Influence of the Length of Time after Hormonal Stimulation on the Milt Quality of African Catfish *Clarias gariepinus* Brood Stock

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**ABSTRACT**

**Background:** African catfish, *Clarias gariepinus*, is a popular fish species not only in Nigeria but in West Africa. Unlike many aquacultural fish species that milt can be collected from the male by stripping, milt from *C. gariepinus* can only be collected by sacrificing the male broodstock. To reduce the number of males to be sacrificed in an artificial reproduction process, the male has to be stimulated with hormone so as to enhance the quantity and quality of milt. As a result, knowledge of the best time to sacrifice the fish after hormonal stimulation is very essential to optimize the use of the male broodstock. **Objective:** This study was conducted to analyse the quality of *C. gariepinus* milt with regards to varied time following hormonal stimulation with ovaprim [(D-Arg6, Pro9Net) - sGnRH]. Milt was collected from 15 male *C. gariepinus* broodstocks, with the first portion obtained 8 hours post injection with ovaprim (0.25 ml kg⁻¹ b.w.). Subsequent portions of milt were obtained in batches at 5 hours interval i.e. after 13, 18 and 23 hours. Milt of the control group was obtained along with the first portion. Selected parameters such as volume of milt per kilogram of body weight (VOM, ml kg⁻¹ b.w.), sperm concentration (×10⁶ mL⁻¹ b.w.), number of spermatozoa in milt (×10⁶ kg⁻¹ b.w) sperm motility and percentage of sperm motility (MOT %) were analyzed by microscopic method. **Results:** The concentration of spermatozoa (10⁶ mL⁻¹), motility of sperm (%), number of spermatozoa (10⁶), number of spermatozoa in milt (10⁶ kg⁻¹ b.w) and sperm duration were highest at 13 hours after hormonal stimulation. **Conclusion:** This study shows that the best time to obtain milt after hormonal stimulation in male *C. gariepinus* is 13 hours and that hormonal stimulation with ovaprim has a positive impact on their milt quality since the values obtained from the treatment groups were better than the control group.

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**INTRODUCTION**

Nigerians are large consumer of fish and fish remains one of the main products consumed in terms of animal protein. Only about 50% of the demand for fish is currently being met by local supply. The fisheries sector is estimated to contribute 3.5% of Nigeria’s G.D.P and provides direct and indirect employment to over six million people (FAO, 2004).

The aquaculture industry in Nigeria has grown tremendously in the last three decades. The reason for the exceptional growth in aquaculture is mainly due to depletion of marine stock as there has been a consistent downward trend since 1974 in the proportion of over-exploited and depleted stocks from about 10% in the mid 1970’s to close to 25% in the early 2000’s (FAO,2004).

As aquaculture is gaining attention all over the world as a means of improving world’s fish production which is currently on decline due to dwindling output from capture fishery (FAO, 2009). One problem facing fish culturist is that fish may exhibit reproductive dysfunction when reared in captivity. Alavi et al. (2012) reported that in captivity, GnRH particularly LH secretion from pituitary, functions are disrupted in all sturgeon species, therefore synthetic or natural reproductive hormones should be applied for inducing final sperm maturation.
Meanwhile, the successful large-scale cultivation of any organism for human consumption demands that the resource be easily renewable (Harvey and Hoar, 1979). It is clearly disadvantageous to cultivate any organism when the supply of the young cannot be easily replenished.

*Clarias gariepinus* is suitable for culture, readily acceptable in Nigeria among fish farmers and consumer alike and as such command good commercial value. With so many people to feed in Nigeria and highly socialised citizenry that patronise night club, pepper soup joints and other places where catfish is cooked fresh. Today, a kilogram of catfish goes for more than US$ 3.75. Qualities which makes African catfish suitable for culture includes: ability to withstand poor water quality, omnivorous mode of feeding, fast growth rate (Rad et al., 2003) and quality of its meet (Pruszynski, 2003)

Therefore, in commercial production of *C. gariepinus*, the evaluation of milt quality is essential in order to increase the efficiency of artificial fertilization, as supply of fertile milt of high quality is essential in order to increase the efficiency of artificial propagation. Supply of fertile milt of high quality will give high survival and grow rate of the fish seed to meet the ever growing demand of the fish seed. When a low number of male broodstock is used, it is especially important to ensure that the sperm quality is good enough for high percentage fertilization. The correct or irregular functioning of male gonads based on the sperm concentration can be determined indirectly (Krol et al., 2006). The capacity of spermatozoa to fertilize an egg is determined by the sperm motility, duration of motility and sperm number. These are often used to estimate milt quality (Suquet et al., 1995; Chereguini et al., 1999; Rurangwa et al., 2004).

The maturation of spermatozoa in fish takes place in the spermatic ducts and it is hormonally controlled while the entire process of spermatogenesis is determined by environmental factors and linked to the species reproduction strategy (Billard, 1986). Hormonal stimulation with ovaprim [(salmon GnRH analogue plus a dopamine antagonist)] has been applied to a wide variety of families and species of fish to promote ovulation and spermatiation (Haniffa et al., 2007). The basic parameters determining milt quality are motility and concentration of spermatozoa (Krol et al., 2006; Hajirezaee et al., 2010a).

Although various researches has been carried out on the culture of *Clarias gariepinus*, but less study has been done on male broodstocks. Therefore, this study was to investigate whether extending the time of obtaining milt from 8 hours to 13 hours, 18 hours and 23 hours after hormonal stimulation with ovaprim would influence the qualitative parameters of *C. gariepinus* milt, since the success of artificial reproduction is greatly influenced by the quality of the gametes produced by the fish.

**MATERIALS AND METHODS**

**Experimental site:**

The research was carried out in the Fish Hatchery Unit of the Research and Teaching Laboratory of the Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba-Akoko, Ondo. Nigeria. Forty five male 1-year old *C. gariepinus* broodstocks were used. All the male broodstocks were divided into five experimental groups, i.e. group 1- control and group 2, from which milt was collected after 8 hours; group 3, from which milt was collected after 13 hours; group 4, from which milt was collected after 18 hours; and group 5, from which milt was collected after 23 hours from hormonal stimulation (n=3 per group).

Hormonal stimulation was performed with ovaprim at the rate of 0.25ml/kg of fish with the aid of hypodermal needle. Ovaprim is a complex, synthetic substance containing salmon hypothalumus hormones analogue [(D-Arg⁶, Pro⁹Net)-sGnRH] and domperidone, a dopamine receptor antagonist (Haniffa et al., 2007). Before milt collection, individuals in each group were weighed and the quantity of milt obtained from them was measured and stored at 4°C for further analyses.

**Evaluation of milt quality:**

At the end of the stipulated treatment period (i.e. after 8 hours, 13 hours, 18 hours and 23 hours of ovaprim induction respectively), the fish were killed and the testes removed, the milt was collected paying close attention to avoid contamination with blood in batches (T1, n=3, control; T2, n=3) after 8 hours. After 13 hours (T3, n=3), another batch was taken after 18 hours (T4, n=3) under the same conditions as the previous batches. The last batch of milt (T5, n=3) was obtained after 23 hours. The milt quality parameters determined include, Milt volume, motility of sperm, Number of spermatozoa, Concentration of sperm and Sperm duration.

For the Milt Volume determination, small incision was made into the cream coloured lobes of the testes, the milt squeezed out into a Petri dish and its volume was measured with plastic syringe in (ml).

Each sample was estimated for sperm motility using light microscope at 400× magnification immediately after dilution with 20µl distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted and when the activation stopped, whole sperm cell (WSC) was counted (Canyurt and Akhan, 2008). The motile sperm cell (MC) was calculated as;

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MC = WSC - ISC\] while the percentage motility was calculated as

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\%MC = \frac{MC}{WSC} \times 100
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The Concentration of sperm was determined by counting the number of spermatozoa in sample diluted with distilled water (1:100) in a Burk er haemocytometer (Improved Burk er haemocytometer B.S.748 Hawksley England), under 400X magnification (Rainis et al., 2003).

Sperm Duration was determined by placing 1 C. gariepinus µl of milt for each fish sample on a Neubauer haemocytometer (Improved Neubauer haemocytometer. B.S.748 Hawksley England), a drop of distilled water was added and covered with a slip. The milt was viewed under Olympus microscope at 100X magnification to see when all the sperm motility got stopped. This was determined in seconds (Mims, 1991).

To evaluate the Number of Spermatozoa, Sperm counts were conducted on a Neubaur haemocytometer by diluting the already diluted milt (1: 50) a further 1: 2 in cold catfish extender (10µl diluted milt + 10µl extender) in a microfuge tube. A 15µL aliquot of this diluted milt was allowed to flood one chamber of the Neubauer haemocytometer (using a hand held pipette). After 20 minutes (for the cells to settle), sperm cells were counted on an Olympus microscope (with a 40X objective) connected to a camera and television monitor.

Analysis of data:

The data obtained for each parameter were subjected to descriptive statistics using arithmetic mean and standard deviation and compared by one way analysis of variance (ANOVA) with SPSS 1.70 software package to determine the significant difference, thereafter the data was subjected to Duncan’s multiple range test to further verify the effect of length of time after hormonal stimulation with ovaprim on milt quality of C. gariepinus.

Results:

After the administration of ovaprim to male C. gariepinus, milt was obtained from all individual in each experimental group. The largest milt volume was obtained from fish group 2 (FG 2) (1.50±0.01), 8 hours after hormonal stimulation. It was also the largest volume of milt (2.14±0.99) per kilogram of body weight of males. The lowest milt volume was obtained from males of fish group 1 (FG 1) i.e. Control (0.70±0.01). It was also the lowest volume of milt (0.88±0.16) per kilogram of body weight (Table 1).

A statistically significant difference was found in milt volume samples obtained in the entire fish groups (P< 0.05). When the milt volume was expressed per kilogram of body weight, no significant difference was found in all the fish groups (P> 0.05).

The percentage of motile spermatozoa after activation was highest in fish group 3 (FG 3), 13 hours after hormonal stimulation while it was lowest in fish group 5 (FG 5) (51%). In the other fish groups, spermatozoa motility was at the level of 75%, 75% and 51% in group 1, 2 and 4 respectively (Fig. 1). There was significant difference in motility of Spermatozoa between group 1, 4 and 5 (P< 0.05).

The highest number expressed as a million of Spermatozoa per kilogram body weight (Fig.3) was found after 13 hours of hormonal stimulation with ovaprim, i.e. in FG 3 (120.33±1.02 × 106 kg⁻¹ B.W), while the lowest number was recorded in the control, i.e. FG 1 (40.01±0.01 × 106 kg⁻¹ B.W). The result in this parameter shows a significant difference in all the fish groups (P< 0.05).

The concentration of spermatozoa in milt expressed in millions of spermatozoa per mL⁻¹ (Fig.2) varies in all the fish groups (45.73±0.02; 54.47±1.01; 70.19±0.01; 65.98±0.01; 55.52±0.02) in groups 1-5 respectively. Significant difference in this milt quality parameter was found between tested fish groups (P< 0.05).

The motility duration reached very similar level in all the fish groups (62secs, 70secs, 72secs, 70secs and 70secs) in group 1-5 respectively. The lowest motility duration was obtained in the control (FG 1) (62secs). Significant difference in this parameter was found between the control (FG 1) and other fish groups at (P<0.05)

| Table 1: Mean (± S.D) of selected parameters of Clarias gariepinus milt quality obtained after hormonal stimulation with ovaprim (n = 3). |
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| PARAMETERS | CONTROL | TREATMENTS |
| | FG 1 | FG 2 | FG 3 | FG 4 | FG 5 |
| Body Weight (g) | 800.1±0.03<sup>a</sup> | 700.26±0.99<sup>a</sup> | 700.26±1.01<sup>b</sup> | 700.23±0.05<sup>a</sup> | 700.20±0.19<sup>c</sup> |
|Volume of milt (ml) | 0.70±0.01<sup>a</sup> | 1.50±0.01<sup>a</sup> | 1.20±0.01<sup>b</sup> | 0.86±0.01<sup>c</sup> | 0.91±0.01<sup>d</sup> |
|Motility of Sperm (%) | 75.07±0.02<sup>a</sup> | 75.24±1.00<sup>a</sup> | 75.41±0.99<sup>a</sup> | 50.81±0.03<sup>a</sup> | 50.70±0.99<sup>a</sup> |
|Concentration of Sperm (10<sup>6</sup> mL⁻¹) | 45.73±0.02<sup>a</sup> | 54.47±1.01<sup>b</sup> | 70.19±0.01<sup>a</sup> | 65.98±0.01<sup>a</sup> | 55.52±0.02<sup>a</sup> |
|No of Spermatozoa (10<sup>9</sup>) | 32.01±1.01<sup>a</sup> | 80.20±2.84<sup>b</sup> | 84.23±2.01<sup>b</sup> | 56.68±3.62<sup>b</sup> | 50.52±2.02<sup>b</sup> |
|Volume of milt (ml, kg⁻¹ b.w) | 0.86±0.01<sup>a</sup> | 2.14±0.99<sup>a</sup> | 1.71±1.01<sup>b</sup> | 1.21±1.01<sup>c</sup> | 1.29±0.09<sup>c</sup> |
|No of Spermatozoa in milt (10<sup>9</sup>, kg⁻¹ b.w) | 40.01±0.01<sup>a</sup> | 114.57±1.01<sup>b</sup> | 120.33±1.02<sup>b</sup> | 80.11±1.02<sup>b</sup> | 72.17±0.01<sup>d</sup> |
|Sperm Duration (seconds) | 62.00±1.00<sup>a</sup> | 70.00±1.00<sup>a</sup> | 72.00±1.00<sup>a</sup> | 70.00±1.00<sup>a</sup> | 70.00±1.73<sup>b</sup> |

Note: Values on the same row with different superscript are significantly different (P<0.05)
Fig. 1: Percentage sperm motility of *Clarias gariepinus* treated with Ovaprim hormone at various times of assessment.

Fig. 2: Sperm concentration (10^6 mL^-1) of *Clarias gariepinus* treated with Ovaprim hormone at various times of assessment.

Fig. 3: Number of Spermatozoa (10^6) of *Clarias gariepinus* treated with Ovaprim hormone at various times of assessment.

**Discussion:**

The use of hormones to stimulate milt production in teleost is a common practice in aquaculture (Zohar and Maylonas, 2001). Various marine and freshwater fish species have been successfully treated with GnRHα and found to be an effective method of improving sperm quantity and quality (Mylonas *et al.*, 1997a; Clearwater and Crim, 1998; Zohar and Mylonas, 2001). In Nigeria, the use of ovaprim (salmon GnRH + domperidone) is a popular agent for ovulation and induction in African catfish (*C. gariepinus*). In teleost fish, sperm motility is one of the parameters for the assessment of sperm quality (Lahnsteiner *et al.*, 1998). Krol *et al.* (2009) observed increase in motility duration in European smelt (*Osmerus eperlanus*) treated with ovaprim + domperidone hormone; the author also reported that GnRH can regulate stimulation of physiological events by directly or indirectly affecting the release of other hormones necessary for successful spermiation and milt production.
Seifi et al. (2011) reported that using carp pituitary gland (cPG) and ovaprim (GnRHa) treatments increased percentage of motile spermatozoa in cultured and wild carp compared to control. Also, GnRHa implants increased sperm motility in Atlantic halibut (Hippoglossus hippoglossus). An increase in percentage motile sperm in European catfish (Silurus glanis) was reported by Linhart and Billard, (1994). Similar results was obtained in this study, where males of African catfish (C. gariepinus) stimulated with ovaprim resulted in non-significant (P> 0.05) increase in percentage of motile spermatozoa compared to the control but duration of sperm motility was significantly (P< 0.05) higher in stimulated groups than the control. The duration of motile spermatozoa in each group was high and none was lower than 70% except the control group. The high values of motile spermatozoa were not surprising in that there were no contamination with urine because the milt was not obtained by massaging the abdominal parts. Also, duration of motility increased with time till 13 hours after stimulation and began to fall; this might be due to the presence of uncontrolled factors or anomalies which could indicate the beginning of milt ageing (Cejko et al., 2010).

Kucharczyk et al. (1999) reported that hormonal stimulation of male ide during the reproductive season using CPE and Ovopel caused a double increase in the volume of obtained milt as compared to the control group. Chub, Leuciscus cephalus L. treated with CPE and Ovopel resulted in increased milt volume and better biological quality of ejaculate was obtained as compared with the non-stimulated control group (Krejzef et al., 2008). Sperm volume significantly increased with (cPG) and ovaprim (GnRHa) treatments of cultured carp than wild carp (Hajirezaei et al., 2011). Caille et al. (2006) observed increase in sperm volume of tench (Tinca tinca) after inducing with carp pituitary extract compared to control group injected with rings solution. Similar results were reported in paddle fish (Polyodon spatula) (Linhart et al., 2000), and European cat fish (Silurus glanis) (Linhart and Billard, 1994). In this study, milt volume significantly (P < 0.05) increased in ovaprim treated fish groups compared with the control group. This result was in agreement with that of Zheng and Stacey (1996) who reported significant increase in the sperm volume of gold fish 6 hours post injection with hCG. Also, Cejko et al. (2010) reported that increased milt volume was found in each of the test groups after treatment with Ovopel, and it was highest 84 hours after injection in ide (Leuciscus idus L.)

Significant differences (P < 0.05) in concentration and number of spermatozoa emerged between the treatment groups and the control with these parameters been higher in the treatment groups than the control. The concentration and number of spermatozoa increased with time gradually to 13 hours after hormonal stimulation, then decreased steadily. It should be noted that the high milt volume recorded 8 hours after stimulation is followed by a decrease in concentration of sperm.

**Conclusion:**

These results supported the use of ovaprim hormone as an effective agent for improving spermiation and increasing sperm production in C. gariepinus. Also, it suggests that its milt should be collected 13 hours after ovaprim hormonal stimulation to ensure high quantity and quality milt.

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