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Trial for Induced Spawning with Histological Evaluation of Gonads in Forskal Catfish *Bagrus bayad*

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Abstract

Sixteen mature *Bagrus bayad* of both sexes (8 males and 8 females) were used to evaluate a trial for spawning induction using GnRHa combined with domperidone during spawning season. The experiment was carried out during May 2014. Fish were divided into 4 groups. Group 1 was control males and females *Bagrus bayad*, group 2 was females *Bagrus bayad* treated with dopamine antagonist (domperidone) alone, group 3 was females *Bagrus bayad* treated with domperidone and gonadotropin releasing hormone analogue (GnRHa), and group 4 was males *Bagrus bayad* treated with GnRHa and domperidone. GnRHa was used in the present study by dose of 20 ug/kg and dose of domperidone was 5 mg/kg. Results showed that no evidence for spawning induction. Histological examination showed structural changes in ovary of group 2 and group 3 in comparison to control ovary but there was no obvious change between structure of ovary of group 2 and group 3. In addition to, histological examination showed structural changes in the testis of group 4 in comparison to control testis. In both sex, histological examination did not identify any evidence of spawning. In conclusion, domperidone and/or GnRHa induced histological changes in the ovary and testis of *Bagrus bayad* but did not induce spawning. Further studies are needed with higher doses of GnRHa and/or carrying out the experiment during mid or late spawning season of *Bagrus bayad*.

Keywords: Forskal catfish; *Bagrus bayad*; Gonadotropin releasing hormone analogue (GnRHa); Domperidone; Ovary; Testis

Introduction

Bagrus bayad is an important food fish, with flesh of good eating and of economic importance in Egypt. It has high growth rate and attains maximum weight of 12.5 kg [1] but it has not been cultured due to limited information on their breeding under captivity as they, like other catfishes, are not able to exhibit their natural spawning behavior in artificial ponds [2].

Recently, gonadotropin releasing hormone analogue (GnRHa) has been used in the induced spawning of several fish species [3,4]. In some fish, dopamine prevents the release of gonadotropin through inhibitory actions on the pituitary gland, thereby making it impossible for fish to ovulate and spawn. Pre-spawn fish exhibiting dopaminergic tone were induced to spawn using GnRHa in combination with dopamine antagonist like domperidone. Successful spawning of some fish species were achieved using GnRHa and domperidone [5,6].

Therefore, the aim of this study was to investigate the effects of using gonadotropin releasing hormone analogues (GnRHa) with dopamine antagonists (domperidone) on the spawning performance of *Bagrus bayad* and to evaluate the histological changes occurred in the gonads in relation to the hormonal treatment.

Materials and Methods

Experimental location

The present study was conducted in the Fish Hatchery, Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt during natural early spawning season (May 2014) but at that time, there was long winter.

Experimental design

16 apparently healthy *Bagrus bayad* were divided into 4 randomly groups as shown in Table 1. The fish were selected from over-wintered *Bagrus bayad* maintained in 6.5 faddan (4200 m) earthen ponds that had 1.5 meter depth. In late days of March, the spawners were caught, selected, sexed and transferred to a quarter faddan ponds with 1 meter depth. At the early spawning season, female *Bagrus bayad* which showed spawning signs were randomly divided and distributed in tanks of 3.5 cubic meter volume filled with water supplied with aerator. After acclimatization of fish for 24 hrs. The water quality was measured daily according to Dewis and Freiles [7]. The fish were maintained in the laboratory under normal photoperiod (12.5 h L: 11.5 h D) and temperature (20 ± 4°C). The fish were subjected to the treatments as in Table 1. In general, injection was intra-peritoneal and the second dose injected after 8 hours from the first dose.

Group	Number of fish	Drugs	1st Dose	2nd Dose after 8 hrs	Specimens for histology
Group 1	2 females 2 males	----------	----------	----------	Testes and ovaries at zero time
Group 2	3 females	DOM	5 mg/kg		Ovaries after 8 hrs
Group 3	3 females	GnRHa DOM	2 ug/kg 5 mg/kg	18 ug/kg 2.5 mg/kg	Ovaries after 11 hrs from 2nd dose
Group 4	6 males	GnRHa DOM	20 ug/kg 5 mg/kg	----------	Testes after 18 hrs

DOM: Domperidone; GnRHa: Cystorelin

Table 1: Showing GnRHa and domperidone doses used in different groups.

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Preparation of GnRHa and dopamine antagonists

Gonadotropin releasing hormone analogue GnRHa: Cystorelin® (vail 20 ml) each 1 ml contains 50 ug synthetic Gonadorelin (GnRH) diacetate tetrahydrate manufactured by (CEVA SANTLE ANIMAL – France).

Domperidone tablets: Each tablet contains 10 mg from the active principle (under trade name Gastromotil® manufactured by EIPICO Campany, Cairo, A. R. E.) were powdered and then re-suspended in physiological saline 0.9% when needed according to Alok [8].

Histological examination

Specimens from ovaries and testis of all groups of *Bagrus bayad* were taken and fixed in Bouin's fluid for 24 hr. Specimens were dehydrated in alcohols, cleared in xylene and blocked in paraffin. Sections of 5 µm were cut and stained with hematoxylin and eosin stain, Masson's trichrome stain, Alcian blue stain and periodic acid Schiff technique. Fixation and staining methods and techniques were carried out according to Banchroft [9].

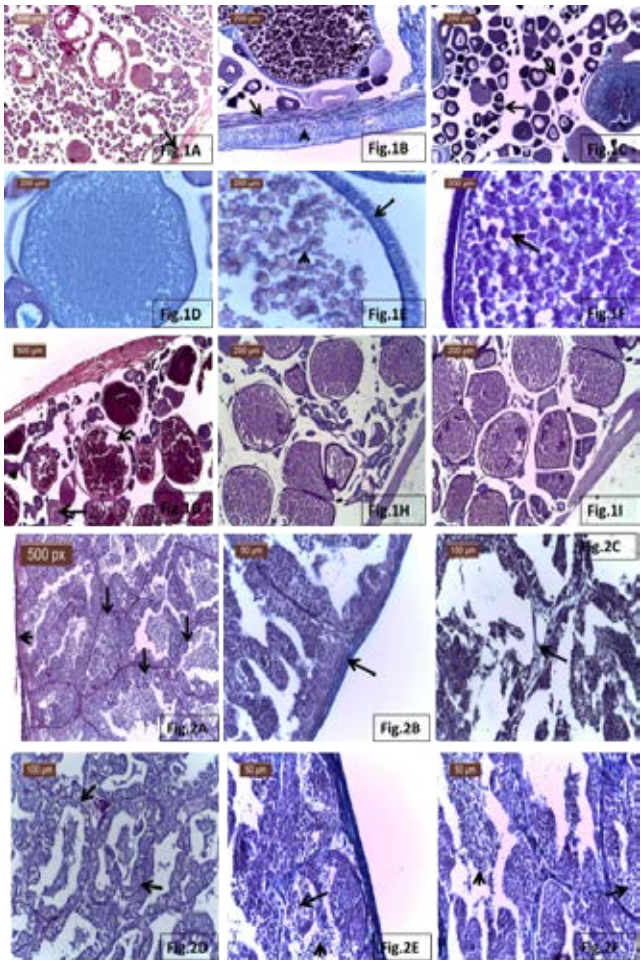
Results

Both sex of *Bagrus bayad* in the current study do not show noticeable spawning after injection of GnRHa (20 ug/kg) and domperidone (5 mg/kg).

Histologically, the ovary of control *Bagrus bayad* (in the early spawning season) was surrounded by connective tissue capsule known as tunica albuginea which is measured 209 µm thickness (Figure 1A). The tunica albuginea is fibromuscular layer as it consists of dense collagenous connective tissue which contains outer longitudinal and inner circular smooth muscle layers (Figure 1B). From tunica albugenia, collagenous ovigerous lamellae are extended into the ovary and enclose different developmental follicular stages; previtellogenic (immature), vitellogenic, and posvitellogenic (mature), with predominance of previtellogenic ones (Figure 1C). Vitellogenic follicles show alcinophilic yolk granules (Figure 1D) while postvitellogenic follicles show non alcianophilic yolk globules (Figure 1E) but yolk globules show PAS positive reaction (Figure 1F). The follicular epithelium shows PAS and AB positive (Figures 1E and F).

In group 2 which treated with domperidone alone, the ovarian structure of *Bagrus bayad* has clearly changed. Tunica albugenia becomes thicker and is measured 294 µm. The vitellogenic and postvitellogenic follicles are relatively increased in number, with predominance of postvitellogenic (mature) follicles (Figure 1G). Moreover, all vitellogenic and postvitellogenic follicles shows PAS positive yolk granules, yolk globules and follicular epithelium (Figure 1H). In group 3 which treated with combination of domperidone and GnRHa, ovaries show no obvious changes in comparison to ovaries of group 2 except slightly increase in the number of postvitellogenic follicles per field (Figure 1I).

The testis of control *Bagrus bayad* is covered with connective tissue capsule known as tunica albuginea that is measured 155 µm and it is divided into several testicular lobules (Figure 2A). Tunica albuginea consists of dense collagenous connective tissues with (Figure 2B). From tunica albuginea, interlobular connective tissues are arisen and collected in between seminiferous lobules (Figure 2C). The basement membranes of the seminiferous lobules show strong PAS positive (Figure 2D). There are all different developmental stages from spermatogonia to spermatozoa (Figures 2A-D). In group 4 which treated with combination of domepridone and GnRHa, tunica



Figures 1 and 2: (1A) Photomicrograph of control ovary of *Bagrus bayad* showing tunic albuginea (arrow) and different developmental stages of eggs. H&E stain. (1B) Photomicrograph of ovary of *Bagrus bayad* showing muscular layers in tunica albuginea. Outer longitudinal (arrow) and inner circular smooth muscle layers (arrowhead). Masson's trichrome stain. (1C) Photomicrograph of ovary of *Bagrus bayad* showing collagen fibers in the ovigerous lamellae (arrows). Masson's trichrome stain. (1D) Photomicrograph of vitellogenic follicle in ovary of *Bagrus bayad* showing alcianophilic yolk granules. Alcian blue stain. (1E) Photomicrograph of postvitellogenic follicle in ovary of *Bagrus bayad* showing alcian blue negative yolk globules (arrowhead) but alcianophilic follicular epithelium (arrow). Alcian blue stain. (1F) Photomicrograph of postvitellogenic follicle in ovary of *Bagrus bayad* showing PAS positive yolk globules (arrow) and follicular epithelium. PAS technique. (1G) Photomicrograph of ovary of *Bagrus bayad* treated with domepridone showing increase number of vitellogenic (short arrow) and postvitellogenic follicles (long arrow) in comparison to control ovary. Compare to (1A) H&E. (1H) Photomicrograph of ovary of *Bagrus bayad* treated with domepridone showing PAS positive yolk granules and globules in the vitellogenic and postvitellogenic follicles respectively. PAS technique. (1I) Photomicrograph of ovary of *Bagrus bayad* treated with domepridone and GnRHa showing thicker tunica albuginea in comparison to (1A) and (1H). PAS technique. (2A) Photomicrograph of control testis of *Bagrus bayad* showing tunica albuginea (short arrow) and many seminiferous lobules (arrows). H&E stain. (2B) Photomicrograph of control testis of *Bagrus bayad* showing that tunica albuginea consists of dense collagenous connective tissue (arrow). Masson's trichrome stain. (2C) Photomicrograph of control testis of *Bagrus bayad* showing collagen fibers in the interlobular connective tissue (arrow). Masson's trichrome stain. (2D) Photomicrograph of control testis of Bagrus bayad showing PAS positive basement membranes (arrows) of the seminiferous lobules. PAS technique. (2E) Photomicrograph of testis of *Bagrus bayad* treated with domperidone and GnRHa showing spermatid cyst (arrow) and spermatozoa (arrowhead). Masson's trichome stain. (2F) Photomicrograph of testis of *Bagrus bayad* treated with domperidone and GnRHa showing increase of interlobular connective tissue. Masson's trichome stain.

albuginea becomes thicker and is measured 165 μm in addition to, cysts of spermatid and spermatozoa are increased in number in comparison to control testis (Figure 2E). Moreover, the interlobular connective tissues are increased (Figure 2F).

Discussion

The effect of GnRH in induction of fish spawning is controversial. Our results demonstrated that GnRHa (20 ug/kg) in combination with domperidone (5 mg/kg) was not sufficient to induce spawning during early spawning season of *Bagrus bayad*. However, Sahoo et al. [6] reported that ovaprim (sGnRHa (20 μg/kg) and domperidone (10 mg/kg) was ideal for induction of spawning in the Asian catfish. On the contrary, Tan-Fermin et al. [10] stated that 50 μg/kg BW GnRHa could not induce ovulation in the Asian catfish. On the other hand, Silverstein et al. [11] recorded that 100 μg/kg GnRHa was used in channel catfish *Ictalurus punctatus*, more hatching rate was recorded.

Histologically, the ovary of *Bagrus bayad* was consisted of tunica albuginea and different developmental follicular stages (previtellogenic, vitellogenic, and postvitellogenic stages) that was similar to ovarian structure in most of teleost fish as tilapia [12-16]. The ovarian tunica albuginea of *Bagrus bayad* was supported by muscular layer which differentiated into an outer circular layer and inner longitudinal layer as reported in *Bagrus bayad* by Khallaf et al. [17] and Gaber [18], while Gaber [18] did not differentiate two muscular layers in the ovarian wall of Bagrus docmac.

The testis of *Bagrus bayad*, like most of teleost fish, was of the lobular type as it was consisted of anastomosing seminiferous lobules which were separated by connective tissue stroma as stated by (Abd El-Aziz [19]; Mousa [12]; El-Gohary [13]) in tilapia and (Oteme et al. [20]; Emam and Abugherin [16]) in catfish. Spermatogenic cells were in synchronous development inside cysts in the testicular lobules of *Bagrus bayad* that was similar to testicular structure of teleost fish (Abd El-Aziz [19]; Schulz et al. [21]; El-Zoghby et al. [15]; Emam and Abugherin [16]). In the present study, the spermatozoa begun to appear within lumen of testicular lobules in group 4 after injections of domeperidone and GnRHa in comparison to testis of group 1. The testis of group 4 did not show any signs for spawning where the spent testis or post-spawning testis should show some empty testicular lobules indicating spawned testes as they discharge their content during spawning as reported by Resink et al. [22] in Clarias gariepinus, Gaber [18] in *Bagrus bayad* and El-Zoghby et al. [15] and Emam and Abugherin [16] in Clarias lazera. In addition to, our results revealed relatively decrease the number of previtellogenic (immature) follicles and increase number of postvitellogenic (mature) follicles in groups 2 and 3 after injection of domperidone and/or GnRHa in comparison to ovary of group 1 that supports the findings of Chowdhury et al. [23] in the Indian catfish indicating potential role of GnRHa in induction of spawning but it may be of dose dependent. But there were no signs for spawning where the spawned ovary should show postovulatory and atretic follicles as mentioned by Gaber [18] in *Bagrus bayad*, and El-Zoghby et al. [15] and Emam and Abugherin [16] in Clarias lazera.

Conclusion

From all previous findings, we concluded that GnRHa (20 ug/kg) in combination with domperidone (5 mg/kg) is not sufficient to induce spawning during early spawning season of *Bagrus bayad*. Therefore, a further study is needed for the use of higher doses of GnRHa and/or doing the experiment during mid or late spawning season of *Bagrus bayad*.

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