# Study of steroid hormones as a factors to determine the appropriate age group for reproductive female grass carp (Ctenopharyngodon idella(

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**Abstract:**
Field part of the study was conducted at a private hatchery as named as Al- Hilla fish hatchery, location in Babylon Governorate. The laboratory part of the study carried out in the laboratory of reproductive physiology and artificial insemination, department of Animal Production Techniques, Al- Musaib Technical College, during April and May 2024. female's grass carp fish were selected , and divided into five groups , including the T1 , T2 , T3 , T4 and T5 groups , each group containing five females. The results showed a significant increase ($p<0.05$) in the concentration of estradiol hormone in the older age group (groups, T3, T4 and T5), they also recorded the highest concentration of estradiol hormone compared to the younger age groups (groups, T2 and T1). A non-significant decrease in estradiol concentration from April to May. A significant ($p<0.05$) increase in the concentration of testosterone for T3 group (2.64±0.25 ng/ml) compared to other groups, A non-significant decrease in testosterone concentration from April to May. The concentration of progesterone hormone differs between groups on age and weight. The T3 group recorded a significant increase ($p<0.05$) in progesterone hormone concentration (19.90±0.86ng/ml) compared to the other groups, a signification decrease ($p<0.05$) in progesterone concentration was observed form April to May. In conclusion, based on this data we can conclude that the T3 age group was appropriate age group for use in artificial reproduction programs of female grass carp because showed highest reproductive efficiency.

**Keywords: estradiol, grass carp, progesterone, testosterone.**

## Introduction

Steroid hormones play a crucial role in various physiological processes, particularly reproduction, in many teleosts, including carp. These hormones, categorized in to three types, estradiol, testosterone and progesterone are regulated by gonadotropin – releasing hormone (GnRH). They are vital for development of male and female gametes [1]. The relationship between gonadal development and serum steroid hormones levels serves as valuable tool for understanding the endocrine of reproduction in teleosts [2]. Regulation of vitellogenesis and the final maturation of the egg by GnRH via steroid hormones secreted by granulose and theca cells of the developing mature eggs in teleosts [24]. Estradiol hormone is produced in teleosts by the ovarian follicle and is essential for the growth and development of female reproductive function during vitellogenesis [6]. The high level of estradiol hormone in the reproductive cycle confirms the importance of this hormone in development of the gonads

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and the positive relationship with the weight and age of the fish [9]. Reproductive activity in female fish begins with a low level of estradiol during pre-vitollgenesis and then gradually increase until the time of vitellogenesis [21]. Testosterone serves as for estradiol production, contributes to yolk synthesis in liver, and positively influence gonadotropin secretion from pituitary gland [5]. Testosterone is playing a role during ripe maturation of the eggs, ovulation, and egg growth before vitellogenesis and maintaining secretion of GnRH [14]. Progesterone is identified as the maturation-inducing hormone (MIN) in most teleosts. It plays a pivotal role in stimulating final Oocyte maturation [25]. Progesterone is important for the growth of the egg before the vitellogenesis [13]. In fishes, estradiol and testosterone were identified during gonadal development, and progesterone during ovarian and testicular maturation [24,15]. The aim of this work was to study of steroid hormone as a factor to determine the appropriate age group for reproduction females grass carp (Ctenopharyngodon idlla.(

Material and Methods

Place of study:

Field part of the study was conducted at a private hatchery as named as Al- Hilla fish hatchery, location in Babylon Governorate. The laboratory part of the study carried out in the laboratory of reproductive physiology and artificial insemination, department of Animal Production Techniques, Al- Musaib Technical College, during April and May 2024.

Fish handling:

Fish were introduced into indoor tanks in the hatchary three day before the start of experiment as an adaptation period, the water temperature in the tanks was 23ºC, and the dissolved oxygen was 7-7.8 mg/L. The total weight of each fish was measured, a total length between the tips of the snout to the end of the tail was measured. The age of fish was estimated by fish scales (operculum) [20]. Based on these measurement, female's grass carp fish were selected, and divided into five groups, including the T1, T2, T3, T4 and T5 groups, each group containing five females (Table 1.(

**Table 1. Mean (±S.D.) total length, weight and age of fish selected for study**

| Groups | Total length (cm) | Total weight (gm) | Age (years) |
|---|---|---|---|
| $T_1$ | 36.13±4.38 | 1432±12.9 | 1+ |
| $T_2$ | 50.40±4.22 | 2008±70.8 | 2+ |
| $T_3$ | 53.17±3.18 | 3160±107.6 | 3+ |
| $T_4$ | 65.30±4.12 | 4010±212.8 | 4+ |
| $T_5$ | 67.00±5.21 | 5050±416.7 | 5+ |

Blood sampling:

To analyzed the 17 β – estradiol, testosterone and progesterone hormones concentration in female's grass carp. Every middle of the month a sample was taken from each fish. Blood samples were collected from the heart in the morning, nearly 9 – 10 Am, using heparinized needle, and sampling placed in tubes, those tubes were carried on icebox and transported to the laboratory. In the laboratory the blood samples were putted at 37 ºC for one hours in order to clot , then centrifuged at 4000 rpm for 10 minutes, and serum collected

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by pipette and putted in Eppendorf tubes and stored at – 20 C until analyzed.

Analysis of steroid hormones:Serum samples were analyzed to estimate of steroid hormones (17B estradiol, testosterone and progesterone) using appropriate commercial Kits The steroid hormones measured by microplate enzyme immune assay method using Enzyme linked Immune sorbent Assay (ELISA) reader (Awareness , ELISA – Japan) by commercial kits (Human kit, Germany) [13.[

Statistical analysis:

Statistical analysis system (SAS) [17] program was used in data analysis, to study the significant of the difference in the average values (Mean± S.E.). Significant differences average were compared with Duncan's multiple rang test [3] (DMPT) at 5% probability.

Results and Discussion

The results showed a significant increase ($p<0.05$) in the concentration of estradiol hormone in the older age group (groups, T3, T4 and T5), they also recorded the highest concentration of estradiol hormone compared to the younger age groups (groups, T2 and T1). A non- significant decrease in estradiol concentration from April to May (Table 2).These results confirm the increase in reproductive activity with age and Wight .The increase in the concentration is attributed to its important role in process as the precursor for egg yolk protein, which is synthesis in liver cells under influence of estradiol hormone [6]. [9] Noted that the concentration of estradiol hormone increase gradually with age and reaches its peak during stage of vitellogenesis. [15] Pointed out that the concentration of estradiol hormone recorded its highest concentration during development and growth of gonads in fish. [10] Explained that there was significant positive effect of estradiol hormone level on gonadosomatic index (GSI), which indicates the importance of this hormone in the maturation of the ovaries. The accumulation of yolk in the egg in response to the increase in the estradiol hormone is the reason for (GSI) increased. The results of our study are consistent with many previous studies that observed high estradiol concentration during the reproductive activities , as high concentration were recorded during the maturation and vitellogenesis stages , as indicated by [4] and [21]. These results contribute to understanding the close relations between estradiol concentration development in female's grass carp, as there is estradiol and advancement of the maturation stage. Studies that addressed the decline in estradiol concentration after the vitellogenesis stage , such as the study by [11] and [18], confirm that the hormone concentration decrease after the end of the vitellogenesis stage and the beginning of the atresia stage , indicating to supporting the vitellogenesis stage and initial maturation of the egg.

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**Table 2. Mean (±S.D.) serum estradiol concentration pg/ml in April and May in female grass carp**

| Groups | No. of fish | Serum estradiol concentration pg/ml | |
|---|---|---|---|
| | | April | May |
| $T_1$ | 5 | $_c$408.16±14.41 | $_b$379.00±12.73 |
| $T_2$ | 5 | $_b$505.12±23.77 | $_b$465.08±14.61 |
| $T_3$ | 5 | $_a$621.00±25.82 | $_a$585.34±17.02 |
| $T_4$ | 5 | $_a$612.09±19.54 | $_a$580.00±14.79 |
| $T_5$ | 5 | $_a$598.10±17.87 | $_a$571.13±14.94 |

Values with different letters differ significantly ($p<0.05$ (

The results showed a significant ($p<0.05$) increase in the concentration of testosterone for T3 group (2.64±0.25 ng/ml) compared to other groups, A non- significant decrease in testosterone concentration from April to May (Table 3.(

This results indicated that the T3 group was approaching full sexual maturity, which is consistent with the role of testosterone hormone in stimulating egg maturation and supporting of ovulation stages and early egg development in female's grass carp. These results support previous studies that have shown that the testosterone concentration gradually increase as fish progress through the stages of final maturation, (Ripe maturation) of the egg and vitellogenesis [22]. Some studies have indicated that increase in testosterone concentration during the month of April corresponds to the peak of gonadal activity, and that the highest concentration of testosterone was during the spring season, which corresponds to the active sexual reproductive phase [4,19].Some studies have shown different results, which recorded the highest concentration of testosterone in the summer and autumn seasons [8,23]. However, this variation can be explained by environmental and climatic factors and the techniques used in the studies, such as analysis techniques and sampling methods, in addition to the deference in fish species and breeding conditions [12.[

**Table 3. Mean (±S.D.) serum testosterone concentration ng/ml in April and May in female grass carp**

| Groups | No. of fish | Serum testosterone concentration ng/ml | |
|---|---|---|---|
| | | April | May |
| $T_1$ | 5 | $_d$1.10±0.14 | $_c$0.98±0.06 |
| $T_2$ | 5 | $_c$0.63±0.19 | $_b$1.52±0.17 |
| $T_3$ | 5 | $_a$2.64±0.25 | $_a$2.50±0.26 |
| $T_4$ | 5 | $_a$2.30±0.33 | $_a$2.17±0.22 |
| $T_5$ | 5 | $_b$2.18±0.21 | $_a$2.10±0.18 |

Values with different letters differ significantly ($p<0.05$ (

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The results showed that the concentration of progesterone hormone differs between groups on age and weight. The T3 group recorded a significant increase ($p<0.05$) in progesterone hormone concentration (19.90±0.86ng ml) compared to the other groups (Table 4). A signification decrease ($p<0.05$) in progesterone concentration was observed form April to May .These results indicate that group T3 clearly reached the final oocyte maturation stage compared to the older and younger groups supporting the importance of as a major stimulator of final oocyte maturation in grass carp. The results are consistent with the study of [4] who showed that the progesterone concentration increase to a peak during stage of ovarian maturation and reached 20.20 ng/ ml. A similar studies reported fluctuations in the concentration of steroid hormone during annual reproductive cycle. These hormones peak during ovulation season, the progesterone concentration begins to rise from month of March and reaches its maximum concentration in the month of April and reached 21.80±1.70ng/ml when female reach ovarian maturity [8, 19].Our results are consistent with those of [7].who reported that progesterone concentration increased during the final maturation of oocyte and ovulation phases in female fish.

**Table 4. Mean (±S.D.) serum progesterone concentration ng/ml in April and May in female grass carp**

| Groups | No. of fish | Serum progesterone concentration ng/ml | |
|---|---|---|---|
| | | April | May |
| $T_1$ | 5 | $_b$13.00±0.76 A | $_b$7.80±0.34 B |
| $T_2$ | 5 | $_{ab}$15.02±0.81 A | $_{ab}$8.90±0.41 B |
| $T_3$ | 5 | $_a$19.90±0.86 A | $_a$11.80±0.64 B |
| $T_4$ | 5 | $_{ab}$18.20±0.72 A | $_{ab}$10.60±0.58 B |
| $T_5$ | 5 | $_{ab}$17.80±0.67 A | $_{ab}$9.90±0.55 B |

a,b,c values with different letters in the same column differ significantly ($p<0.05$(
A, B values with different letters in the same row differ significantly ($p<0.05$(

## Conclusions

Based on the result, estradiol plays a major role in supporting reproductive activity in grass carp, especially older age groups, which showed increased estradiol concentration during vitellogenesis. The testosterone has an importance role in regulating reproductive development in crass group, especially in middle age group (3 years) which showed the highest reproductive efficiency. Progesterone concentration is a crucial factor in choosing the appropriate season and age groups for artificial reproduction. The T3 group in the most suitable for artificial reproduction due to the high concentration of progesterone at this age. Finally, the T3 age group was appropriate age group for use in artificial reproduction programs of female grass carp because showed highest reproductive efficiency

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