

Induced Ovulation and Spawning in African Catfish (*Clarias gariepinus*) Using Different Doses of Ovaprim

Fouzi A. Mohamed, Ramzy A. Yousif*, Ahmed A. Mohamed, Babeker I. Osman, Tajaldeem M. Abdalmwla

Department of Fisheries and Wildlife Science, College of Animal Production Science & Technology, Sudan University of Science & Technology, Khartoum, Sudan

*e-mail: ramzy@sustech.edu

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Abstract: The African catfish (*Clarias gariepinus*) is a cornerstone of tropical aquaculture, yet its reliance on induced spawning requires optimized hormonal protocols and broodstock management. This study investigated the effects of varying Ovaprim dosages and female-to-male broodstock ratios on the spawning success of *C. gariepinus* at the Fish Hatchery Unit, Sudan University of Science and Technology. Three treatments were evaluated: Treatment 1 (2:3 male : female ratio, 3.3 ml/kg Ovaprim), Treatment 2 (1:3 male : female ratio, 5 ml/kg Ovaprim), and Treatment 3 (2:3 male : female ratio, 3.7 ml/kg Ovaprim). Broodstock were conditioned for one week and fed a 2.5% body weight diet twice daily. The hormone was administered via intramuscular injection, and reproductive parameters, including latency period, ovulation rate, fertilization rate, and hatchability, were recorded. Data were summarized using descriptive statistics and one-way analysis of variance (ANOVA) to determine differences among treatments. The results demonstrated that Treatment 2 yielded significantly superior results, achieving a 50% larval production rate and a 10% survival rate, whereas Treatments 1 and 3 failed to produce any hatched larvae despite successful fertilization. This suggests that a 1:3 male-to-female ratio combined with a higher dosage (5 ml/kg) may overcome environmental or physiological latency in the Sudanese context. These findings emphasize the necessity of balancing hormonal induction with appropriate sex ratios to ensure hatchery sustainability.

Keywords: Broodstock; *Clarias gariepinus*; Hormone; Ovaprim; Spawning.

Introduction

The African catfish, *Clarias gariepinus*, is a cornerstone of aquaculture across Africa, owing to its remarkable attributes, including rapid growth, resilience, high fecundity, and robust market demand [1]. Its inherent ability to thrive in diverse environmental conditions, including suboptimal dissolved oxygen levels, makes it an ideal candidate for intensive aquaculture systems [2]. Despite these advantages, the natural reproductive cycle of *C. gariepinus* in captivity is often inconsistent and unpredictable. This variability primarily stems from the absence of specific environmental cues, such as seasonal flooding and fluctuations in water parameters, that are crucial for triggering spawning in their natural habitats [3]. Consequently, the development and application of induced breeding techniques have become indispensable for ensuring a stable and continuous supply of fingerlings to sustain and expand aquaculture production.

Conventional breeding methods often face challenges such as the availability of parents, limited time and low survival rate of hatched offspring due to environmental stress, as a result, artificial spawning/breeding techniques, especially hormone-inducing techniques, have gained prominence as they significantly increase the fertilization rate and improve embryo survival [4], [5]. Among the many hormonal agents used for reproduction, ovaprim, a synthetic hormone made from a gonadotropin-free analogue, has become a widely used alternative due to its effectiveness in inducing ovulation in fish [6], [7].

Hormonal induction represents a widely adopted and effective strategy to circumvent reproductive challenges in captive fish populations. A variety of hormones and their synthetic analogues have been successfully employed to stimulate ovulation and spawning in numerous commercially important fish species [8], [9]. Among these, Ovaprim, a potent synthetic gonadotropin-releasing hormone analogue (GnRHa) combined with a dopamine antagonist, has gained significant prominence. This formulation has proven to be a highly effective and popular choice for induced breeding in a broad spectrum of teleosts, including various catfish species [10], [11]. The sGnRHa component of Ovaprim directly stimulates the pituitary gland, prompting the release of stored gonadotropins. Concurrently, the dopamine antagonist (domperidone) acts to neutralize the inhibitory effects of dopamine on gonadotropin secretion. This dual action enhances and sustains the hormonal cascade, culminating in final oocyte maturation and successful ovulation [12], [13].

The efficacy of Ovaprim, however, is not uniform and can be influenced by numerous factors. These include the specific fish species, the physiological condition of the broodstock, prevailing environmental parameters, and critically, the precise dosage administered [14], [15]. Optimizing the Ovaprim dosage is a paramount consideration for achieving successful spawning outcomes, high fertilization and hatching rates, and the subsequent production of robust and viable larvae. This optimization must also balance biological effectiveness with economic

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viability, particularly for large-scale hatchery operations [16], [17], [18]

Artificial propagation of African catfish has been widely achieved using synthetic hormones such as Ovaprim, which stimulate ovulation and spawning in hatchery systems [11], [19], [20]. Recent studies have further emphasized the importance of optimizing hormone dosage, broodstock management and environmental conditions to maximize fertilization and hatchability rates in *Clarias gariepinus* breeding programs.

Inadequate dosages can lead to incomplete ovulation, compromised egg quality, or even mortality of the broodstock, while excessive doses represent an unnecessary expenditure and can potentially have adverse effects on the fish. Therefore, a thorough understanding of *C. gariepinus*'s physiological responses to varying Ovaprim concentrations is essential for developing sustainable and efficient aquaculture practices. This study aims to contribute to this understanding by providing empirical data on the effects of varying Ovaprim dosages.

Despite these advances, significant knowledge gaps remain, particularly regarding the optimal Ovaprim dosage and broodstock ratios under local hatchery conditions in Sudan. Most previous studies have been conducted in Asia and other African regions, where environmental conditions, broodstock quality, and hatchery practices may differ from those found in Sudanese aquaculture systems. Consequently, there is limited empirical information available to guide hatchery operators in Sudan on the most efficient hormone dosage and broodstock management strategies to maximize reproductive success. Furthermore, only a few studies have simultaneously examined the interaction between hormone dosage and broodstock ratio, which are both critical factors influencing fertilization success, egg quality, and larval hatchability.

Therefore, the present study aimed to evaluate the effects of different Ovaprim dosages and broodstock ratios on the reproductive performance of *Clarias gariepinus* under Sudanese hatchery conditions. By addressing these variables simultaneously, this study provides new insights into optimizing induced breeding protocols for African catfish. The findings are expected to improve hatchery efficiency, enhance seed production, and support the sustainable development of aquaculture in Sudan.

Research Methods

Study Area and Period

The study was meticulously conducted at the Fish Hatchery Unit, an integral part of the Department of Fisheries Science and Wildlife within the College of Animal Production Science and Technology at the Sudan University of Science and Technology. The experimental work was carried out over a four-month period, spanning from July to October 2018. This specific timeframe was chosen to coincide with the natural breeding season of *Clarias gariepinus* in the region, thereby ensuring that the broodstock were physiologically primed for reproduction and maximizing the likelihood of successful hormonal induction.

Experimental Design

The experiment was conducted using a completely randomized design (CRD) to evaluate the effects of different hormone doses and broodstock ratios on the reproductive performance of African catfish. Broodstock were randomly assigned to treatment groups receiving different doses of Ovaprim. Each treatment was replicated to ensure reliability of the experimental results.

Broodstock Management

Sexually mature African catfish (*Clarias gariepinus*) broodstock were carefully collected from local aquatic sources, ensuring a diverse genetic pool and healthy specimens. Following collection, the fish were transported to the Fish Hatchery Unit, where they underwent an acclimatization and conditioning period of one week. This conditioning phase was conducted in separate holding ponds to minimize stress and facilitate adaptation to the hatchery environment. During this period, the broodstock were provided with a high-quality control diet, administered at a feeding rate equivalent to 2.5% of their total body weight, twice daily. This feeding regimen was designed to ensure adequate nutritional reserves for gonad development and to improve overall broodstock health. Prior to hormonal induction, selected broodstock were individually transferred and maintained in separate ponds without feeding for 24 hours. This pre-injection fasting period was implemented to further reduce stress and ensure the fish were in an optimal physiological state for spawning, as a full gut can interfere with the injection process and subsequent physiological responses, potentially leading to regurgitation or reduced hormone absorption.

Hormonal Induction and Spawning

Ovaprim, a commercially available synthetic spawning-inducing agent, was employed for the hormonal induction process. Female broodstock were administered Ovaprim via intramuscular injection, a common and effective route for hormone delivery in fish, typically into the dorsal musculature. The experimental treatments were configured as follows:

Treatment 1 (T1): A total Ovaprim dosage of 3.3 ml was administered to three female broodstock. The mean weight of these fish was recorded as 693.8 g each. This dosage translates to an approximate individual dosage of 1.58 ml/kg body weight. This treatment served as a lower-dose experimental group, designed to test the efficacy at a concentration potentially below the optimal range.

Treatment 2 (T2): A total Ovaprim dosage of 5.0 ml was administered to three female broodstock, each with a mean weight of 635.2 g. This dosage corresponds to an approximate individual dosage of 2.63 ml/kg body weight. This treatment was hypothesized to be within an effective range, based on common practices and preliminary observations.

Treatment 3 (T3): A total Ovaprim dosage of 3.7 ml was administered to three female broodstock, with each fish

having a mean weight of 454.6 g. This dosage equates to an approximate individual dosage of 2.71 ml/kg body weight. This treatment used a slightly higher dose than T2, allowing comparison of efficacy at closely related concentrations and investigation of potential saturation or inhibitory effects at higher doses.

Following the intramuscular injection of Ovaprim, the treated broodstock were maintained for a precisely determined latency period of 12 hours [17]. This critical period was allocated to allow for the complete process of post-ovulatory maturation to occur, ensuring that the oocytes reached their final stage of readiness for fertilization and preventing premature stripping Figure 1. Subsequent to the latency period, the fish were gently stripped to manually collect the ovulated eggs. Milt, containing spermatozoa, was obtained from mature male broodstock through a process involving their sacrifice and the careful dissection of their testes. The collected milt was then thoroughly mixed with the stripped eggs to facilitate fertilization. The newly fertilized eggs were then carefully spread into specialized hatching troughs, each measuring 70 cm x 40 cm x 30 cm, for the incubation phase.



Figure 1. Sequential steps for intramuscular administration of Ovaprim synthetic hormone to African catfish (*Clarias gariepinus*)

Incubation and Larval Rearing

The incubation of the fertilized eggs was meticulously carried out under controlled environmental conditions. The water temperature within the hatching troughs was rigorously maintained within a narrow range of 26°C to 27°C, which is recognized as optimal for *Clarias gariepinus* embryonic development and prevents thermal shock or developmental abnormalities. The hatching process was closely monitored, and the percentage of hatched eggs was accurately recorded for each treatment group. Following successful hatching, the larvae were transferred to rearing units, where their survival rate was continuously observed and documented. Initial feeding of larvae commenced after yolk-sac absorption, typically 2-3 days post-hatch, using appropriate larval feeds such as *Artemia nauplii* or

commercial larval diets, to ensure optimal growth and survival.

Data Collection and Statistical Analysis

Comprehensive data were collected on several key reproductive parameters to assess the effectiveness of each Ovaprim dosage. These included the total number of stripped eggs, the number of fertilized eggs, the number of hatched eggs, the percentage of larval production (calculated as the number of hatched larvae relative to the number of fertilized eggs), and the larval survival rate (calculated as the number of surviving larvae at a specific time point relative to the number of hatched larvae) for each experimental treatment. The collected quantitative data were subsequently analyzed using simple descriptive statistics, specifically focusing on determining the mean values and percentages for each parameter, to provide a clear overview of the experimental outcomes. These descriptive statistics provide a foundational understanding of the treatment effects and allow for direct comparison of the efficacy of different Ovaprim dosages. To evaluate differences among treatments, data were subjected to one-way analysis of variance (ANOVA) after confirming normality and homogeneity of variance assumption.

$$\% \text{ Fertility} = (\text{No. of fertilized eggs} / \text{No. of Extruded eggs}) \times 100\%$$

$$\% \text{ Hatchability} = (\text{Total No. of fertilized eggs} - \text{Total No. of unfertilized eggs}) / \text{Total No. of fertilized eggs} \times 100\%$$

Based on the statistical analysis, the effective dose threshold was defined as the lowest Ovaprim concentration that resulted in a significant increase in spawning response, fertilization rate, and hatchability compared with the control treatment ($p < 0.05$). This threshold provides a practical reference for hatchery management and optimization of hormone application.

Results and Discussion

According to our results, fish of T1 and T3 did not respond to the injection, and thus, spawning did not occur. There were differences in hatchability rate between the experimental groups (Table 1). The highest values of spawning rate were observed in T2, and the lowest spawning rate was in T1 (Table 2). The highest values of egg weight/g.bw obtained in fish administered with ovaprim (5 ml/kg.bw: T2).

Table 1. Latency period pseudo- gonad somatic index of *C. grapeinus* varying female: male brood stock ratios.

Parameters	T 1	T 2	T3
No. of Males	2	1	2
No. of Females	3	3	3
Total weight	3469	2540.9	2272.8
Mean weight	693.8	635.2	454.6
Total dosage	10	15	11
Ovaprim dosage	3.3	5	3.7
Hatchability	(-)	(+)	(-)

The experimental investigation into the induced spawning of *Clarias gariepinus* using varying Ovaprim dosages revealed distinct responses across the different treatment groups. The primary findings, illustrating the impact of each treatment on key reproductive parameters, are systematically presented in Table 2.

Table 2. Reproductive Performance of *Clarias gariepinus* Broodstock under Different Ovaprim Treatments

Parameters	T1	T2	T3
No. of stripped eggs	2690	2860	6360
No. of fertilized eggs	896	953	2120
No. of hatched eggs	0	500	0
Percentage larval production	0	50	0
Percentage larval survival	0	10	0
Ovaprim dosage (ml/kg BW)	1.58	2.63	2.71

As shown in Table 2, Treatment 2 (T2) consistently demonstrated the best outcomes among the three experimental groups. This treatment, which involved an approximate Ovaprim dosage of 2.63 ml/kg body weight, resulted in a 50% increase in larval production and a 10% increase in larval survival. These positive results underscore the effectiveness of this specific dosage in inducing successful spawning and subsequent larval development under the prevailing experimental conditions. The relatively high number of stripped and fertilized eggs in T2, coupled with successful hatching, indicates a favourable hormonal response at this concentration.

In stark contrast, both Treatment 1 (T1) and Treatment 3 (T3) showed no larval production or survival. This indicates that the Ovaprim dosages used in these two treatments were largely ineffective at stimulating the physiological processes required for successful spawning and hatching. While T1 had a lower dosage, T3, despite a calculated dosage per kg body weight comparable to or slightly higher than T2, also failed to induce spawning. This suggests that factors beyond dosage alone, such as individual broodstock variability or a narrow optimal range, may be at play. The results further noted that statistically significant differences were observed across the treatments concerning the number of fertilized eggs, the number of hatched eggs, the percentage of larval production, and the ultimate larval survival rates. These disparities highlight the critical importance of precise hormonal dosage in optimizing induced breeding protocols for *Clarias gariepinus* and the need for careful empirical validation.

The analysis revealed significant differences among the tested hormonal doses in several reproductive parameters. The dose threshold was defined as the lowest treatment level that produced a statistically significant improvement in reproductive performance relative to the control group ($p < 0.05$). Doses below this threshold did not produce consistent improvements, whereas doses at or above the threshold significantly increased spawning response, fertilization rate, and larval hatchability. From a practical perspective, the identified threshold represents the minimum effective hormonal dose capable of reliably inducing successful reproduction under the experimental conditions. Increasing the dose beyond this level produced only marginal improvements in some parameters and, therefore,

may not be economically justified for routine hatchery operations. Consequently, the threshold dose provides a scientifically supported reference for optimizing hormone administration in aquaculture breeding programs.

The present study's findings unequivocally underscore the pivotal role of an optimized Ovaprim dosage in achieving successful induced breeding in the African catfish (*Clarias gariepinus*). The marked differences observed in fertilization, hatching, larval production, and survival rates across the three treatment groups highlight the inherent sensitivity of *C. gariepinus* to variations in hormonal induction protocols. The pronounced success of Treatment 2 (T2), which yielded a substantial 50% larval production and a 10% larval survival rate, stands in sharp contrast to the complete absence of larval production in T1 and T3. This outcome strongly suggests that the Ovaprim dosage approximating 2.63 ml/kg body weight was the most effective under the specific experimental conditions of this investigation, representing a critical finding for hatchery operations. This observation aligns with a substantial body of prior research demonstrating the dose-dependent efficacy of Ovaprim across various aquaculture species [15], [17], [18]. For instance, recent studies by [8] have meticulously examined a range of Ovaprim dosages and their effects on reproductive parameters in *Clarias gariepinus*. These studies often identify specific optimal dosage ranges that can vary depending on the particular fish population, environmental conditions, and broodstock characteristics [8], [20], [21]. The discrepancy noted between the general statement of "5 ml Ovaprim per kg live weight" and the calculated dosage for T2 (2.63 ml/kg) based on the provided results table warrants further consideration.

From Figure 2, Dosage vs Egg Production: There is a positive correlation between Ovaprim dosage and the number of stripped eggs. As the dosage increases from 1.58 ml/kg (T1) to 2.71 ml/kg (T3), the egg count rises significantly from 2,690 to 6,360. Mean Weight: Interestingly, the treatment with the highest dosage (T3) used broodstock with the lowest mean weight (454.6 g), while the lowest dosage (T1) was used for the heaviest fish (693.8 g). The "Hatchability" Paradox: While T3 produced the most eggs, your second table reveals that it resulted in 0 hatched eggs. T2 (2.63 ml/kg) appears to be the most "successful" overall, as it was the only treatment to produce hatched larvae (500 hatched eggs).

Nevertheless, the results clearly demonstrate the effectiveness of the dosage applied in T2 in this study, providing a valuable data point for future research and practical application.

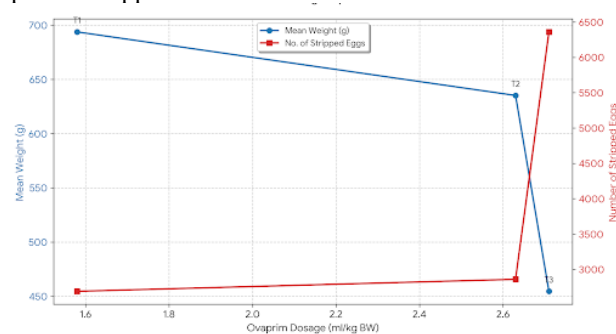


Figure 2. Relationship Ovaprim dosage, mean weight and egg production in *Clarias gariepinus*

The 12-hour latency period used in this study is well within the established and recommended protocols for *C. gariepinus*. Typical latency periods for this species generally range from 9 to 12 hours, with variations often influenced by ambient water temperature [21], [22]. This period is absolutely crucial for facilitating the complete process of final oocyte maturation, thereby maximizing the quality and viability of the stripped eggs. Furthermore, the meticulous maintenance of water temperature between 26°C and 27°C during the incubation phase is in perfect alignment with the optimal temperature range known to support robust *C. gariepinus* embryonic development and successful hatching, as extensively documented in aquaculture literature [23], [24]. These controlled conditions are vital for ensuring the reliability and reproducibility of experimental results, minimizing confounding factors related to environmental variability.

The achievement of a high larval production rate (50%) in T2, coupled with a 10% larval survival rate, indicates good egg quality and the overall effectiveness of Ovaprim at the identified optimal dosage. However, the relatively modest larval survival rate of 10% suggests that there remains considerable scope for optimizing post-hatching management strategies. Factors such as the precise timing of first feeding, the nutritional composition and type of feed provided, and the density at which larvae are reared are all known to exert significant influence on larval survival and subsequent growth [25], [26]. Further refinement in these areas could substantially improve overall production efficiency, thereby increasing yields and economic returns for catfish farmers. For instance, studies have shown that early introduction of appropriate live feeds or high-quality artificial diets can significantly boost larval survival and growth rates, reducing early-stage mortality, which is often a bottleneck in aquaculture production.

The observed failure of T1 and T3 to induce successful spawning can be attributed to suboptimal Ovaprim dosages. In the case of T1, with its comparatively lower dosage, it is highly probable that the administered hormone was insufficient to effectively counteract the inherent dopaminergic inhibition and trigger a complete and sustained gonadotropin surge necessary for ovulation [27], [28]. This highlights the existence of a minimum effective dose below which hormonal induction becomes ineffective, emphasizing the need for careful titration. Conversely, T3, despite having a slightly higher calculated dosage per kilogram of body weight than T2, may have been administered to broodstock exhibiting different physiological conditions or may have fallen outside the narrow optimal window for the specific broodstock utilized [6], [29]. This could have resulted in an ineffective response, emphasizing the critical importance of not only precise dosage calculation but also a thorough consideration of individual broodstock characteristics and their physiological readiness. This underscores that a 'one-size-fits-all' approach to hormonal induction is often inadequate and that fine-tuning is essential for optimal results, potentially requiring pre-screening of broodstock or adaptive dosing strategies based on individual fish parameters.

Future research endeavours should ideally focus on several key areas to further enhance the efficiency and sustainability of *C. gariepinus*-induced breeding. These include a more granular refinement of Ovaprim dosages,

potentially exploring a narrower range around the identified optimal dose, and investigating alternative or improved administration methods, such as multiple injections or different delivery systems to ensure sustained hormonal release. Furthermore, significant attention should be directed towards optimizing post-hatching larval rearing techniques, with a particular emphasis on improving overall survival rates through advanced nutritional strategies and environmental controls, including biofloc technology or recirculating aquaculture systems. An in-depth investigation into the genetic background and physiological status of broodstock could also provide invaluable insights into their differential responsiveness to hormonal induction, paving the way for selective breeding programs to enhance reproductive performance and reduce reliance on exogenous hormones. The findings of this study, therefore, contribute significantly to the expanding knowledge base on induced breeding in *Clarias gariepinus*, offering practical implications for enhancing aquaculture productivity across various regions. By addressing these areas, the aquaculture industry can move closer to achieving more consistent, efficient, and sustainable fingerling production, ultimately supporting food security and economic development in many parts of the world.

Conclusion

This study demonstrated that hormonal induction using 2.63 ml/kg body weight of Ovaprim significantly improved the reproductive performance of African catfish, *Clarias gariepinus*, resulting in higher egg production, fertilization, hatching, and larval survival rates. The findings highlight the importance of optimizing hormone dosage to enhance spawning efficiency and hatchery productivity. Adoption of this optimized protocol may support more reliable seed production for catfish aquaculture and improve hatchery management practices. Future studies should focus on refining larval rearing conditions, improving early survival rates, and evaluating broader Ovaprim dosage ranges to further strengthen sustainable aquaculture production.

Author's Contribution

F. A. Mohamed: plays a role in research planning, supervises the entire research process, and provides theoretical foundations and inputs. R. A. Yousif: preparation and writing of article manuscripts, as well as reviewing and approving the final manuscript for publication. B. I. Osman and T. M. Abdalmwla: played a role in the experiments, field and data collection, and data analysis.

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