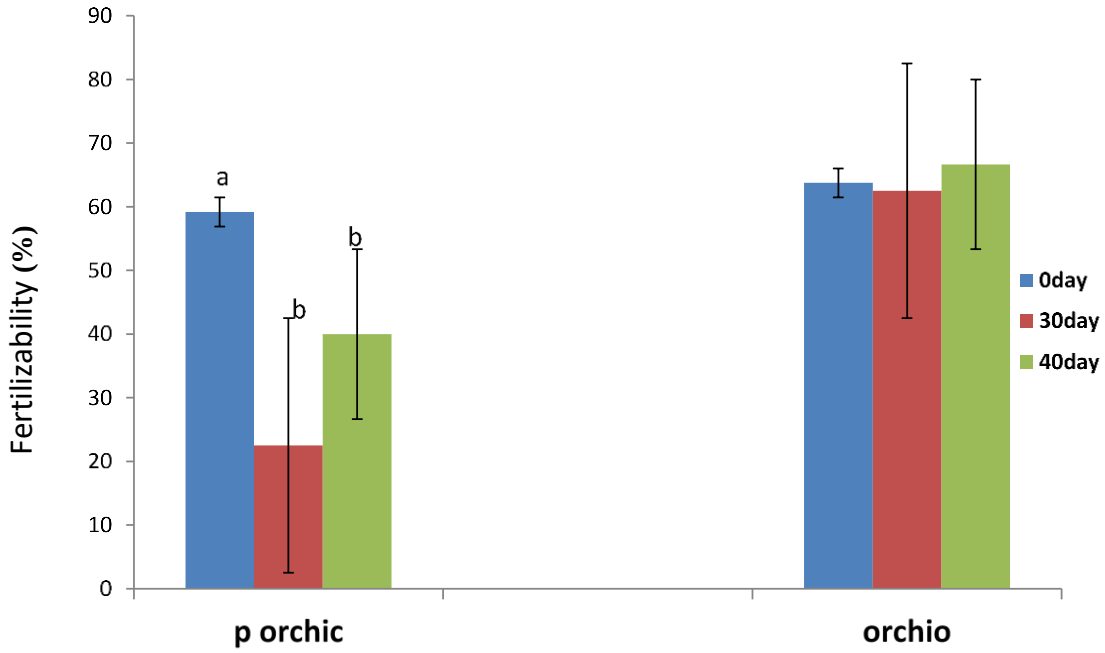

Figure 3. Fertilizability (%) at 0, 30 and 40days for partial orchiectomy and orchiotomy method. Bars with different superscript (^{a,b}) showed statistically significant difference at ($p<0.05$).

30 and 40 days post-surgery compared to day 0.

DISCUSSION

The water quality parameters recorded during this study were within the recommendations of Towers (2014) that temperature (26-32ºC), dissolved oxygen (4-9 mg/L) and pH (6.5-7.5) were the acceptable values for the rearing of African catfish. Healing of the incision site occurred within 14 days, but the suture material was absorbed within 21 days post-surgery in this study. This was in accordance with the work of Diyaware *et al.* (2010b), who reported that cicatrization (healing) of the incision occurred within 14 days in male *Clarias angularies.*

The present study provides compelling evidence that orchiotomy is a superior non-lethal technique for milt collection in *Clarias gariepinus*, particularly when repeated

collections are required for sustained breeding programmes. While both orchiotomy and partial orchiectomy yielded comparable milt quality at the initial sampling (day 0), marked differences emerged during subsequent collections, highlighting the importance of testicular integrity in maintaining reproductive performance over time. The lack of significant differences at initial collection suggests that both surgical approaches allow adequate access to viable spermatozoa. This observation is consistent with earlier findings that direct surgical exposure of the testes in teleosts can provide high-quality milt irrespective of technique, provided the testes are physiologically mature (Diyaware *et al.,* 2010a; Adebayo *et al*., 2012). However, the progressive decline in milt volume, sperm concentration, and fertilisation capacity observed in the partial orchiectomy group at day 30 and 40 post-surgery underscores the detrimental impact of tissue excision on spermatogenic potential. Removal of testicular tissue likely reduces the population of spermatogonia and disrupts the seminiferous architecture necessary for continuous sperm production, a phenomenon also reported in studies involving gonadal ablation in fish (Pronina and Petrushin, 2019).

In contrast, the stability of milt parameters observed in the orchiotomy group across all sampling periods demonstrates the advantage of preserving gonadal structure. Ochiotomy permits recurrent milt extraction without seriously hindering spermatogenesis by making a small incision in the testicular tissue. This finding aligns with the general principle in reproductive biology that conservation of germinal epithelium is critical for sustained gamete production (Woelders and Hiemstra, 2005). Furthermore, the rapid healing, absence of visible scarring, and minimal adhesion reported in this study suggest that orchiotomy induces less inflammatory response and tissue trauma compared to partial orchiectomy, thereby facilitating faster functional recovery. As observed in the study at day 40, there was a significant decrease in the sperm concentration and milt volume for the partial orchiectomy method (26.50 × 109 /ml-1, 0.37 ml) compared to the orchiotomy method. This is because the testes regenerated more at 40 days than at 30 days.

The anatomical peculiarities of *C. gariepinus* further contextualise these findings. The dorsal positioning of the testes, coupled with the presence of large seminal vesicles and viscous secretions, has been widely reported to hinder effective milt release through stripping (Viveiros *et al.,* 2001; Eduardo *et al.,* 2001). These constraints explain the historically low success rates of non-surgical milt collection techniques, including hormonal induction and syringe aspiration (*Nguenga et al*., 1996; Idahor, 2014). The orchiotomy method circumvents these limitations by enabling direct and controlled release of milt from the proximal testicular region, where the suspensory ligament permits easier manipulation. This targeted approach likely accounts for the consistently higher milt volume and sperm concentration observed in the present study.

Sperm concentration, fertilizability, and milt volume significantly decreased in the partial orchiectomy procedure at 30 and 40 days as compared to day 0. This is because it takes longer for the testes to regrow after a portion of them was removed. Sperm concentration, fertilizability, and milt volume did, however, increase between day 30 and day 40; this increase was not statistically significant. This disagrees with the findings of Guerra *et al.* (2008), Diyaware et *al.* (2010b), Adebayo *et al.* (2012), and Pronina and Petrushin (2019) that partial orchiectomy could not alter the quality of sperm production in European catfish, confirming data on African catfish. This is because Diyaware *et al.* (2010b) kept the fish for 3 months while Pronina and Petrushin (2019) kept the fish for 1 year. Therefore, the sperm derived from regenerated testes performed effectively for the fertilisation of eggs. This is because in this study, a shorter regeneration period (30 and 40 days) was used for partial orchiectomy, which is why lower milt volume was obtained.

The significantly reduced fertilisation rates associated with partial orchiectomy at 30 days further indicate that not only sperm quantity but also sperm quality may be compromised following tissue removal. Surgical trauma and repeated reopening of the abdominal cavity may lead to vascular disruption, oxidative stress, or contamination with blood and tissue debris, all of which can negatively affect sperm motility and viability. Similar observations have been reported in hormonally induced milt of poor quality, characterised by low motility and reduced fertilisation success (Viveiros *et al*., 2002). Although the difference in fertilisation rate at 40 days was not statistically significant, the consistently higher mean values in the orchiotomy group reinforce its biological and practical superiority.

From a fish welfare perspective, the rapid healing observed in this study indicates that minimally invasive surgical approaches such as orchiotomy may be compatible with repeated broodstock use, provided that appropriate anaesthesia, aseptic procedures, and post-operative management are applied. At the initial milt collection (day 0), no significant differences were observed between partial orchiectomy and orchiotomy in terms of sperm concentration, fertilisation rate, and milt volume. This indicates that both techniques are capable of yielding viable milt during the first collection. However, the absence of differences at this stage likely reflects the fact that both methods relied on mature testes with fully developed sperm reserves. The comparable fertilisation success obtained at day 0 confirms that surgical manipulation did not immediately impair sperm viability. This finding is important for artificial propagation programs because it demonstrates that milt obtained through surgical extraction can maintain fertilising capacity comparable to conventional testicular extraction methods commonly used in hatcheries.

In the orchiotomy method, there was an increase in the sperm concentration at 30 and 40 days compared to day 0, and also day 40 is slightly higher than day 30, but not significant. The fertilizability decreased but insignificantly at 30 days compared to 0 days; however, at 40 days the fertilizability increased but is insignificant compared to 0 days. The milt volume decreased in 30 and 40 days, but not statistically significant. In this method, there is no significant difference between days of collection; this is because the parenchyma of the testis is not affected; thus, no longer is a healing process required, and the spermiation period in catfish is 7 days. Due to that, similar volume, sperm concentration and fertilizability were obtained on all the days of collection. This is similar to the findings of Lacerda *et al*. (2019), who reported that the combined duration of meiotic and spermiogenic phases in catfish was estimated to be approximately 7 days.

In contrast, the orchiotomy method involved only a small incision in the proximal portion of the testes without removal of testicular tissue. Because the structural integrity of the testes remained largely intact, spermatogenic activity could continue with minimal disruption. This explains the relatively stable sperm concentration, fertilisation capacity, and milt volume observed at 30 and 40 days in the orchiotomy group. The preservation of testicular tissue is particularly important in fish reproductive management because continuous spermatogenesis allows broodstock males to be reused multiple times within a breeding season. These findings have important implications for sustainable aquaculture practices. In many African catfish hatcheries, the routine sacrifice of male broodstock during artificial fertilization to obtain milt has significant implications, including the loss of valuable genetic material and increased costs associated with broodstock replacement. The findings in this study, accentuates the importance of adopting methods that allow for repeated milt collection without necessitating the sacrifice of males.

Notwithstanding these encouraging results, it is important to recognise some limits. The study's brief length and comparatively small sample size may have limited its ability to adequately capture the long-term consequences of recurrent orchiotomy on reproductive physiology. Additionally, factors such as seasonal variation, nutritional status, and individual genetic differences could influence milt quality and should be considered in future investigations. The evidence base for the widespread use of this method would be further strengthened by longitudinal studies evaluating endocrine responses, cumulative surgical effects, and reproductive lifetime.

Conclusion

The findings of this study demonstrate that the orchiotomy method is superior to partial orchiectomy for milt collection, as it consistently yielded a greater milt volume, higher sperm concentration, and enhanced fertilisation capacity. In addition to improved reproductive output, orchiotomy was associated with favourable postoperative outcomes, including the absence of testicular adhesions to surrounding tissues, minimal or no scar formation, and accelerated wound healing. Notably, milt regeneration occurred more rapidly following orchiotomy compared with partial orchiectomy. Furthermore, milt collection at 40 days post-procedure proved more effective than at 30 days, as earlier reopening was associated with increased bleeding, which may compromise fish welfare and milt quality. Collectively, these results support orchiotomy as a more efficient and less invasive technique for sustainable milt harvesting in fish reproductive management.

COMPETING INTERESTS

The authors declare no conflict of interest.

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