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EFFECT OF THE DOPAMINE ANTAGONIST, DOMPERIDONE ALONE AND IN COMBINATION WITH THYROXINE, CLOMIPHENE, hCG OR GnRH ON SERUM SEX STEROIDS PROFILE, GONADAL MATURATION AND SPAWNING OF NILE CATFISH, *CLARIAS LAZERA*.

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SUMMARY

The present investigation was carried out on one hundred and twenty mature Clarias lazera fish during the prespawning season (March - April). Fish were allocated into two main groups (sixty fish of each sex). Fish weight ranged between 200-220 g. b. wt. Each main group was subdivided into six equal groups (Ten fish each). Fish of each group; both males and females were subjected to the same regimen of intraperitoneal exogenous treatment, as follows:

1st Group: Saline"0.65% NaCl solution."(Control).

2nd Group: Domperidone 5µg/g. b. wt. in saline.

3rd Group: Domperidone 5µg/g. b. wt. plus thyroxine 1ng/g. b. wt. in saline.

4th Group: Domperidone 5µg/g. b. wt. plus clomiphene citrate 1µg/g. b. wt. in saline.

5th Group: Domperidone 5µg/g. b. wt. plus hCG 2 IU/g. b. wt. in saline.

6th Group: Domperidone 5µg/g. b. wt. plus GnRH 10µg/kg. b. wt. in saline.

The present study revealed that " Domperidone" administration alone, in each sex of Clarias fish; increased serum sex steroid hormones (testosterone and 17-β estradiol), gonadal weights (testes and ovaries), final gonadal maturation and gonadosomatic indices of both sexes as compared to respective controls.

Exogenous administration of domperidone plus gonadotropin releasing hormone (GnRH) was the most effective treatment for production of sex hormones, final gonadal maturation and spawning in both sexes of Clarias lazera fish, followed by domperidone plus thyroxine; domperidone plus clomiphene citrate and domperidone plus hCG., respectively.

In conclusion, the exogenous administration of dopamine antagonist plus GnRH is the more efficient regimen for enhancement of gonads maturation (gonadal recrudescence), spawning and spermiation of Clarias lazera fish throughout the prespawning season.

INTRODUCTION

Reproduction in teleosts is controlled by the hypothalamic - pituitary - gonadal axis (Donaldson, 1973). This sequential mechanism allows for intervention at several levels to promote or interfere with the gonads maturation process. The first generation technique was applied by Donaldson et. al. (1972) via the use of partially purified piscine gonadotropin (homologous or heterologous pituitary extract) for induction of final gonadal maturation and spawning in teleosts. The use of fish or mammalian pituitary preparations involving the collection and processing of materials from biological sources. The size and complexity of the typical gonadotropin molecule makes it unlikely that synthetic piscine gonadotropin will be available in the foreseeable future, unless it can be produced in quantity by genetic engineering in microorganisms as has recently been achieved for human growth hormone and insulin.

Search for synthetic alternatives to gonadotropin had led to the development of second generation techniques for the induction of final gonadal maturation, ovulation and spermiation in fish (Kumar, 1985; Chang et. al. 1992 and Patino, 1997). These techniques either operated at a higher level in the axis by stimulating the production and/or release of gonadotropins in the pituitary gland using anti-estrogens and gonadotropin releasing hormones (Kumar, 1985 and Chang et. al., 1992); or they operated at a lower level of the axis by suppling ovarian hormones (17α–OH, 20 β dihydroprogesterone and prostaglandins) which would normally be stimulated by endogenous or exogenous gonadotropin (Epler et. al., 1985). These second generation spawning inducers are all small molecules relative to gonadotropins (Goetz, 1997), that can be produced synthetically and are relatively free of species specificity.

Thyroid hormones are playing a role in fish reproduction. Thyroxine clearly stimulates ovarian development in the intact goldfish but has no influence on hypophysectomized regressed adult fish. (Fontaine, 1976).

The ovarian response of goldfish, carassius auratus and brown trout, to salmon gonadotropins and GnRH analogue was enhanced by thyroid hormones (Hurlburt, 1977, Higgs et. al., 1982 and Mylonas et. al., 1994). Moreover, thyroid hormones has been a stimulatory effect on testicular activity, spermatogenesis and androngens production in male teleosts (Cyr and Eales, 1996).

Advanced manipulation technique that applied on fish aquaculture is based on usage of exogenous

chemical agents particularly "Dopamine antagonists". Catecolamines, dopamine and its agonist apomorphine (Chang and Peter 1982; Chang, et. al. 1983 & 1984a,b) reduce the increase in plasma gonadotropin concentration associated with preoptic lesions, suggested that a gonadotropin release - inhibitory factor (GRIF) in goldfish may be dopamine (Chang and Peter, 1982, 1983a &; Chang et. al., 1984a,b). Injection of the dopamine into gonadotropin - releasing hormone analogue (LH-$RH_{a\ D}$ - Ala^6) - treated goldfish blocked the normal increase in plasma gonadotropin associated with ($LHRH_{a\ D}$-Ala^6 injection) (Chang and Peter, 1983a). Furthermore, injection of the dopamine antagonist, (pimozide) into intact goldfish; caused an increase in plasma gonadotropins concentration (Chang & Peter, 1983b and Peter, et. al. 1993). Therefore, in teleosts, where GRIF plays a significant role in the regulation of gonadotropin release from the hypophysis (Peter, 1982), injection of dopamine antagonists as pimozide; metoclopramide or domperidone; either alone or in combination with a suitable potent gonadotropin, gonadotropin - releasing hormone (GnRH) or GnRH analogue (Peter et. al., 1993; Patino, 1997 and Kucharczyk, et. al 1998) was proved to be an effective means for gonadal maturation, ovulation and spermiation of teleosts.

The present investigation aimed to clarify the effect of dopamine antagonist "domperiodone" administration alone or in combination with exogenous chemical and hormonal agents on: a) serum levels of sex steroid hormones (testosterone and estradiol), b) final maturation of gonads (testis and ovary). Another goal was to study the histological structure of gonads in Nile Catfish, (Clarias lazera).

MATERIAL AND METHODS

The present investigation was carried out on one hundred and twenty mature Clarias lazera fish during the period extended from 27th March to 10th April, 1998 "Prespawning period of Clarias Lazera" (El-Bolock, 1973 and Dowidar et. al., 1985). Clarias fish were purchased alive from Giza - Fish Market. Fish weight were ranged between 200-220 g. and from 312 - 330mm total length as a maturity index (Hogendoorn & Vismans, 1980). Fish were transferred with a minimal delay into tanks containing dechlorinated water to the laboratory.

Fishes were allocated into two equal main groups. First main group including sixty mature male Clarias fish and the second main group included sixty mature female fish. Each main group of both sexes was subdivided into six (6) equal groups, of ten fish each. Fish of each group of both sexes were subjected to the same regimen of treatment. Each group of fish was kept in a separate glass aquarium (100 X 40 X 40cm). The dechlorinated aquarium water was oxygenated using pumping aerator. The aquarium water temperature was subjected to atmospheric environmental alterations

(Aquarium temperature ranged from 24-26°C and day light: dark hours were 12:12 hours). Fish were left in the aquarium water for 7 days for acclimatization and fed a commercial pelleted diet.

Each group of both sexes was exposed to the following regimen of treatment:

* **The first group**: was injected intraperitoneally (i. p.) with 0.2ml of 0.65% NaCL solution and served as a control group.
* **The second group**: was injected i. p. with 0.2ml saline containing dopamine antagonist (Domperidone)* at a dose of 5µg/g.b.wt. (Redondo et. al., 1989 and Fermin 1991).
* **The third group:** was injected i. p. with 0.1ml saline containing domperidone at a dose of 5µg/g. b. wt. plus 0.1ml saline containing thyroxine (Eltroxin)** at a dose of one ng/g. b. wt. (Higgs et. al., 1982 and Hurlburt, 1977).
* **The fourth group**: was injected i. p with 0.1ml saline containing domperidone (5µg/g. b. wt.) plus 0.1ml saline containing antiestrogen as (clomiphene citrate)*** at a dose of oneµg/g. b. wt. (Singh &Singh, 1976 and Ueda & Takahashi, 1977a, b).
* **The fifth group:** was injected i. p. with 0.1ml saline containing domperidone (5µg/g. b. wt.) plus 0.1ml saline containing Human Chorionic Gonadotropin "hCG" as (Pregnyl)****as a dose of 2 IU/g. b. wt. (Miura et. al., 1991 and Eding et. al., 1982).
* **The sixth group:** was injected i. p. with 0.1ml saline containing domperidone (5µg/g. b. wt.) for two weeks (day after day) and at the last three days of treatment, each fish was injected daily i. p. with Gonadotropin Releasing Hormone "GnRH" as (Fertagyl)***** at a dose of 10µg/g. b. wt. (Peter et. al., 1988).

All injections were done at alternative days for two successive weeks.

Blood Sampling:

At the end of the experimental period (2 weeks), all fishes were taken separately, dried externally by filter paper to avoid hemolysis and then weighed. Blood samples were taken individually by caudal puncture technique into a polyethylene tube for clotting. Serum was separated and stored at -20°C till hormonal assay was carried out.

Gonadosomatic Index (GSI):

Each fish was sacrified, gonads (testes or ovaries) were taken, weighed and thoroughly examined for gonadal maturation and spawrning. The gonadosomatic index was computed as a percentage of weight of gonads to body weight of fish, according to Tan - Fermin (1985).

$$GSI = \frac{\text{Gonads weight}}{\text{Fish body weight}} \times 100$$

* Domperidone : Janseen Pharmaceutica / Beerse / Belgica.
** Extroxin: Glaxo Wellcome, Cairo-A.R.E.
*** Clomiphene: Arab. Drug Company, Cairo-A.R.E.
**** Pregnyl: Nile Pharmaceutical Co.,- A.~R.E.
***** Fertagyl: Intervet International B.V., Boxmeer, Holland.

Checking of gonadal maturation, ovulation and spermiation in Clarias, Catfish:

Fish were checked at the end of the present investigation for maturation of gonads (ovaries and testes) & ovulation by gently pressing the abdomen (Stripping). Fish that yielded a copious stream of mature green brownish eggs "act of ovulation "(Eyo, 1997) and fish that produce a large quantity of mature spermatozoa in seminiferous tubules and releasing of milt by GnRH administration "act of spermiation" (Kelly and Kohler, 1996).

Histological technique:

Ovaries and testes of both control and treated groups were dissected, isolated and preserved in 10% neutral buffered formaline. The fixed specimens were then subjected to routine histological technique. Paraffin sections of 5 microns thickness were stained with Harris hematoxylin and eosin for histological study according to George (1981).

Hormonal assay:

Radioimmunoassay technique using (RIA Kits) was used for determination of sexual steroid hormone levels:

1- Serum testosterone level was determined according to the method of Jaffe and Behrman (1974).

2- Serum 17-β estradiol level was determined according to the method adopted by Xing et. al. (1983).

Data Analysis:

Data was subjected to statistical analysis according to "t" test procedure reported by Snedecor and Cochran (1980) to evaluate the difference between mean values of various groups. Values are expressed as mean ± S. E.

RESULTS

A- Effects of exogenous administration of domperidone alone and in combination with thyroxine, clomiphene citrate, hCG or GnRH on:

1-Serum testosterone:

Data presented in Table (1) and Fig. (1) revealed that there is a significant increase in sera testosterone levels of all treated groups as compared to control groups ($P<0.01$).

2-Testes weights and gonadosomatic index:

Data presented in Table (1) and Figs. (2&3) revealed that there is a significant increase in testes weights and gonadosomatic indices in all treated groups as compared to their respective control values ($P<0.0.01$ or $P<0.05$).

3- Data presented in Table (1) and Figs. (1, 2 &3) revealed that there are statistical significant alterations among sera testosterone levels; testes weights and gonadosomatic indices in all respective - treated groups ($P<0.01$ or $P<0.05$).

B- Effects of exogenous administration of domperidone alone and in combination with thyroxine, clomiphene citrate, hCG or GnRH on:

Serum 17-β estradiol:

Data presented in Table (1) and Fig. (4) showed that there is a significant increase in sera 17-β estradiol levels of all treated groups as compared to control group (P<0.01).

2- Ovarian weights and gonadosomatic index:

Data presented in Table (2) and Figs. (5&6) showed that there is a significant increase in ovarian weights and gonadosomatic indices in all treated groups when compared to the respective control values (P<0.01).

3- Data presented in Table (2) and Figs. (4, 5, & 6) showed that there are statistical significant alterations among sera 17-β estradiol levels, ovarian weights and gonadosomatic indices of all respective - treated groups (P<0.01 or P<0.05).

I. Histological changes of Clarias testes associated with exogenous administration of domperidone alone and together with thyroxine, clomiphene citrate, hCG or Gn-RH are recorded as follow (PLATE I):

1- Control "saline" Group: (Fig. 7a)

The testes at this period (prespawning season) showed numerous interstitium connective tissue (C. T.), small seminiferous tubules "convoluted tubules" with narrow lumen (LST) and lined by less mitotically active spermatogonia (SPG).

2- Domperidone - injected Group: (Fig. 7b)

The testes showed more wider seminiferous tubules; signs of spermatogenesis (SPG) with formation of spermatids and spermatazoa.

3- Domperidone plus thyroxine - injected Group: (Fig. 7c)

The testes showed numerous mature spermatozoa (SPZ) filling the lumen of the seminiferous tubules (ST).

4- Domperidone plus clomiphene - injected Group: (Fig. 7d)

The testes showed signs of spermatogenic activity (mitotic multiplication of spermatogonia SPG, fewer number of spermatids; SPD, without formation of mature spermatozoa.

5- Domperidone plus hCG - injected Group: (Fig. 7e)

The testes showed active process of spermatogenesis and less distinct signs of spermiogenesis.

6- Domperidone plus GnRH - injected Group: (Fig. 7f).

The testes showed completed spermiogenesis with the formation of fully mature spermatozoa (SPZ) with signs of highly active"ICS" or "Leydig Cells" embeded in "C. T" of the interstitium.

II. Histological changes of Clarias ovary associated with exogenous administration of domperidone alone and together with thyroxine, clomiphene citrate, hCG or GnRH are recorded as follow (PLATE II):

1- Control "Saline" Group: (Fig. 8a)

The ovary at this period (Prespawning season) showed numerous perinuclear oogonia (PN) and growing ova or oocytes (G. OV.); representing the previtellogenic stage (Stages 1 and 2).

2- Domperidone - injected Group: (Fig. 8b)

The ovary showed many growing oocytes (G. OV.), immature ova (IM. OV.); and fewer number of mature ova (M. OV.); representing, previtellogenic , vitellogenic stages. (Stages 2 and 3) and early posvitellogenic stage (early stage 4) respectively..

3- Domperidone plus thyroxine - injected Group (Fig. 8c)

The ovary showed many mature ova (M. OV.) representing the postvitellogenic stage (Stage 4).

4- Domperidone plus clomiphene - injected Group: (Fig. 8d)

The ovary showed numerous maturing ova (MG. OV.) and fully mature ova (M.OV.) representing the vitellogenic and late postvitellogenic stages. (Stage 3 and late Stage 4).

5- Domperidone plus hCG - injected Group: (Fig. 8e)

The ovary showing many mature ova (M. OV.), centrally located in the ovary representing the late postvitellogenic stage (late Stage 4) with yolk globules (YO. GLO) deposition in full mature egg.

6- Domperidone plus GnRH - injected Group: (Fig. 8f)

The ovary showed many ripe mature ova (M.OV.), that are larger in size with centrally located nucleus. The mature egg has a definitive egg membrane with external C. T. sheath. The C.T. stroma (interstitum) becomes more loosely preparing the ovary to spawn " ovulation Stage" (Stage 5).

The act of spawning and spermiation of Clarias fish:

After domperidone - GnRH injection into the female Clarias lazera fish; gentle squeezing of the female abdomen led to stripping of mature ova (greenish eggs), but in male Clarias fish; it is suggested that the sexual interaction (enfolding) of the male to female is required for spawning and to extrude mature spermatozoa via the milt (spermiation) in Clarias lazera fish.

Table (1): Effect of domperidone with thyroxine; clomiphene; human chorionic gonadotropin (hCG); and gonadoropin releasing hormone (GnRH) on serum testosterone level, testicular weight and gonadosomatic index of mature male Nile Catfish, Clarias lazera.

Parameter / Group	Serum testosterone ng ml^{-1}	Testes weight (gram / fish)	Gonadosomatic Index (G.S.I.)
Control (March - April)* Saline (0.65% NaCl) (n) = 10	A,B,C,D,E 0.69±0.08	A,B,C,D,E 0.67±0.07	A,B,C,D,E 0.29±0.05
Domperidone (5µg/g.b.wt.) (n)=10	A,F,G,H,I 1.25±0.05	A,F,G,H 1.14±0.06	A,F,G,H 0.55±0.02
Domperidone (5µg/g.b.wt.) + plus Eltroxin (1n ng/g.b.wt.) (n)=10	B,F,j,K,L 2.69±0.09	B,F,i,J,K 1.71±0.04	B,F,I,J, 0.81±0.02
Domperidone (5µg/g.b.wt/) + plus Clomiphene Citrate (1µg/g.b.wt.) (n)=10	C,G,j,M 2.33±0.11	C,G,,i,l,M 1.54±0.06	C,G,k,L 0.73±0.04
Domperidone (5µg/g.b.wt.) + plus hCG (2 IU/g.b.wt) (n)=10	D,H,K,N 2.02±0.16	D,J,l,N 1.26±0.11	D,i,k,M 0.56±0.05
Domperidone (5µg/g.b/wt.) + plus GnRH (10µg/kg.b.wt) (n)=10	E,I,L,M,N 3.74±0.19	E,H,K,M,N 1.99±0.08	E,H,J,L,M 0.97±0.02

- Mean ± S.E.
- (n) = Number of fishes
- Mean values having the same capital latter (s) of the same column are significantly different from each others at P<0.01.
- Mean values having the same small letter (s) of the same column are significantly different from each others at P<0.05.

* (March-April) = Prespawning season of Claries lazera Fish.

Table (2): Effect of domperidone with thyroxine; clomiphene; human chorionic gonadotropin (hCG); and gonadoropin releasing hormone (GnRH) on serum 17-β estradiol level, ovarian weight and gonadosomatic index of mature female Nile Catfish, Clarias lazera.

Parameter / Group	Serum 17-β estradiol pg ml^{-1}	Ovarian weight (gram / fish)	Gonadosomatic Index (G.S.I.)
Control (March - April)* Saline (0.65% NaCl) (n) = 10	A,B,C,D,E 864±16.2	A,B,C,D,E 3.69±0.13	A,B,C,D,E 1.76±0.06
Domperidone (5µg/g.b.wt.) (n)=10	A,F,G,H 1164±20.6	A,F,G,H 4.50±0.22	A,F,G,H 2.15±0.10
Domperidone (5µg/g.b.wt.) + plus Eltroxin (1 ng/g.b.wt.) (n)=10	B,F,I,J,K 1482±23.3	B,F,I,j 5.95±0.31	B,F,I,J, 2.79±0.13
Domperidone (5µg/g.b.wt/) + plus Clomiphene Citrate (1µg/g.b.wt.) (n)=10	C,G,I,L,M 1378±17.8	C,G,k,L 5.54±0.20	C,G,k,L 2.64±0.11
Domperidone (5µg/g.b.wt.) + plus hCG (2 IU/g.b.wt) (n)=10	D,J,L,N 1200±15.5	D,I,k,M 4.87±0.16	D,I,k,M 2.34±0.07
Domperidone (5µg/g.b/wt.) + plus GnRH (10µg/kg.b.wt) (n)=10	E,H,K,M,N 1698±30.6	E,H,j,L,M 6.89±0.14	E,H,J,L,M 3.27±0.06

- Mean ± S.E.
- (n) = Number of fishes
- Mean values having the same capital latter (s) of the same column are significantly different from each others at P<0.01.
- Mean values having the same small letter (s) of the same column are significantly different from each others at P<0.05.

* (March-April) = Prespawning season of Claries lazera Fish.

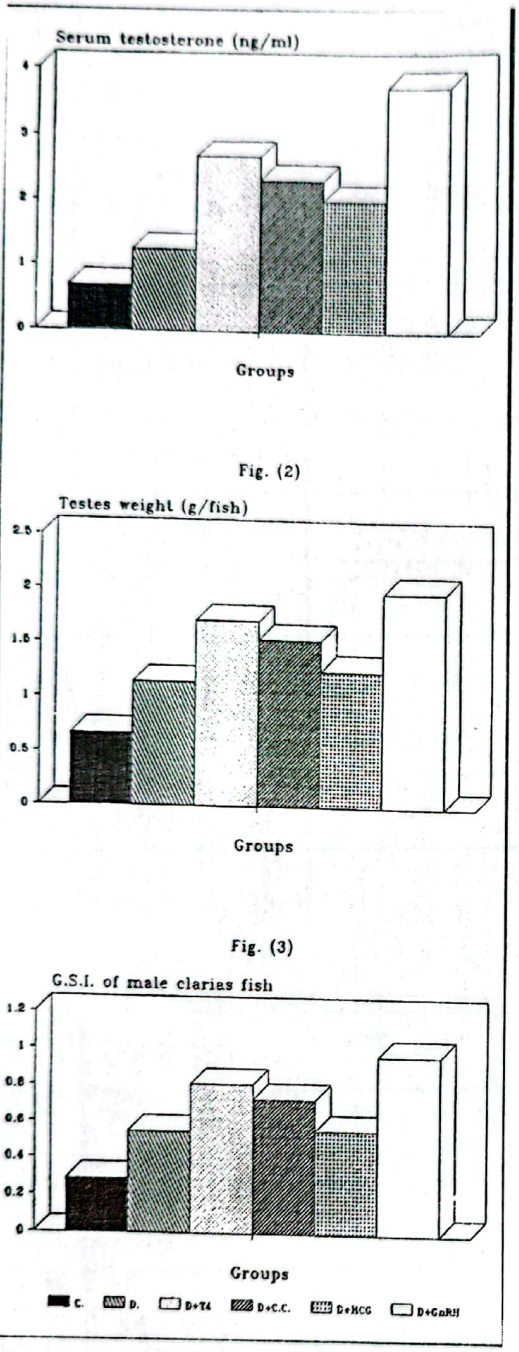


Fig. (2)

Fig. (3)

Figs.(1,2&3): Effect of domperidone (D) alone and in combination with thyroxine (T4), clomiphene citrate (C.C), hCG or GnRH on serum testosterone levels, testes weight and gonadosomatic index (G.S.I.).

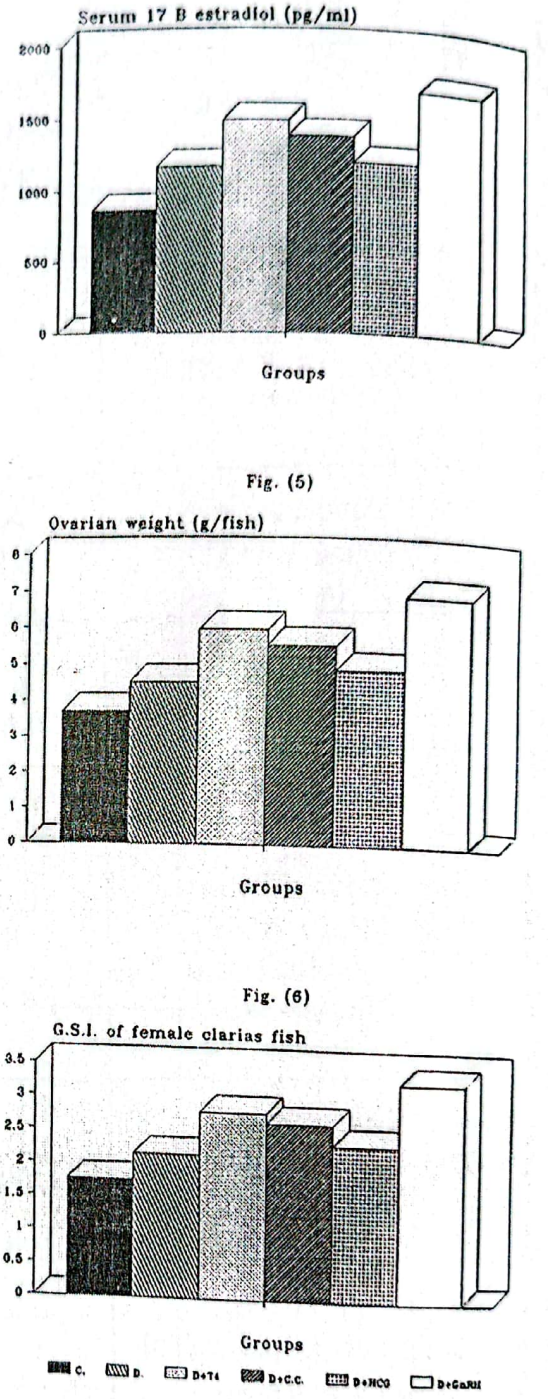
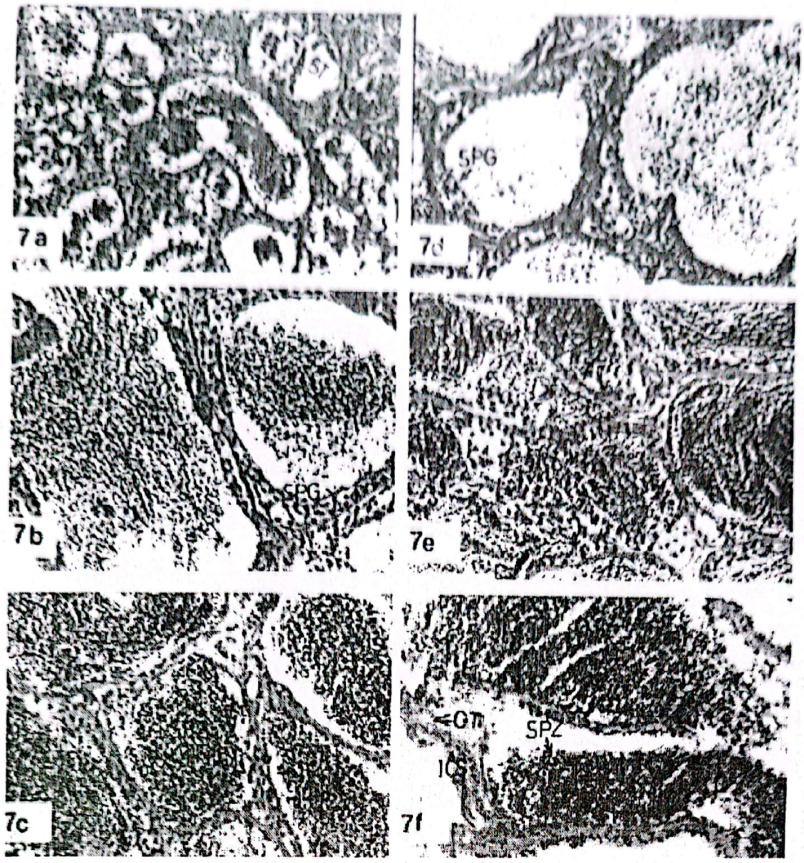


Fig. (5)

Fig. (6)

Figs.(4,5&6): Effect of domperidone (D) alone and in combination with thyroxine (T4), clomiphene citrate (C.C), hCG or GnRH on serum17β-estradiol levels, ovarian weight and gonadosomatic index (G.S.I.).



Figs.(7) (a-f): Photomicrographs of histological sections of Clarias lazera testes . H & E X 100.

-7a): Control " Saline" Group: Numerous connective tissue (C.T.), narrow lumen seminiferous tubules (LST) and active spermatogonia (SPG).

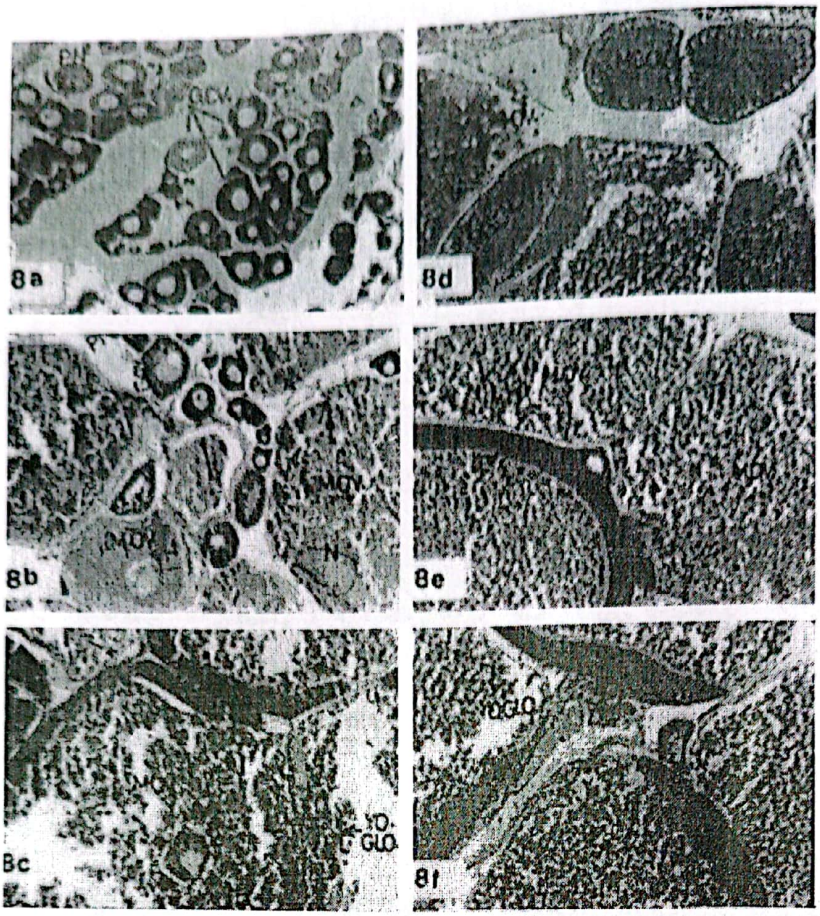
-7b): Domperidone-Group: Wider seminiferous tubules, active spermatogonia (SPG)& fewer mature spermatozoa (SPZ).

-7c): Domperidone + T4 Group: Mature spermatozoa (SPZ) filling the lumen of the seminiferous tubules.

-7d): Domperidone + clomiphene Group: Less active spermetogonia (SPG) and fewer No of spermatids (SPD).

-7e): Domperidone + hCG Group: Active spermatogonia (SPG) and less spermiogenesis.

-7f): Domperidone + GnRH Group Mature spermatozoa (SPZ) & active Leydig cells (ICS) in C.T. interstitium.



Figs.(8) (a-f): Photomicrographs of histological sections of Clarias lazera ovaries. H & E X 25.

-8a): Control " Saline" Group: The ovary showing numerous perinuclear oogonia (PN) and growing ova (G.OV.).

-8b): Domperidone-Group: The ovary showing many growing ova (G.OV.) and fewer mature ova (M.OV.).

-8c): Domperidone + T4 Group: The ovary showing many fully mature ova (M.OV.).

-8d): Domperidone + clomiphene Group: The ovary showing numerous maturing ova (MG.OV.) and fully mature ova (M.OV.).

-8e): Domperidone + hCG Group: The ovary showing many mature ova (M.OV.) with yolk globules (YO.GLO.) deposition.

-8f): Domperidone + GnRH Group: The ovary showing many ripe ova (M.OV.) with loose C.T. interstitum.

DISCUSSION

Concerning the administration of dopamine antagonist, "domperidone", the results of the present study (Tables 1 & 2 and Figs. 7b & 8b) revealed significant increase in sera testosterone and 17-β estradiol levels with enhancement of both testicular and ovarian maturation as compared to the control group (Tables 1 & 2 .and Figs 7a & 8a) in Clarias lazera fish.

Present results are in agree with Patino (1997) and Kucharczyk et. al., (1998); who proved that dopamine antagonist either alone or in combination with exogenous gonadotropins (GTH) or GnRH had a role in gonadal maturation, ovulation and spermiation in teleost fish.

Regarding thyroid hormones application in fish aquaculture, Present results (Tables 1 & 2 and Figs. 7c & 8c). confirm previous observations of Fontaine (1976) & Dettaff and Davydova (1979) in Sturgeon, Acipenser Stellatues, who concluded that injection of triiodothyronine (T_3) with pituitary extract facilitated maturation and spawning of fish on a production scale. In goldfish, carassius auratus, T_3 and T_4 were required for normal gonadal maturation, probably as a factor regulating gonadal metabolism rather than gametogenesis (Hurlburt, 1977).

Concerning interrelationship between thyroid hormones and sex steroid production in fish Eales (1982) and Ueda et. al. (1984) suggested that there was a direct relationship between thyroxine and elevation of sera sex steroids in fish. Present results are in agreement with those of Ueda, et. al. (1984)who found that serum T_4 level was highest in females and males Chum salmon, at the pre-spawning time and decreased during the spawning season. They added that, ♀♀ in Chum Salmon, the level of estradiol-17 beta was very high during oocyte maturation, and decreased significantly at the time of ovulation, in ♂♂ chum salmon, high level of androgens was detected throughout pre-spawning time and then declined sharply around the spermiation time. Similarly, Mackenzie, et. al. (1989) concluded that plasma estradiol concentration of mature channel catfish, Ictalurus punctatus; showed significant increase in February (early prespawning), and remained at a higher level in April (late prespawning) period. This increase in plasma oestradiol level was accompained by oocyte growth (↑↑oocyte diameter and GSI). Moreover, in Chum salmon, Oncorhynchus keta, at varions stages of sexual maturity, Tagawa et. al. (1994) found that both plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) were high in both sexes during the spawning season and were relatively lower throughout early gonadal maturation phase (GSI are low).

Nevertheless, thyroxine clearly stimulated ovarian development in the intact Goldfish, it had no influence on hypophysectomized regressed adult fish (Hurlburt, 1977). The ovarian response to

salmon gonadotropin was enhanced by T4, indicating that thyroid hormones might act synergestically with exogenous GTH rather than acting directly on stimulating pituitary function because of the increase of ovarian response to exogenous GTH even after hypophysectomy (Hurlburt, 1977; Higgs et. al., 1982 and Mylonas et. al., 1994). Moreover, Cyr and Eales, (1996) and Timmermans et. al. (1997) found that thyroid hormones stimulated the testicular activity "spermatogenesis & spermiogenesis" and androgen production in male teleosts. The exposure of female freshwater catfish, Clarias batrachus to sublethal concentrations of carbaryl (anti-thyroids) led to inhibition of T_4 as well as T_3 synthesis and also the extrathyroidal conversion of T4 to T3; that reflect a depression in gametogenesis, maturation and release of mature gametes of Clarias fish (Sinha, et. al. 1991).

Immersion (or) injection of T_4 or T_3 into dam broodstock fish (Lam, 1994) or injection of Goldstriped amberjack, Seriola lalarndi broodstock fish with triiodothyronine (T_3) primed by hCG plus salmonid pituitary homogenate (Tachihara et. al., 1997) led to improvement of the survival and growth rates of offsprings, but the fertilization and hatching rates were not affected.

Concerning the applying of antiestrogens into male teleosts for regulation of GTH secretion, testicular activity and androgenic efficacy. Present results of exogenous administration of antiestrogen "Clomiphene citrate" into male Clarias fish, revealed improvement of spermatogenic activity (Table, 1 & Fig. 7d). Our results are in agreement with Kumar (1985) who found that injection of clomiphene in male catfish, Clarias batrachus; for 3 months (Feb-Apr.), led to increase in the testis weight, mean number of spermatid per seminiferous tubules; mature spermatozoa and artificial spermiation. However, exogenous salmon gonadotropin treatment had a stimulating effect on testes activity of adult male catfish (higher testes weight, larger nuclear diameter of Leydig cells and spermatogenesis) that previously arrested by 17beta-estradiol injection (Shetty and Rao, 1990).

The gonadal steroids (androgens & estrogens) exered their effects by interacting with specific steroid binding sites in hypothalamus and pituitary that were involved in the control of GTH secretion in fish (McEwen and Krey, 1984 and Peter, 1983). The androgenic steroids e. g. Testosterone are mediated by their localized conversion within the teleost fish brain e. g. Goldfish by aromatase enzyme to oestradiol (Pasmanik & Callard 1988). So, aromatization of testosterone to estrogen, rather than inhibition of biosynthesis of endogenous androgens may led to testicular hypofunction or even feminization of coho salmon fish (Pifferrer and Donaldson, 1991). Steroid negative feedback regulation of GTH secretion in fish had proved by several investigators; in rainbow trout (Bommelear et. al., 1981), african catfish (Habibi et. al., 1989) and goldfish (Kobayashi and Stacey,

1990) are suppressible by testosterone or estradiol.

Results of the present investigation confirmed previous observations of Billard and Peter (1977) who found that implantation of antiestrogens into the brain and pituitary of intact male goldfish stimulated the GTH release, that may have disrupted inhibitory dopaminergic inputs into the pituitary (Peter, et. al. 1986) and caused GTH release.

Present results are in disagreement with those of Crim et. al. (1981) and Trudeau, et. al. (1991) who concluded that positive feedback action of aromatizable types of androgens on induced GTH secretion was blocked by 1,4,6-androstatrien-3,17-dione aromatose inhibitor, suggesting that testosterone, through aromatization to E2 could increase pituitary responsiveness to LHRH analogue in mature male goldfish and consequently increase serum GTH secretion.

Regarding the use of antiestrogens into female teleost fish for enhancement of ovarian maturation and ovulation. The present results (Table, 2 & Fig. 8d) revealed that administration of domperidone plus clomiphene is more potent than domperidone alone in ovarian maturation and ova vitellogenesis. Results of the present study are in agreement with those of Singh and Singh (1976) who found that treatment of intact female Catfish, Heteropneustes fossilis, with clomiphene induced final ovarian maturation and ovulation in fishes within 6 days, similar treatment of hypophysectomized fish did not induce oocyte development or ovulation. Ueda and Takahashi, (1977a) succeeded in induction of ovarian maturation and spawning in loach, Misgurnus anguillicaudatus by injection of clomiphene in the prespawning time.

Action of non steroidal antiestrogens (clomiphene and tamoxifen) might be due to the ability of these synthetic compounds for competing with estrogen binding sites on estrogen receptors resulting in negative feedback increase of plasma gonadotropins (Billard and Peter, 1977; Patterson, 1981 and Adashi et. al., 1981). In addition, Donaldson et. al (1981) concluded that induction of final maturation and ovulation in Pacific salmon could be achieved by antiestrogen tamoxifen only.

Present results are in agreement, with those of Donaldson et. al. (1981), Worthington et. al. (1981 & 1982) and Jafri (1989) who indicated that either antiestrogens (clomiphene & tamoxifen) alone or salmonid gonadotrphin-tamoxifen combination was effective for synchronization of ovulation in teleosts. Chang and Yuen (1990) and Chang et. al. (1992) proved that LHRH analog injection with antiestrogens in female Black Porgy and Ayu, Plecoglossus altivelis fish was more effective in induction of ovulation rather than usage of antiestrogens (enclomiphene, zuclomiphene and clomiphene) alone. However, clomiphene and

enclomiphene are more efficient than zuclomiphene on final oocytic maturation.

Concerning administration of hCG with domperidone. The present results (Table 1 & Fig. 1) in male and (Table, 2 & Fig. 4) in female Clarias catfish, revealed that hCG had two different effects on serum sex steroids levels i. e., testosterone in male and 17- beta estradiol in female Clarias fish. Additionally, inspite of elevation in serum testosterone level in males injected with domperidone plus hCG as compared to domperidone-injected group (Table, 1 and Fig. 1); complete spermiogenesis and spermiation are not achieved (Fig. 7e). However, the same treatment into female fish led to complete oocytic maturation " late post vitellogenic stage" of the Clarias ovary (Fig. 8e) as compared to domperidone-injected group "previtellogenic and vitellogenic stages" (Fig. 8b).

As, the gonadotropins bind specifically to receptor sites in the gonads, Schulz, 1995 and Schulz et. al. (1997) proved that catfish had two subunits of LH glycoproteins (alpha and beta). The LH beta subunit were stimulated by a signal of testicular origin which reflected on gonadotropin (GTH II) secretion, steroidogenesis and onset of spermatogenesis. Moreover, the common and stronger type was the LH glycoprotein alpha subunit (Schulz et. al.,1997 & Liu et. al., 1997); proposing that in the absence of FSH-like gonadotropin in catfish; the LH glycoprotein alpha subunit had FSH-like action (GTH I) suggesting that LH (i.e. hCG) covers all functions requiring gonadotropin regulation; gonadal maturation and spawning in catfish species.

Regarding the effect of the dopamine antagoinst plus GnRH on gonad recrudescence, maturation, spawning and spermiation in fish; results of the present investigation (Table 1 & 2 and Figs. 7f & 8f) revealed that domperidone plus GnRH was the most efficient regimn for elevation of sex steroid hormones, final gonadal maturation and spawning in both sexes of Clarias fish.

The present results are in agreement with Patino (1997) who demonstrated that final stages of gonadal growth and spawning could usually be achieved by implanting a GnRH analogue, which in some species of fish had to be applied in combination with dopamine antagonists to increase the responsiveness to the GnRH analogue. Moreover, results in the present study, also are in agreement with Deragon et. al. (1997) and Pati & Habibi (1998) who concluded that synthetic LHRH of teleosts and mammals are chemically different but had a pronounced stimulating effect on GTH release and final gonadal maturation in fish.

Simultaneous injection of domperidone with GnRH into Clarias fish, not only had an elevating action on serum sex steroids, but also a maturation effect on gonads (testes and ovaries). In this

respect, the results of the present investigation are in agreement with Glubokov et. al. (1994) who demonstrated a final maturation of ovary of Pacific mullet by GnRH plus 4 types of neuroleptics dopamine antagonists (pimozide, sulpiride, metoclopramide and isofloxythepin).

Additionally, enhancement of gametogenesis (oogenesis and spermatogenesis) and spawning induction could be achieved in carp fish by injection of GnRH analogue plus metoclopramide (Yaron, 1995); also acceleration of testes maturation, spermiation and elevation plasma levels of testosterone in male black porgyfish were induced by LHRH analoge (Chang et. al. 1995). However, Degani et. al. (1995) induced acceleration of oocytic maturation and elevation of oestradiol 17 beta in female Trichogaster fish by LHRH analogue with pimozide . Donaldson, (1996) added that regulation of broodstock maturation, ovulation induction and spermiation of teleosts could be induced by GnRH with and without dopamine antagonists. Induction of oocytic maturation, ovulation and spawning in Asian catfish by LHRH analogue with pimozide were reported by (Tan-Fermin, 1992; Rath & Kalita, 1996 and Tan-Fermin, 1997).

In conclusion, exogenous administration of the dopamine antagonist " domperidone " together with GnRH has the most effective role on enhancement of final gonadal maturation , sperm production and spawning in Clarias lazera fish.

REFERENCES

Adashi, E.Y., Hsuech, A.J., Bambino, T.H. and Yen , S.S. (1981): Disparate effect of clomiphene and tamoxifen on pituitary gonadoropin release in vitro. Am.J. Physiol. Endocrinol. Metab. 3, E: 125-130.

Billard, R.; Bieniary, K., Popek, W.; Epler, P., Berton, B. and Alagarswami, K. (1987): Stimulation of gonadotropin secretion and spermiation in carp by pimozide-LHRH-A treatment: Effects of dose and time of day. Aquaculture, 62: 161-170.

Billard, R., and Peter, R. E. (1977): Gonadotropin release after implantation of antiestrogens in the pituitary and hypothalamus of goldfish, Carassius auratues. Gen. Comp. Endocrinol. 32, 213-220.

Bommelear, M. C.; Billard, R.; Berton, B. (1981): Changes in plasma gonadotropin after ovariectomy and estradiol supplementation at different stages at the end of the reproductive cycle in the rainbow trout (Salmo gairdneri R.). Reprod. Nutr. Develop. 21: 989-997.

Chang, C. F.; Hu, H. J.; Tang, H. C. and Sun. L. T. (1992): Stimulation of ovulation in ayu, Plecoglossus altivelis, by treatment with antiestrogens and lutenizing hormone-releasing hormone analog. Aquaculture, 101 (3/4): 329-336.

Chang, C. F. and Yueh, W. E. (1990): Oocyte maturation in protandrous black porgy, Acanthopagrus schlegeli, Stimulated by enclomiphene and luteinizing releasing hormone analogue. Bull. Instit. Zool. Acad. Sinica, 29 (3): 173-179.

Chang, C. F.; Yueh, W. S; Lee-M. F. and Schally, A. V. (1995): A microencapsulated analog of LHRH accelerates maturation but without stimulating sex reversal in the protandrous black porgy, Acanthopagrus schlegli. Repord. Nutr. Derelop. 35 (3): 339-350.

Chang, J. P., Cook, A. F. and Peter, R. E. (1983): Influence of catecholamines on gonadotropin secretion in goldfish, Carassius auratus. Gen. Comp. Endocrinol. 49, 22-31.

Chang, J. P.; Mackenzie, D. S. Gould, D. R. and Peter, R. E. (1984a): Effect of dopamine and norepinephrine on vitro spontaneous and gonadotropin releasing hormone induced gonadotropin release by dispersed cells or fragments of goldfish pituitary. Life Sci., 35: 2027-2033.

Chang, J. P. and Peter, R. E. (1982): Action of dopamine on gonadotropin release in goldfish, Carassius auratus. Proc. Int. Symp. Repord. Physiol. Fish. 1982, P. 51-57.

Chang, J. P. and Peter, R. E. (1983a): Effects of dopamine on gonadotropin release in female goldfish, Carassius auratus. Neuroendocrinology, 36: 351-357.

Chang, J. P. and Peter, R. E. (1983b): Effects of pimozoide and des-Gly^{10} (D-Ala^{6}) luteinizing hormone ethylamide on serum gonadotropin concentrations. Gen. Comp. Endocrinol., 25: 302.

Chang, J. P.; Peter, R. E. and Crim, L. W. (1984b): Effects of dopamine and apomorphine on gonadotropin release from transplanted distalis in goldfish. Gen. Comp. Endocrinol., 55: 347-350.

Crim, L. W., Peter, R. E. and Billard, R. (1981): Onset of gonadotropic hormone accumulation in the immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. Gen. Comp. Endocrinol. 44: 374-381.

Cyr, D. G. and Eales, J. G. (1996): Interrelationships between thyroidal and reproductive endocrine systems in fish. Reviews in Fish Biology and Fisheries, 6 (2): 165-200.Degani, G.; Jackson, K. and Marmelstein, G. (1995): The effect of LHRH analogue on sex steroid profiles and oogenesis in female Trichogaster trichopterus. J. Aquaculture in the tropics, 10: 4, 297-307.

Deragon, K. I.; Grazourian, L., Pati, D.; Habibi, H. R. and Sower, S. A. (1997): Characteristics of GnRH binding in the gonds and effect of lamprey GnRH-I and II on reproduction in the adult sea lampery. Gen comp. Endocrinol. 108: 327-339.

Dettlaf, T. A. and Davydova, S. I. (1979): The effect of triiodothyronine on the maturation of the oocytes of the Sevryuga (Sturgeon), Acipenser stellatus, under the influence of the gonadotropic hormones. J. Ichthyol. 19, 105-110.

Donaldson, E. M. (1973): Reproductive endocrinology of fishes. Am. Zool. 13: 909-927.

Donaldson, E. M. (1996): Manipulation of reproduction in farmed fish. Anim. Reprod. Sci. 42 (1/4): 381-392.

Donaldson, E. M.; Funk, J. D. Withler, F. C, and Morley, R. B. (1972): Fertilization of pink salmon (Oncorhynchus gorbuscha) ova by spermatozoa from gonadotropin injected juveniles. J. Fish. Res. Board Can. 29: 13-18.

Donaldson, E. M.; Hunter, G. A. and Dye, H. M. (1981): Induced ovulation in the coho salmon (Oncorhynchus Kisutch). III. Preliminary study on the use of antiestrogen tamoxifen. Aquaculture 26, 143-154.

Dowidar, N. M.; Zaki, M. I. and Abdalla, A. (1985): Sexual maturity, gonadal cycle and fecundity of Clarias lazera in lake Manzalah. First. Int. Conf. App. Sci. Vol. IV.

Eales, J. G. (1982): Circannual cycles of thyroid hormones in plasma of winter flounder (Pseudopleuronectes. A.) Can J. Zool. 60: 304-309.

Eding, E. H.; Jonssen, J. A.; Kleine, S. G. and Richter, C. J. (1982): Effect of human chorionic gonadotropin (HCG) on maturation and ovulation of oocyte in the ovary of the African catfish, Clarias lazera (C & V) In: C. J. J. Richter and H. J. Th. Goos (Editors), Proc. Int. Symp. Reproductive Physiology of Fish, Wageingen. Netherlands, 2-6 Agust, 1982. PUDOC, Wageingen, P. 195.

El-Bolock, A. R. (1973): Studies on spawning and spawning behaviour of Clarias lazera, Cuv. & Vol. In fish farms of A. R. E. (Egypt). Bull. Zool. Soc. Egypt, 25: 25-32.

Epler, P.; Bieniarz, K. and Marosz, E. (1985): Effect of temperature, 17 alpha-hydroxy-20 beta-dihydroprogesterone and prostaglandin F2 alpha on carp oocyte maturation and ovulation in vitro. Gen. Comp. Endocrinol. 58 (2): 192-201.

Eyo, J. E. (1997): Effects of in vivo crude human chorionic gonadotropin on ovulation and spawning of the African catfish. Clarias gariepinus. J. Applied Ichthyology, 13 (1): 45-46.

Fermin, A. C. (1991): LHRH-a and domperidone induced oocyte maturation and ovulation in Bighead carp, Aristichthys nobilis (Richardson). Aquaculture, 93 (1): 87-94.

Fontaine, M. (1976): Hormones and the control of reproduction in aquaculture. J. Fish. Res. Board Can. . 33: 922-939.

George, C. (1981): Staining procedures, 4th Ed., Williams and Wilkins, Baltimore, London.

Glubokov, A. I.; Kouril, J.; Mikodina, E. V. and Barth, T. (1994): Effect of synthetic GnRH analogues and dopamine antagonists on the maturation of Pacific mullet, Mugil So-iuy Bas. Aquacult. & Fisher. Manag. 25 (4): 419-425.

Goetz, F. W. (1997): Follicle and extrafollicular tissue interaction in 17 alpha, 20 beta-dihydroxy-4-pregnen-3-one stimulated ovulation and prostaglandin synthesis in the yellow perch, (Perca flavescens) ovary. Gen. Comp. Endocrinol. 105 (1): 121-126.

Habibi, H. R.; Deleeuw, R.; Nahorniak, C. S.; Goos, H. J. and Peter, R. E. (1989): Pituitary gonadotropin-releasing hormone (GnRH) receptor activity in goldfish and catfish: Seasonal and gonadal effects. Fish. Physiol. Biochem. 7: 109-118.

Higgs, D. A.; Fagerlund, U. H.; Eales, J. G. and McBride, J. R. (1982): Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp. Biochem. Physiol. 73: (B)/1: 143-176.

Hogendoorn, H. and Vismans, M. M. (1980): Controlled propagation of the African catfish, Clarias lazera (C. & V.) II. Artificial reproduction. Aquaculture, 21: 39-53.

Hurlburt, M. E. (1977): Role of the thyroid gland in ovarian maturation of the goldfish, Carassius auratus L. Can. J. Zool., 55: 1906-1913.

Jaffe, B. M. and Behrman, N. R. (1974): Methods of hormone radioimmunoassay. Academic Press; New York, U. S. A.

Jafri, S. T. H. (1989): The effect of some exogenous hormones and an antiestrogen on spawning in the roach (Rutilus rutilus L.) (Cyprimdae: Teleostei). Pakistan, J. Zoology, 21 (3): 213-219.

Kelly, A. M. and Kohler, C. C. (1996): Manipulation of spawning cycles of channel catfish in indoor water-recirculating systems. Progressive Fish-Culturist, 58 (4): 221-228.

Kobayashi, M. and Stacey, N. E. (1990): Effects of ovariectomy and steroid hormone implantation on serum gonadotropin levels in female goldfish. Zool. Sci. 7: 715-721.

Kucharczyk, D.; Kujawa, R.; Mamcarz, A.; Skrzypczak, A. and Wyszomirska, E. (1998): Induced spawning in Perch, Perca fluviatilis L., using FSH + LH with pimozide or metoclopramide. Aquaculture-Research, 29: 2, 131-136.

Kumar, S. (1985): Induction of artificial spermiation in the freshwater Clarias batrachus (Linn) by clomiphene citrate. Exp. Clin. Endocrinol. 85 (3): 289-296.

Lam, T. J. (1994): Hormones and egg/larval quality in fish. J. World Aquaculture Society, 25 (1): 2-5.

Liu, Z.; Li, P.; Argue, B. J. and Dunham, R. A. (1997): Gonadotropin alpha-subunit glycoprotein from channel catfish (Ictalurus punctatus) and its expression during hormone-induced ovulation. Mol. Mar. Biol. Biotechnol. 6 (3): 217-227.

Mackenzie, D. S.; Thomas, P.; Farrar, S. M. (1989): Seasonal changes in thyroid and reproductive steroid hormones in female channel catfish (Ictalurus punctatus) in pond culture. Aquaculture, 78 (1): 63-80.

McEwen, B. S. and Krey, L. C. (1984): Metabolism of hormonal steroids in the neuroendocrine structures. In: Celotti, F., Naftolin, F. and Martini, L. (eds.), Properties of Estrogen Sensitive Neurons: Aromatization, Progestin Receptor Induction and Neuroendocrine effects. New York: Raven Press; 1984: 117-138.

Miura, T.; Yamauchi, K.; Takahashi, H.; Nagahama, Y. (1991): Human chorionic gonadotropin induces all stages of spermatogenesis in vitro in the male Japanese eel (Anguilla japonica). Developmental Biology, 146 (1): 258-262.

Mylonas, C. C.; Scott, A. P.; Vermeirssen, L. M. and Zohar, Y. (1997): Changes in plasma gonadotropin II and sex steroid hormones, and sperm production in Stripped bass after treatment with controlled-release gonadotropin-releasing hormone agonist delivery systems. Biol. Repod. 57, 669-675.

Mylonas, C. C.; Sullivan, C. V. and Hinshaw, J. M. (1994): Thyroid hormones in brown trout (Salmo trutta) reproduction and early development. Fish Physiology and Biochemistry 13 (6): 485-493.

Pasmanik, M. and Callard, G. V. (1988): Changes in brain aromatase and 5 α- reductase activities correlate significantly with seasonal reproductive cycles in goldfish (Carassius auratus). Endocrinology, 122: 1349-1356.

Pati, D. and Habibi, H. R. (1998): Presence of salmon GnRH and compounds with GnRH-like activity in the ovary of goldfish. Endocrinology, 139: 2015-2024.

Patino, R. (1997): Manipulations of the reproductive system of fishes by means of exogenous chemicals. Progressive-Fish-Culturist, 59 (2): 118-128.

Patterson, J. S. (1981): Clinical aspects and development of antiestrogens therapy: A review of the endocrine effects of tamoxifen in animals and man. J. Endocrinol. 89, 67-75.

Peter, R. E. (1982): Neuroendocrine control of reproduction in teleosts. Can. J. Fish. Aquat. Sci., 39: 48-55.

Peter, R. E. (1983): The brain and neurohormones in teleost reproduction. In: Howar, W.; S., Randall, D. J. and Donaldson E. M. (eds.), Fish Physiology (Reproduction, Part A), Vol. IX. New York. Academic Press, 1983: 97-135.

Peter, R. E.; Chang, J. P.; Nahorniak, C. S.; Omeljaniuk, R. J.; Sokolowska, M., Shih, S. and Billard, R. (1986): Interaction of catecolamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog. Horm. Res. 42: 513-548.

Peter, R. E.; Lin, H. R.; Kraak, G. Van-der; Little, M.; and Van-der-Kraak. G. (1993): Releasing hormones, dopamine antagonists and induced spawning. Recent-Advances-In Aquaculture, 4: 25-30.

Peter, R. E.; Lin. H. R. and Vander Kraak, G. (1988): Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture, 74: 1-10.

Pifferrer, F. and Donaldson, E. M. (1991): Dosage-dependent differences in the effect of aromatizable and nonaromatizable androgens on the resulting phenotype of coho selmon (Oncorhynchus kisutch). Fish Physiol. Biochem. 9 (2): 145-150.

Rath, R. and Kalita, K. (1996): Induction of precocious ovarian maturation by LHRHa treatment in the Asian catfish, Clarias batrachus. J. Ecotoxicol. Environm. Monitor. 6: (3/4): 203-208.

Redondo, C.; Heymann, A.; Billard, R. and Cosson, M. P. (1989): Evaluation of quality in carp after stimulation of spermation with pituitary homogenates and LHRH-A comined with domperiodone, European Aquaculture Society, 10: 209-210.

Schulz, R. W. (1995): Physiology, morphological and molecular aspects of gonadotropins in fish with special reference to the African catfish, Clarias gariepinus. In: Goetz, F. W. Thomas, P. (eds.), Proceeddings of the Fifth International Symposium on the Reproductive Physiology of Fish. Austin, TX: Fish Symposium, 2-6.

Schulz, R. W.; Zandbergen M. A.; Peute, J.; Bogerd, J., Van-Dijk, W. and Goss, H. J. (1997): Pituitary gonado-trophs are strongly activated at the beginning of spermatogenesis in African catfish, Clarias gariepinus. Biol. Reprod. 57 (1): 139-147.

Shetty, V. K. m and Rao, G. P. (1990): Stimulation of spermatogenesis with salmon gonadotropin and ovine luteinizing hormone in 17 beta-oestradiol-treated male catfish, Clarias batrachus (Linn). Indian J. Anim. Sci. 60 (7): 900-907.

Singh, A. K. and Singh, T. P. (1976): Effect of Clomid, Sexovid and prostaglandins on induction of ovulation and gonadotropin secretion in a freshwater catfish, Heteropneustes fossils (Bloch). Endokrinologie, 68: 129-136.

Sinha, N.; Lal, B. and Singh, T. P. (1991): Carbaryl-induced thyroid dysfunction in the freshwater catfish, Clarias batrachus. Ecotoxicol and Environm. Safety, 21 (3): 240-247.

Snedecor, G. W. Cochran , W. G. (1980): Statistical Methods. Oxford and J. B. H. Publishing Co., USA.

Tachihara, K.; El-Zibeh, M. K. Ishimatsu, A., and Tagawa, M. (1997): Improved seed production of goldstriped amberjack, Seriola lalandi under hatchery conditions by injection of triiodothyronine (T3) to broodstock fish. J. World Aquaculture Society, 28 (1): 34-44.

Tagawa, M.; Ogasawara, T.; Sakamoto, T.; Miura, T.; Yamauchi, K., and Hirano, T. (1994): thyroid hormone concentrations in the gonads of wild, Chum salmon during maturation. Fish Physiol. Biochem. 13 (3): 233-240.

Tan-Fermin, J. D. (1985): A histological study of the hypo-physeal gonadal system during sexual maturation and spawning in milkfish, Chanos chanos (Forskal). J. Fish. Biol., 20: 657-668.

Tan-Fermin, J. D. (1992): Induction of oocyte maturation and ovulation in the freshwater Asian catfish, Clarias macrocephalus, by LHRH a and pimozide. J. Applied Ichthyology, 8 (4): 90-98.

Tan-Fermin, J. D.; Pagador, R. R.; Chavez, R. C. (1997): LHRH a and pimozide-induced spawning of Asian catfish, Clarias macrocephalus (Gunther) at different times during an annual reproductive cycle. Aquaculture, 148 (4): 323-331.

Timmermans, L. P; Chmilevsky, D. A. Komen, H. and Schipper, H. (1997): Precocious onset of spermatogene-sis in juvenile carp (Cyprinus L.) following treatment with low doses of L-thyroxine. Eur. J. Morphol. 35 (5): 334-353.

Trudeau, V. L. (1997): Neuroendocrine regulation of gonadotrophin II release and gonodal growth in the goldfish, Carassius auratus. Reviews of Reproduction, 2, (1): 55-68.

Trudeau, V. L.; Peter, R. E. and Sloley, B. D. (1991): Testosterone and estradiol potentiate the serum gonadotropin response to gonadotropin-releasing hormone in goldfish. Biol. Reprod. 44 (6): 951-960.

Ueda, H.; Hiroi, O.; Hara, A.; Yamauchi, K. and Nagahama, Y. (1984): Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon, Oncorhynchus keta. Gen. Comp. Endocrinol. 53 (2): 203-211.

Ueda, H. and Takahashi, H. (1977a): Promotion of ovarian maturation accompanied with ovulation and changes of pituitary gonadotrophs after ovulation in the loach, Misgurnus angullii; treated with clomiphene citrate. Bull. Fac. Fish. Hokkaido Univ. 28: 106-117.

Ueda, H. and Takahashi, H. (1977b): Promotion by clomiphene citrate of gonadal development and body growth in immature and maturing goldfish, Carassius auratus. Bull. Fac. Fish., Hokkaido Univ. 28: 181-192.

Warthington, A. D.; MacFarlane, N. A. and Easton, K. W. (1981): The induced spawning of roach (Rutilus rutilus L.) with carp pituitary extract and oestrogen-antagonists. Gen. Comp. Endocrinol. 31, 1-11.

Warthington, A. D.; MacFarlane, N. A. and Easton, K. W. (1982): Controlled reproduction in the roach (Rutilus rutilus L.). Proc. Int. Symp. Reprod. Physiol. Fish. 1982, P. 220.

Xing, S.; Lekan, S. Z. and Diczfalusy, U. (1983): Validation of radioimmunoassay for estradiol 17–β by isotope dilution-mass spectrometry and by a test of radioc hemical purity. Clinica. Chimica Acta, 135: 189.

Yaron, Z. (1995): Endocrine control of gametogenesis and spawning induction the carp. Aquaculture, 129 (1/4): 49-73.

Young, G.; Ueda, H. and Nagahama, Y. (1983): Estradiol 17 beta and 17 alpha, 20 beta-dihydroxy-4-pregnen-3-one production by isolated ovarian follicles of amago salmon (Oncorhynchus rhodurus) in response to mammalian pituitary and placental hormones and salmon gonadotropin. Gen. Comp. Endocrinol. 52 (2): 329-335.