INDUCTION OF SPAWNING IN AFRICAN CATFISH (*Clarias gariepinus*) BY USING HUMAN CHORIONIC GONADOTROPIN

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ABSTRACT

This work was designed to study the effect of Human chorionic gonadotropin (HCG) on induction of spawning of African catfish (*Clarias gariepinus*). In this experiment a total number of 90 catfish (745.8±9.2g body weight) were divided into three experimental groups (30 each). The first group was considered as a control group and injected intramuscularly with carp pituitary extract (0.3ml/kg b.w.). The second group was injected with HCG (1200 IU / kg body weight ). The third group was injected with both carp pituitary extract and HCG (0.15 ml/kg and 600 IU/kg body weight, respectively.

The results of the present experiment showed significant differences in survival rate, fertilization rate (%), hatching rate (%), latency period, egg weight (g), egg diameter (mm) and total protein (g/dl). However, insignificant differences were detected in initial weight (g), condition factor and specific growth rate, body weight before spawning, body weight after spawning, egg number/g, gonadosomatic index, hepatosomatic index, cortisol (ug/dl) and estradiol17β (pg/m). Females treated with HCG had the highest values of fertilization rate, hatching rate, latency time and survival rate.

Conclusively, the results of the present study indicated that at a level of 1200 IU from HCG was the most effective agent in inducing spawning of African catfish (*Clarias gariepinus*) females.

Key words: Spawning, African catfish, *Clarias gariepinus*, HCG

INTRODUCTION

With increasing human populations all over the world and increasing food demands especially protein source, fish is considered to be an important and more valuable source of animal protein characterized by high nutritional value.
African catfish (*Clarias gariepinus*) is one of the most widely produced food fish in the world (Fasakin, *et al.*, 2003; Al Dohail, 2005 and Sutriana, 2007) and it considered a native fish in all freshwater bodies of Egypt (Saleh, 2007). The attributes that make this species a farmers choice include faster growth rate and its bigger maturity size, easy to reproduce, accepts artificial feeds, tolerates to high stocking densities, adapting with poor water quality, high resistance to disease, lucrative in local regional and international markets, and its economical feasibility in pond culture systems, the most common culture system in East African Community (Teugels, 1986). However, lack of constantly available seed, low egg fertilization rate and low hatching rate are considered as the main obstacles (El–Sayed, 1999) and are not yet be solved.

The present study was designed to investigate the effect of human chorionic gonadotropin (HCG) on induction of ovulation in female African catfish (*Clarias gariepinus*).

**MATERIALS AND METHODS**

The present study was carried out in the Fish Hatchery belonging to the General Authority for Fish Resources Development, Abbassa, Abou-Hammad, Sharkia Governorate, Egypt. During year 2016.

In this experiment, a total number of 90 females of African catfish (745.8±9.2 initial body weight) was divided into three experimental groups (30 each). All fish were distributed in nine hapa (10 fish/hapa) and 27 females were used at the spawning season. The first group was considered as a control group and injected with carp pituitary extract at a level of 0.3ml/kg b.w. The second group was injected with HCG at a level of 1200 IU / kg body weight. The third group was injected with both carp pituitary extract (CPE) and HCG (0.15ml / kg plus 600 IU/kg body weight, respectively). Growth performance (condition factor, specific growth rate, and hepatosomatic index) and reproductive performance (latency time, ovulation rat, gonadosomatic index, egg parameters such as egg weight, egg number and egg diameter, fertilization rate, hatchability and survival rate) were determined according to the methods of Szabo *et al.*(2002); Adebayo (2006); Phelps *et al.*, (2007) and El-Hawarry *et al.*(2016).

Blood samples (5ml) were collected from tail vein at spawning. Samples were centrifuged immediately at 3000 r.p.m. for 15 minutes. The serum was collected and stored at -20°C until analyzed for cortisol and stradio17β concentrations by using Radioimmuniasiassay (Diagnostic
products corporation kits) as described by Eckert et al., (2001) and Gore-langton and Armstrong (1988). Total protein was also determined as described by Henry, (1974), Histopathological examinations of both liver and kidney of the experimental animals were also studied. 

Data were subjected to analysis of variance according to Snedecor and Cochran (1982). General linear model procedure of the statistical analysis system (SPSS, 2004) was used. Reproductive traits were analysed by the log linear model for the analysis of contingency tables.

Duncans New Multiple Range Test was used for multiple comparisons (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance traits:

The Initial weight (g), condition factor (K\text{factor}), specific growth rate (SGR), gonadosomatic index and hepatosomatic index showed insignificant variation among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and their combination (Table 1). These results are in agreement with those reported by Eman (2008) who found no significant variation in K\text{factor} of common carp fish as a result of hormonal treatment, however, Jayaprakas and Sambthu (1995) reported that fish treated with gonadotropic hormones showed an increase in the growth parameters over the control. The same authors also found that human chorionic gonadotropin promoted better growth rate.

The results presented in Table (2) indicated that the differences in survival rate (SR%) among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant (P<0.05). Females treated with HCG gave higher survival rate than those treated with either pituitary gland of common carp (CPE) CPE plus HCG. However, the differences in body weight before and after spawning (g) were not significant. These results agreed with those reported by EL-Hawary et al., (2016) and Mylonas et al., (1992). They reported an increase in survival rate when HCG was used. However, Ndimele and Owodeinde (2012) found that less survival rate was obtained following using ovaprim as compared with pituitary extract.
Table (1): Means and standard error of initial weight (g), condition factor, specific growth rate, gonadosomatic index%, hepatosomatic index% of African catfish females injected with PG extract of common carp, HCG and their combination.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Initial weight (g)</th>
<th>Condition factor</th>
<th>Specific growth rate</th>
<th>Gonadosomatic Index, %</th>
<th>Hepatosomatic Index %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CPE)</td>
<td>759.11 ± 9.99</td>
<td>0.92 ± 0.03</td>
<td>4.81 ± 0.01</td>
<td>14.79 ± 0.01</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>HCG</td>
<td>741.11 ± 9.75</td>
<td>1.00 ± 0.05</td>
<td>4.79 ± 0.01</td>
<td>14.92 ± 0.57</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>HCG + CPE</td>
<td>737.22 ± 7.78</td>
<td>0.91 ± 0.03</td>
<td>4.79 ± 0.01</td>
<td>14.69 ± 0.56</td>
<td>0.72 ± 0.56</td>
</tr>
</tbody>
</table>

Significance NS NS NS NS NS
NS= Not significant.

Table (2): Means and standard error of survival rate (SR%), body weight before and after spawning (g) of African catfish females after injection with CPE extract of common carp, HCG and their combination.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Survival rate (%)</th>
<th>Body weight before spawning (g)</th>
<th>Body weight after spawning (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CPE)</td>
<td>74.00 ± 2.23</td>
<td>776.67 ± 9.89</td>
<td>706.22 ± 9.63</td>
</tr>
<tr>
<td>HCG</td>
<td>81.78 ± 1.38</td>
<td>762.33 ± 7.61</td>
<td>712.78 ± 7.80</td>
</tr>
<tr>
<td>HCG + CPE</td>
<td>77.11 ± 2.00</td>
<td>755.89 ± 7.08</td>
<td>689.67 ± 6.68</td>
</tr>
</tbody>
</table>

Significance NS NS
- Means in the same column within the same classification having different litter differ significantly (P < 0.05). *= P < 0.05, NS= Not significant

2. Reproductive traits:

The differences in fertilization rate, hatching rate and latency time (Table 3) among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant (P<0.05 or P<0.01). Females treated with HCG had the highest values of fertilization rate, hatching rate and latency period than those treated with PG of common carp (CPE) and their combination. These
Table (3). Means and standard error of fertilization rate, hatching rate and latency time of African catfish females injected with PG extract of common carp, HCG and their combination.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Latency time (hrs)</th>
<th>Fertilization rate (%)</th>
<th>Hatching rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CPE)</td>
<td>11.78 ±.43</td>
<td>84.56 ±1.34</td>
<td>74.78 ±1.99</td>
</tr>
<tr>
<td>HCG</td>
<td>9.22 ±.32</td>
<td>92.11 ±2.38</td>
<td>86.11 ±.69</td>
</tr>
<tr>
<td>HCG+CPE</td>
<td>11.11 ±.56</td>
<td>87.89 ±2.56</td>
<td>79.11 ±2.03</td>
</tr>
</tbody>
</table>

Significance: ** = P < 0.05, * = P < 0.01.

Means in the same column within the same classification having different litter differ significantly (P < 0.05).

Results are in agreement with those reported by Haniffa et al. (2000); Haniffa and Sridhar (2002) and Leonardo et al. (2004). The same authors showed that the highest fertilization rate was obtained when HCG was used. Salami et al. (2006); Haniffa et al. (2000) reported that hatching rate was increased after injection with HCG in spotted murrel (Channa punctatus) and catfish (Heteropneustes fossilis). EL-Hawary et al.(2016) stated that the use of HCG produced higher latency period and less hatching rate. Ndimele and Owodeinde (2012) reported that induction of spawning in catfish (Clarias gariepinus) with synthetic hormone (ovaprim) produced offspring with better qualities than those induced with pituitary extract. Oyeleye et al (2016) found that the application of ovaprim in inducing spawning produced higher fertilization rate and hatching rate as compared with pituitary gland extract injection. However Zairin et al., (1992); Brzuska, (2003) and (2004), Akar, (2006) and Akar and Ali, (2006) and Eman (2008) found that the mean fertilization and hatching percentage were considerably higher after injection with HCG combined with CPE of common carp. In contrary EL-Hawary et al.(2016) stated that the use of HCG produced less hatching rate.

The results of the present study (Table 4) showed that the difference in weight of eggs (g), and diameter of eggs (mm) among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic
Table (4): Means ± SE of egg weight (g), number of eggs /g and diameter of eggs (mm) of African catfish females injected with CPE extract of common carp, HCG and their combination.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Weight of egg (g)</th>
<th>Number of egg/ g</th>
<th>Diameter of egg (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (PG)</td>
<td>70.56±2.83</td>
<td>730.00±22.04</td>
<td>1.46±.11</td>
</tr>
<tr>
<td>HCG</td>
<td>58.33±.94</td>
<td>782.78±13.25</td>
<td>0.94±.04</td>
</tr>
<tr>
<td>HCG+CPE</td>
<td>64.56±1.41</td>
<td>770.00±13.94</td>
<td>1.07±.06</td>
</tr>
</tbody>
</table>

Means in the same column within the same classification having different litter differ significantly (P < 0.05).
- ** =P < 0.01, NS = Not significant.

gonadotropin (HCG) and CPE plus HCG were significant (P<0.01). However, the differences in the number of eggs/g were not significant. Females treated with CPE only produced better weight of egg (g), and diameter of egg (mm) than CPE plus HCG and HCG. These results agreed with those reported by Brzuska,( 2002& 2003); Ndimele and Owodeinnde, (2012); Onoliyi and Akinbola (2013); Oyeleye et al., (2016) and El-Hawary et al , (2016). They also found higher quantity of egg were obtained after the application of synthetic ovulation stimulator than after the treatment with stimulator of natural origin such as carp pituitary or human chorionic gonadotropin. However, Zairin et al (1992) found that the injection of human chorionic gonadotropin in Clarias lazera stimulates ovarian development. It induces increases of weight and size of eggs (Haniffa and Sridhar, 2002). Salami et al. (1994) reported similar increase in egg weight and big sized eggs in Clarias gariepinus. Eman (2008) found that the mean total weight of eggs were considerably higher after injection with HCG combined with CPE of common carp. Oyeleye et al. (2016) and Bruzuska (2004) found that the application of ovaprim resulted in a higher weight of egg / kg body weight and higher quality eggs in comparison with pituitary extract. The same author stated that treatment of female's African catfish (Clarias gariepinus) with two doses or one dose of ovopel as ovulation stimulator had insignificant effects in either weight of eggs or eggs quality.

The results presented in Table 6 showed that the difference in total protein, among the experimental groups injected with pituitary gland of
Table (5). Means and standard errors of total protein (g/dl), cortisol (ug/dl) and estradiol (pg/ml) concentration of African catfish females after injection with PG extract of common carp, HCG and their combination.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total protein (g/dl)</th>
<th>Cortisol (ug/dl)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CPE)</td>
<td>6.29±.11</td>
<td>23.86±2.46</td>
<td>229.00±44.80</td>
</tr>
<tr>
<td>HCG</td>
<td>5.84±.07</td>
<td>19.69±2.95</td>
<td>220.67±39.02</td>
</tr>
<tr>
<td>HCG+ CPE</td>
<td>5.86±.13</td>
<td>23.74±1.68</td>
<td>254.75±44.29</td>
</tr>
</tbody>
</table>

Significance ** NS NS

Means in the same column within the same classification having different litter differ significantly (P < 0.05). ** =P < 0.01, NS= Not significant-

common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant (P<0.01). However, the differences in cortisol and estradiol concentration were not significant in all hormonal treated groups. Females in the control group which was injected with CPE gave higher total protein concentration than those treated with either HCG or CPE plus HCG respectively. However, Eman (2008) found that a very highly significant decrease (P<0.001) in total protein was recorded in fish injected with combination of CPE of carp and HCG.

Histopathological results:

Histological examination indicated that both liver and ovary of catfish treated with human chorionic gonadotropin, common carp pituitary gland extract and their combinations showed normal histo-morphological structures (Figures 1 and 2). No differences were noticed between the treated groups and the control.
**Figure 1:** Fish liver (car pituitary extract, HCG and their combination treated groups) showed normal hepatic vein (arrow), cords, sinusoids and hepatocytes (star) H&E X400.

**Figure 2:** Fish Ovary (car pituitary extract, HCG and their combination treated groups) showed normal mature follicles (thick arrows) which contains yolk globules (star) and oocytes (thin arrow) H&E X400.
Conclusively, the results of the present study indicated that at a level of 1200 IU from HCG was the most effective agent in inducing spawning of African catfish (Clarias gariepinus) females.

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أحداث التفريخ في أسماك القرموم الأفريقي باستخدام الهرمون الكرسي البشري

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أجريت هذه الدراسة في مفرخ الأسماك التابع للهيئة العامة لتنمية النزهة السمالية، عباس، أبو حامد، محافظة الشرقية، مصر.

صممت هذه التجربة لدراسة تأثير الهرمون الكرسي البشري على تفريخ أسماك القرموم الأفريقي. تم استخدام عدد 90 من آتاق القرموم الأفريقي (وزن 745.8 ± 9.2) تم تقسيمهم إلى ثلاث مجموعات تجريبية (30 سماك/ مجموعة).

وتم اعتبار المجموعة الأولى مجموعًا للمقارنة وحققت مستخلصات الغدة النخامية للبروك (3 مل/ كجم)، بينما تم حقن المجموعة الثانية باستخدام الهرمون الكرسي البشري بـ 1200 وحدة دوائية/ كجم من وزن الجسم. تم حقن المجموعة الثالثة وحققت مستخلصات الغدة النخامية للبروك والهرمون الكرسي البشري 15/ كجم و 600 وحدة دوائية/ كجم من وزن الجسم، على التوالي. وتم دراسة معدل اداء النمو والانداة التناسلي.

وقد أظهرت نتائج التجربة الحالية وجود فوائد مفعولية في معدل البقاء على قيد الحياة و معدل الإخصاب (٪) و معدل الفقس (٪) وزن الجسم قبل التفريخ، وزن الجسم بعد التفريخ، عدد الأنثى في الأول، معدل الاحالة، وزن الفقس وزن الكبد وزن الكبد والكروتيزور والاستراداين 17 غرامي في جميع أنواع الهرمون البشري الأول. 

أظهرت نتيجة الدراسة أيضا أن استخدام 1200 وحدة دوائية من الهرمون البشري كان فعالا في تفريخ أسماك القرموم الأفريقي.

النتيجة: قد خلقت نتائج التجربة إلى معاملة الهرمون الكرسي البشري بـ 1200 وحدة دوائية كان أكثر فاعلية في احداث التفريخ في أسماك القرموم الأفريقي.