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Full Length Research Paper

Sperm Quality and reproductive performance of male *Clarias Gariepinus* induced with synthetic hormones (Ovatide and Ovaprim)

Oguntuase Bukola Gbemisola[1]* and Adebayo O. T.[2]

[1]Department of Ecotourism and Wildlife Management, Federal University of Technology, Akure, Ondo State, Nigeria.
[2]Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

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Food, especially food protein source is highly essential for healthy living, therefore, this study was aimed at boosting fish seed production and it compared the effectiveness of two different synthetic hormones: Ovaprim and Ovatide on sperm quality and reproductive performance of male *Clarias gariepinus* and the resulting effects on percentage fertilization, hatchability, and survival. The study was made up of three experimental groups. The first group comprising four males was injected with 0.3 ml/kg of ovatide hormone, the second group with four males was injected with 0.3 ml/kg of ovaprim hormone; and the third group, the control group with four males was not given hormones. Sperm motility duration was higher in males induced with the synthetic hormones (29.5 and 25.5 s respectively) which were significantly different (P < 0.05) from the control (18.5 s). Motility percentage was observed to be higher too in the groups treated with the hormones and was different significantly (P < 0.05) from the control. Percentage egg fertilization was similar for the groups induced with the hormones (94.4 and 87.7% respectively) while the control (63.8%) differed significantly from them. Percentage hatchability was highest in eggs fertilized with milt from ovaprim induced males (66.3%) which was significantly higher than those of Ovatide induced males (59.7%) and the Control (14.4%). Milt volume was highest in ovatide induced males (1.1 ml) and was significantly different from ovaprim induced males (0.60) and the control (0.25). Survival rate was the same for all the treatment. The hormones gave similar results except for the milt volume. Treatment with the hormones yielded better result when compared to the control, it could be inferred therefore that, the use of synthetic hormones is better in reproduction of *C. gariepinus* in order to boost productivity thereby ensuring abundant food protein production.

Key words: Spermiation, reproductive performance, ovatide, ovaprim, male *Clarias gariepinus*

INTRODUCTION

Fish forms an important source of human diet as they provide proteins, fats and especially vitamins A and D. Special importance of fish is that they contain vitamin B, which is not present in the plant food. Fish is the good source of calcium; polyunsaturated fatty acids belonging to linolenic acid series (18:3) are present in fish (Crawford et al., 1989). Fish culture came in as a means of producing adequate fish food for human consumption

*Corresponding author. E-mail: bukolaoguntuase@gmail.com; +2348130927642.

on regular basis, in other words producing fish in and out of season. The practice of fish culture is very old. Huet (1972) traced the art of fish culture in ponds to the Egyptians, the Romans, the people of Indo-Pacific regions, and the Chinese. However the first written account of fish culture in ponds was by a Chinese fish farmer Fan Lai, in 475 BC (Chackroff, 1976).

Also in Nigeria, the first trace of fish farming was the practice by some missionaries in the early 1920's in Ilora, Oyo State, where fish was raised to supplement the protein intake of pregnant women (Bamidele, 2007). In Nigeria, fish alone contributes, on the average, 20 to 25% per caput animal protein intake and could be as high as 80% in coastal and riverrine communities (FAO, 2000). Aquaculture therefore remains the only viable alternative for increasing fish production in order to meet the protein needs of the people. No other group of vertebrates serves man in so many forms as fish (Bamidele, 2007). African catfish (*Clarias gariepinus*) is a hardy species for aquaculture purposes, it is widely accepted in the tropics and commands good commercial value. This species will not readily breed in captivity all year round and hence the need for artificial seed production using hormones.

In order to boost fish production on a level that will be able to serve people as reliable source of protein, there is a need to be able to reproduce fish on a large basis in and out of season to ensure regular supply. Artificial reproduction and selective breeding has become very popular nowadays in the age of science and technology as a means of ensuring large scale production of fish seeds throughout the year. Artificial propagation methods constitute the major practicable means of providing enough quality seed for rearing in confined fish enclosure such as fish ponds, reservoirs and lake (Charo and Oirere, 2000).

Reproduction in fish is controlled by several factors which include sex steroids in the regulation of reproductive processes (Kime, 1993). These reproductive processes are controlled through the brain-pituitary-gonadal axis. The brain is stimulated by environmental cues like water rise, temperature, feeding, rainfall, and photoperiod to release gonadotropin releasing hormones (Zohar et al., 2010). Ovulation and spermiation are effected as a result of the sex steroids that have been produced. Administration of gonadotropin releasing hormone analogue has been reported to increase levels of plasma sex steroids in fish (Zhuo et al., 2011).

The use of synthetic hormones in female fish is now popular as a means of artificially inducing the female fish in order to ovulate. However, Zhuo et al., 2011 has shown from his study that Gonadotropin releasing hormone analogue multiple injection potentially accelerated testicular maturation of male yellow catfish. The use of both synthetic and natural hormones brings about quick ovulation and higher percentage of hatched fish, though synthetic hormone gives higher yield than the natural hormones (Krol et al., 2006).

Gonadotropin Releasing Hormone analogue (GnRHa) is now the best available biotechnological tool for the induced breeding of fish. Ovaprim and Ovatide are both synthetic hormone preparation containing salmon gonadotropin releasing hormone analogue and domperidone (SGnRHa + Domperidone) which are usually used for spawning induction in catfishes to get quality seed (Sahoo et al., 2005). Synthetic hormones, and specifically, Ovaprim are known to significantly increase ovulation in African catfish (Sharaf, 2012).

As shown by researchers, synthetic hormones are best in inducing ovulation in female fish towards the yield of viable seeds on regular basis, there is a need to also cause the same effect of viable seed production by inducing spermiation in male fish. The use of hormones in female fish is gaining popularity each day while there is little work on inducing spermiation in male fish. This study was necessary to test and compare the effects of synthetic hormones on the milt quality of *C. gariepinus* and the resultant effects of the induced milt on seed yield and quality.

MATERIALS AND METHODS

The study was carried out in the Fish Farm of Federal University of Technology, Akure in Ondo State, Nigeria, between May and July, 2009. Ovaprim and Ovatide (SGnRHa + Domperidone) are synthetic hormones containing gonadotropin releasing hormone analogues and Domperidone that were used for the study; the synthetic hormones were from Aqualife Syndel International Inc., Vancouver, BC, Canada and Hemmo Pharma, India respectively. Matured males and female *C. gariepinus* between 8 and 9 months old and weighing between 1.0 to 1.2 kg were procured from the Federal University of Technology Akure Fish farm.

Brooder selection and maintenance

Hormonal treatments of the broodstocks were conducted in the month of May 2009 in order to coincide with the rainy season, which is the natural breeding period of the fish.

The broodstocks consisting of 12 males and 1 female were removed from the ponds and stocked in concrete tanks for a period of 4 weeks for acclimatization prior to induction. The broodstocks were selected on the basis of their morphological or external sexual characteristics (Ayinla et al., 1994).

The selected brood stocks were weighed individually, and the different weight recorded and they were stocked in plastic troughs of about 25 L capacity each.

Preparation of the spawning bowls

The spawning troughs of about 5 L capacity were collected, washed thoroughly and dried. The bowls were labeled as Control (1, 2, 3, and 4), Treatment A1, A2, A3, A4, and Treatment B1, B2, B3, and B4.

Preparation of hormones and injection of brooders

The quantity of Ovaprim and Ovatide used was commensurate with the weight of the male fish to be injected that is, 0.3 ml per kg of male fish.

Treatment A: Ovatide hormone was injected into the dorsal muscle of four male broodstocks serving as Replicate 1, 2, 3, and 4 accordingly.
Treatment B: Ovaprim was injected into the dorsal muscle of four male broodstocks serving as Replicate 1, 2, 3, and 4 accordingly.
Control: Four male broodstocks were not injected with hormones at all, while the female broodstock to be used for reproductive test was induced to ovulate with 0.5 ml/kg Ovaprim hormone. Administration of dosage and observation of latency period was according to Legendre (1986) and the specifications for the use of the synthetic hormones by the manufacturers. The injected area of each fish was massaged gently with a finger in order to distribute the hormone evenly into the muscle while slowly retracting the needle.

Dissection of male broodstock for gonad removal

The male broodstocks after observing the latency period of 12 h were removed from the troughs; they were placed dorsally on a wet towel. The fish was held firmly down to ensure careful removal of the testis. Using a sharp blade, the abdominal cavity of the fish was dissected and testes were carefully removed from where they were lying, at the ventral wall of the abdominal cavity. The testes were removed whole and cleaned with fluffy material.

Stripping and egg fertilization

After observing the 12 h ovulation period, the female broodstock was removed from the trough carefully and held firmly with a wet towel at both ends, the abdomen was then pressed carefully to release the eggs into a dry bowl, and the stripping was done towards the fish vent. After stripping, the spent female was carefully returned into the trough. 1 g each of the egg mass that is, about 700 eggs, (Viveen et al., 1985) was weighed out and the content of the testes (milt) was spilled on the eggs for fertilization, the eggs were then poured inside the labeled spawning troughs already containing 1 L of water for the sperm activation, and the fertilized egg was left in the spawning troughs for incubation with water temperature between 25 and 27°C.

Incubation and hatching

The fertilized egg masses were incubated in the spawning bowls for a period of 24 to 40 h. After this period, most of the larvae emerged and some eggs did not hatch at all. The percentage hatching rate was estimated 40 h after fertilization.

Survival

After hatching and determination of hatching rate, the unhatched eggs were siphoned out of the spawning bowls in order to ensure the survival of the hatched ones and the water was partially changed with utmost care. The larvae were daily observed after hatching for about 4 weeks to determine the rate of survival by estimating the dead and live ones.

Sperm evaluation

The sperm evaluation includes: gross (visual) and microscopic examination (as reviewed by Rurangwa et al., 2004; Cosson et al., 2008a, b; Cabrita et al., 2009)

The gross examination was based on visual and physical observation such as the milt volume by collecting the milt in graduated cylinder and the level was determined in milliliters. While the microscopic examination was carried out using Olympus model CX40, with magnification between X10 and X25 to determine other parameters such as: motility (duration and percentage). Motility percentage and duration were determined by observing water activated milt placed on a glass slide under microscope. The motile sperms were observed and expressed as a percent of non-moving sperm. Motility duration was determined as the period between movements of the sperm to cessation of any progressive movement expressed in seconds.

Analysis of data

The data obtained for each hormone treatment were compared by one-way analysis of variance (ANOVA) test to determine significant difference ($p = 0.05$), and treatment means were subjected to the Duncan's Multiple Range Test.

Calculations

The rates of fertilization (Adebayo, 2006), hatching, survival, and relative fecundity were calculated as shown below:

$$\text{Fertilization rate} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs counted}} \times 100$$

$$\text{Hatching rate} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs in a batch}} \times 100$$

$$\text{Survival rate} = \frac{\text{No. of hatchlings alive up to larvae stage}}{\text{Total no. of hatchlings}} \times 100$$

RESULTS

Significant differences in spermiation success between control and hormonally induced groups were observed.

Sperm evaluation

The motility duration was expressed in seconds, and volume in ml. Milt was collected from all males originating from both experimental groups (Ovatide and Ovaprim treated males, as well as from the control).

Figure 1 shows the result of the motility duration of hormonally induced milt compared with control, motility duration was higher in the induced groups and was significantly different from the control ($p < 0.05$) as well as the motility percentage (Figure 2). Effect of the synthetic hormones was similar in terms of sperm motility percentage in the groups induced, which was different significantly from the control. Milt volume (Figure 3) was recorded to be highest in males treated with Ovatide, and was significantly different from the other treatments ($P < 0.05$).

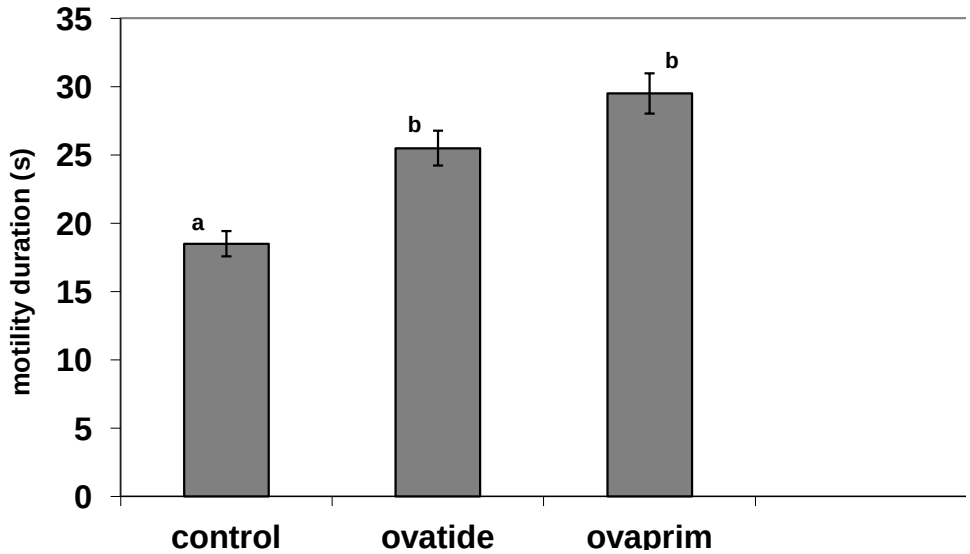

Figure 1. Motility duration of induced male *C. gariepinus* compared with the control.

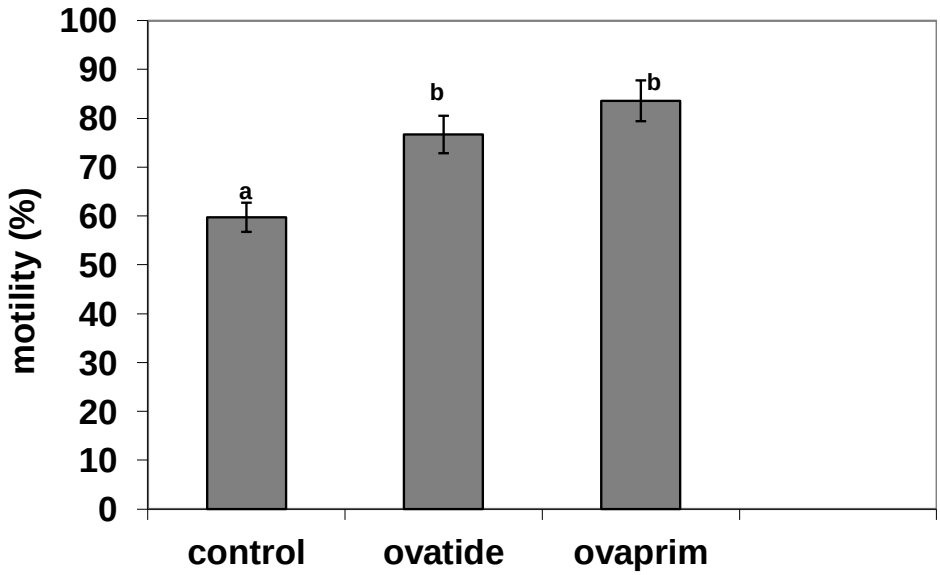

Figure 2. Motility percentage of induced male *C. gariepinus* compared with the control.

Effects of hormonally induced milt on fertilization, hatchability and survival.

Table 1 shows the effects of the milt collected from the hormonally induced groups and the control group on female *C. gariepinus* eggs.

Effects on female eggs showed that milt of male induced with the hormones had higher percentage fertilization, hatchability and survival which were significantly different from the control. The hatching period was the same for all the treatments, while the hatching time varied with the highest time observed from the control male.

DISCUSSION

The quality of fish sperm is as important as quality of female eggs for viable off-springs, various studies have been carried out on induced spawning in female fish with lesser attention on the male counterpart. Sperm morphology, density, volume, motility and fertilizing

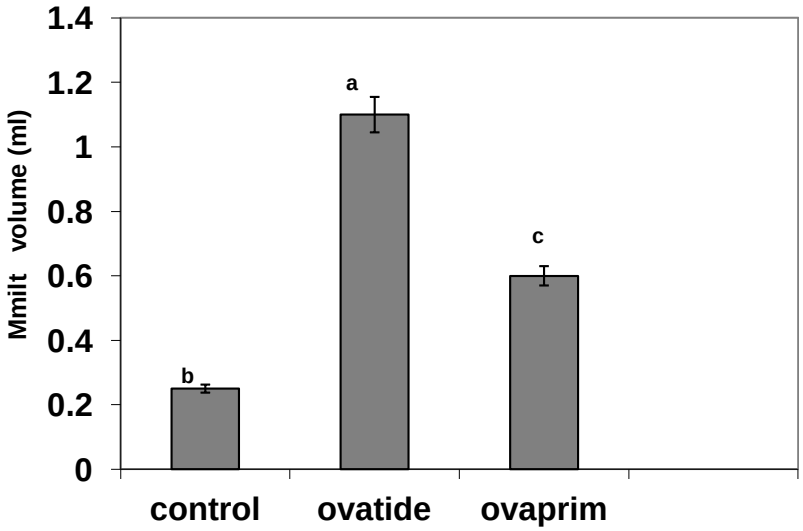

Figure 3. Milt volume of induced male *C. gariepinus* compared with the control.

Table 1. Reproductive performance of induced male sperm on fertility, hatchability and survival percentage of *C. gariepinus* egg.

Parameter	Control	Ovatide	Ovaprim
Fertilization (%)	63.81 ± 5.21[a]	87.73 ± 3.38[b]	94.40 ± 2.03[b]
Hatchability (%)	14.14 ± 2.14[a]	59.70 ± 0.88[b]	66.37 ± 0.74[c]
Survival (%)	61.91 ± 4.77[a]	63.56 ± 13.56[a]	77.38 ± 5.95[a]
Hatching time	26.5 ± 0.50[a]	23.5 ± 0.50[a]	24 ± 00[a]
Hatching period	72 ± 00	72 ± 00	72 ± 00

*Mean values in the same row having the same superscript are not significantly different.

capacity, as well as composition and osmolality of the seminal plasma are parameters commonly measured to assess sperm quality in fish (Alavi et al., 2004).

Fish often respond well to hormonal induction, in atlantic sturgeon, both sexually matured and immature males can be induced to spermiate by injection of gonadotropins. The gonadotropin-releasing hormone stimulates the synthesis and release of pituitary gonadotropin (Melamed et al., 1996), and GTH stimulates the production of steroid hormones in the gonads (Van der kraak et al., 1992). In some species such as *C. gariepinus,* hormonal treatments are the only way for controlling reproduction reliably. Treatment with GnRHa has been tested successfully in various marine and freshwater species and has been shown as an effective strategy for improving milt quantity and quality (Mylonas et al., 1997; Clearwater and Crim, 1998; Mylonas and Zohar, 2001; Zohar and Mylonas, 2001). GnRHa acts at a higher level of the hypothalamus pituitary gonad axis than gonadotropins. Consequently, GnRH can provide a more balanced stimulation of reproductive events and presumably a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful spermiation (Zohar and Mylonas, 2001).

This study has clearly shown that sperm of *C. gariepinus* gained better quality when induced with synthetic hormones as compared to the control, this agrees with the work of Adebiyi et al. (2013) that Ovaprim, a synthetic hormone produced best result in inducing spawning in a river Catfish (*Hemibagrus nemurus*).

Synthetic hormones gave a significant increase in milt volume and other sperm parameters as observed in this study which is in line with the findings of Crim et al. (1988) and Lin et al. (1996) for studies on Rainbow trout *Oncorrchnchus mykiss* and *Paramisgurnus dabryanus* respectively. It is also known, as opined by Mylonas et al. (1997), Zohar and Mylonas (2001) that, the treatment of fish with hormones by injection typically results in a short-term increase in milt volume, and changes in plasma steroids. It is possible that, in smelt, increase in milt volume were partially caused by milt hydration, indicating that seminal plasma volume was being increased at a faster rate than spermatozoa production. Sperm motility in teleost fish, is so far considered the best

biomarker of milt quality (Lahnsteiner et al., 2004). One of the objectives of this study was to examine the quality of the sperm in terms of motility (duration and percentage); and volume. As it was in yellowtail flounder *Limanda ferruginea*, treatment with two different types of GnRHa-delivery systems increased the percentage of motile sperm from 20, 40 to 90% (Clearwater and Crim, 1998). There was an overall improvement in the sperm quality of the induced males in this study which is supported by the findings of Taghi et al. (2010) where male carps induced with synthetic hormones showed improved spermatological properties.

This study showed that there was higher percentage motility observed in induced male when compared to the control, this agrees with the work of Krol et al. (2006) which showed higher percentage of motile sperm stripped smelt spermatozoa of stimulated males than the control group.

The spermatozoa motility and its duration have great influence on successful fertilization.

In this study, percentage fertilization, hatching and survival rate were recorded to be higher in the treated groups, and were significantly different from the control, this agrees with the work of Krol et al. (2006) in which Progeny of smelt hatched with milt from Ovaprim induced male had higher survival than progeny hatched naturally and with the work of Ndimele and Owodeinde (2012) where the induced group of *C. gariepinus* showed significant increase in percentage fertilization, hatching rate, and surviva. Also the findings of Nwokoye et al. (2007) further substantiate the efficacy of synthetic hormones by recording highest mean number of fertilized eggs, hatchability, and survival in clariid catfish (*Heterobranchus bidorsalis*) injected with synthetic hormone (Ovaprim).

CONCLUSIONS AND RECOMMENDATION

The use of synthetic hormone is a better way of improving catfish production with respect to reproductive performance in aquaculture. Stimulation of males by Ovaprim and Ovatide significantly increased volume of milt, motility duration and percentage. The efficacy of these synthetic hormones was evident on the reproductive performance as tested on the female *C. gariepinus*.

The study has shown that, artificial stimulation of male *C. gariepinus* using synthetic hormone is more reliable in reproducing fish. The use of these hormones in male fish is a means of boosting reproductive performance and ensuring good and viable fish seeds. The results arising from this trial is important in encouraging fish seed production through the use of synthetic hormones.

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