International Scholars Journals

African Journal of Agriculture ISSN 2375-1134 Vol. 11 (2), pp. 001-008, February, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

A comprehensive examination of reproduction regulation and hormonal modulation in finfish species

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Accepted 15 November, 2023

Control of reproductive is essential for the sustainability of commercial aquaculture production. The reason for breakage of ovulation and spawning is failure of the pituitary to release gonadotropin hormones. Gonadotropin hormones secretion is under a dual control, by gonadotropin releasing hormone (GnRH), and inhibition by dopamine on the actions of GnRH. The inhibitory actions of dopamine on GTH secretion can vary in potency between species. In some fish it is necessary to use dopamine blocker, such as domperidone, results in potentiation of the actions of GnRH analoge, leading to a large release of GTH-II and ovulation. In some other injection of a high dose of GnRH analoge alone is effective. In several marine species GTH-II secretion is not under dopaminergic inhibitory control but sensitive to exogenous analoge hormones (GnRHa). In many fishes, synthetic analoges of lutenizing hormone-releasing hormone (LHRHa) are widely used for inducing spawning. In several tropical freshwater and marine finfishes the human chorionic gonadotropin (HCG), pituitary homogenate and semi-purified fish gonadotropin are used. Rrecently the replacement of natural GnRH are used and had resulted in a centuple increase in the effectiveness of luteinizing hormones secretion induction.

Key words: Reproduction induced spawning, dopamine antagonists, GTH-II- HCG.

INTRODUCTION

The further development of aquaculture is undoubtly based on the management of fish species. Aquaculture has been practiced for many centuries, but transfer from low and temporary production to intensive and regular approach is to control reproductive processes of fish in captivity, and to acquire high quality fingerlings for grow-out of the marketable product. Most of the fishes when reared in captivity, do not exhibit normal reproduction. Actually they reach reproduction maturity and gonadal growth is normal but the females fail final oocyte maturation (FOM) and do not spawn and male exhibite few or even lack of milt. The example for this subject is female common carp that in their habitat they will reproduce just by change in environmental condition and the precence of male. Therefore, breeding of captive fish may be approached managing the proper environmtal condition suitable for the species. Artificial environments lack natural spawning stimuli (spawning substrate, stream hydraulic, nutrition, water quality, depth etc.) are not able to induce appropriate endogenous response from the fish; the final result is reproductive dysfunction of FOM (Abraham, 1988; Podhorec and kouril, 2009).

Traditional methods of induced spawning for cultured fish are based on the injection of GTH-II from different sources, including crude extract of carp pituitary gland (CPE), partially purified fish GTH-II and mammalian GTH, especially human chorionic hormone (HCG) (Lam, 1982; Donaldson and Hunter, 1983; Peter et al., 1988a). Among these hormones, Fish GTH-II is generally of high species specificity, thus, for example, carp or salmon GTH-II are ineffective in gilthead seabream. HCG is routinely used to ineffective in others, such as grass carp and black carp (Lin and Peter, 1996). In using hormons for artificial reproduction it should be considered that several items such as the side effects of mixture of hormones, the cost of some hormones, also at the time of treatment, the fish should be at an advanced stage of gonadal development otherwise hormone injection at improper time or insufficient

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of the dosage may damage or even will kill the fish. Almost in all cultured fishes, hormonal manipulations may be used as management tools to enhance the efficiency of egg production, increase spermiation and facilitate hatchery operations. Also, hormonal therapies may be employed in artificial collection in order to implement interspecific hybridization, chromosome set manipulation or artificial fertilization for genetic selection programmes (Mylonas et al., 2010).

To have a review of the reproduction control and hormonal manipulations in finfish species, we may first have an investigation of the general hormonal function in reproduction and then the use of different hormone in different fish species. The objective of the present study was a brief study of different hormones used in fish reproduction and listing that are used in different fish species.

THE REPRODUCTIVE CYCLES IN FISH

The endocrine control system of the reproduction in finfish is based on the: hypothalamus-hypophysis-gonads axis. Therefore there is a permanent communication between the brain/pituitary complex and the periphery of the organ (gonads). This communication allows the activity of the different components of the brain–pituitary–gonad axis to be synchronized at all steps of the life cycle, which is crucial for coordinated responses. The rule of GnRH, secretion of dopamine, steroid feed back, LH secration all has their importance alone or in combnation with the others in reproduction. Two types of GtHs, different for structure and chemical role, were identified in the teleosts:

GtH-I (or FSH), involved in the initial stages of gametogenesis (vitellogenesis and spermatogenesis), and GtH-II (or LH), which sets FOM, spermiogenesis and spermiation (Mylonas et al., 1996).

GtH-II increase in plasma just before spermiation and FOM, determining the switch from the steroidogenic and androgenic production to the progestinic production respectively in the interstitial testicular cells and the ovarian theca cells (Rainis and Ballestrazzi, 2005). Studies comparing the relative LH releasing effects of the native GnRH isoforms in teleost species, including goldfish, catfish and seabream demonstrated that the most potent GnRH form in terms of bioactivity is GnRH-2, while the species-specific type 1 isoform is generally the least potent (Johnson et al., 1999; Rebers et al., 2000; Zohar et al., 1995b). In many teleosts, but not all, dopamine was shown to strongly inhibit gonadotropin release through a mechanism that probably has different adaptative significance depending on the species.

Dopamine is a small neurotransmitter that is synthesized from tyrosine through a two step reaction involving the step limiting enzyme tyrosine hydroxylase and DOPA-decarboxylase.

The distribution of dopamine in the brain of fish has been extensively studied using different techniques showing the existence of a well developed dopaminergic system (Nieuwenhuys et al., 1998). Dopamine receptors belong to the G-protein coupled receptor (GPCR) family. There are two main classes of DA receptors that differ in their ability to activate (D1) or to inhibit (D2) the enzyme adenylyl cyclase, with each class containing various subtypes (Cardinaud et al., 1998; Kebabian and Calne, 1979; Zohar et al., 2010). It was shown that dopamine acts directly onto the gonadotrophs through D2 receptors. This was shown first in goldfish and then confirmed in other species such as carp, African catfish, trout, tilapia, eel and gray mullet. In contrast, dopamine inhibition of gonadotropin release was not observed in many other teleost fish, particularly in marine species (see for more detail (Zohar et al., 2010). In fish species as mentioned before the rule of LH is very important in final maturation and ovulation of the egg. The neurodecapeptide GnRH is the central regulator of the reproductive hormonal cascade regulating the synthesis and release of LH secretion from the pituitary gland (Kah et al., 2007). The hypophysiotropic GnRH is processed in the hypothalamic neurons by enzymatic cleavage of a precursor polypeptide and packaged in storage granules (Yaron and Sivan, 2006). Another important factor controlling the reproduction in fish is the secretion and the rule of catecholamine metabolism. The teleost species possess two distinct gonadotropins, GtH-I and GtH-II. This is the case of seabream, striped bass, salmon and trout, whereas only one gonadotropin could be found in catfish (Saligaut et al., 1999).

GtH-I and GtH-II have distinct temporal expression and release profile in teleosts. GtH-I is released over the entire vitellogenesis, whereas GtH-II remains low during vitellogenesis and exhibit a sharp peak before ovulation (Davies et al., 1995; Prat et al., 1996). These different patterns of expression and release demonstrate that there is obviously a differential control of GtH-I and GtH-II (Saligaut et al., 1999). Studying more detail of the effect of GTH showed that GTH-I and GTH-II are equipotent in stimulating 11-ketotestosterone (11KT) and 17α, 20βP production (Planas et al., 1991). By further study it was shown that later, however, GTH-II was more potent than GTH-I in stimulating 17α, 20βP synthesis. Thus, early GnRH treatment may have had a relatively greater impact on GTH-I production while the effectiveness of GnRHa injection at the height of the spawning season is consistent with increase impact on GTH-II production and 17α, 20βP synthesis at that stage of the reproduction cycle. One of the primary factors limiting the early synthesis of 17α, 20βP may also be the lack in availability of its precursor 17α- hydroxyprogesterone 17P

(Sakai et al., 1989; Schulz et al., 1991, 1992) due to the dominance of the androgen biosynthetic pathway. In salmonids, the role of catecholamines remains controversial. In salmon, DA modulated GTH release only after administration of a LHRH analoge (Van der Kraak et al., 1986). However, Billard et al. (1983) demonstrated in trout that pimozide (a DA antagonist) alone could increase plasma GtH.

The efficacy of pimozide and of MPT depends on E2 levels. In immature fish, the increase of GtH-II release is only observed in E2 implanted fish and occurs simultaneously with an increase of the activity of pituitary DA neurones (Linard et al., 1995). In both goldfish and trout, some dopaminergic neurones of the anterior ventral preoptic region project to proximal pars distalis of pituitary (Peter and paulencu, 1980; Kah et al., 1987; Linard et al., 1996). An activation of a DA preoptico-hypophysial pathway could be involved during vitellogenesis in the rainbow trout. Some interesting results concern the catfish (*Heteropneustes fossilis*). It seems that in some air breathing species which coexist in sub-tropical waters, there is seasonality in the dominance of the CA during the reproductive cycle:

DA levels and turnover are high during the resting phase and decrease during the progress of breeding, with a concomitant increase in NE turnover (Senthikumaran and Joy, 1995; Saligaut et al., 1999).

Oocyte maturation and spermiation

Blood levels of GtH-I in immature and vitellogenic trout are much higher than those of GtH-II and can be compared with those found in literature in salmon (Dickey and Swanson, 1995). In contrast GtH-II is high at final stage of gonad development. During vitellogenesis the oocye development is continued. In investigation of “stock identification” and “genetic variation” at Microsatellite Loci of Caspian Sea Salmon (Salmo trutta caspius), genetic differentiation between the male and female, showed to be significant as indicated by Fst analysis, while the pair wise analysis of allele frequencies indicate differentiated at several loci, however we did not find the differences in sex is related to sex genes. (Yousefian, 2010b), at end of vitellogenesis oocyte maturation will be complete but for ovulation a number of cytological and nuclear changes occur. During FOM, the nucleus or germinal vesicle (GV) migrates to the periphery of the oocyte just under the micropyle. During or soon after GV migration, coalescence of the lipid droplets and yolk globules occurs, followed by the breakdown of the GV membrane (GVBD). This is the case that we usually use for testing the stage of sartificially insemination of sturgeon fish in reproduction farms. In inducing artificial reproduction some important note should be considered.

The type of strategy employed for oogenesis by different fishes has important implications in their broodstock management in aquaculture. For example, for synchronous fishes that spawn once in their lifetime (for example, freshwater eels and Pacific salmons), sacrific ing the fish and collecting their artificially matured gametes directly from the brood fish is a very effective and efficient method for producing fertilized eggs. Obviously, the same approach could not be used for fish that reach reproductive maturation at a late age and are expected to have a long reproductive life (for example, tunas, amberjacks or groupers). Similarly, stripping of eggs and artificial insemination may be used in annually spawning synchronous females to obtain the total amount of available eggs from each female, but the same may not be achieved with multiple spawning fishes that mature and ovulate only a small fraction of their total annual fecundity at every spawning event. During OM, some morphological changes occure in the oocyte together with progression of meiosis. In some such as mullet lipid droplet coalescence and yolk globule coalescence which result in the clarification of the oocyte’s cytoplasm, migration of the nucleus (germinal vesicle, GV) to the periphery of the oocyte and dissolution of the nucleus membrane (GV breakdown, GVBD), and the volume of eggs increase due to water uptake. The GV is visible under the microscope after some chemical processing, and disappears when GVBD takes place (Yousefian et al., 2010a).

The problems in reproduction are not restricted to female but also low spermiation or low quality of sperm also may occur during spawing of fish. Lower plasma levels of LH during the spermiation period have been suggested as the cause of the reduced amount of milt produced by some fishes (Mananos et al., 2002; Mylonas and Zohar, 2001a). The amount of LH in the pituitary or the ability of the pituitary to synthesize LH in response to treatment with exogenous GnRHa is not affected in these fishes, suggesting that again the reproductive dysfunction in the males may be identified in the brain control of GtH synthesis and/or release (Mylonas et al., 2010). In case of female as we mentioned before the problem in reproduction is not due to vitellogenesis but the fish fail to undergo oocyte maturation. Steven and his collegues in 2000, stated this problem that LH was synthesized and stored in the pituitary during vitellogenesis, since levels of LH and its mRNA in the pituitary did not differ between wild and captive females, demonstrating that the problem is one of lack of release and not synthesis in captivity. In addition, mRNA levels of the pituitary receptor for the GnRH most relevant to pituitary LH synthesis were similar between wild and captive females. This suggests that the disruption in LH release from the pituitaries of captive fish is not due to a dysfunction in pituitary responsiveness, but may be related to the control of pituitary function by reproductive brain. In fact, differences were observed between wild and captive

females undergoing OM, when comparing the pituitary content of the endogenous GnRHs.

The GnRH mRNA levels within the brain, however, were similar between the two groups, indicating that the altered pituitary content of GnRH in captive fish may be a result of altered release from the hypothalamus, rather than deficient synthesis (Steven, 2000; Steven et al., 2000; Mylonas et al., 2010).

Hormonal therapies and maturation induction

There are several methods available for spawning induction:

Injection of pituitary extracts, human chorionic gonadotropin (HCG), gonadotropin (GTH), gonadotropin-releasing hormone (GnRH) and GnRH agonists, luteinizing hormone releasing hormone (LHRH) and LHRH agonists (LHRHa).

GnRH and its agonists for spawning induction therapies have important advantages over the use of GTH preparations. GnRH can provide a more balanced stimulation of reproduction events and, better integration of the events with other physiological functions (Lam, 1982; Yaron, 1995; Mylonas et al., 2010). Supplying fresh extracts of the pituitary gland are expensive, difficult availability, variability of the product quality and to obtaine the hypophises the fish should be killed. Also there is possible interference of the hormone administered with the endocrine path of the animal. Besides, they were active only on fish phylogenetically close to the donors. For this reason, GtHs were replaced with human chorionic gonadotropin (hCG), that were characterised by a wide availability on the market and a higher chemical purity, ensuring a better efficacy. In the other hand, luteinizing hormone-releasing hormone analogue des-Gly10(D-Ala6)LHRH- Ethylamide (LHRHa) has been sucessfully used to induce final maturation and synchronize ovulation of many commercially cultured fish (Donaldson and Hunter, 1983; Park et al., 1997; Mylonas et al., 2010).

Administration methods of GnRHa to broodstocks include injection of GnRH or GnRHa sustained release preparations (Crim and Bettles, 1997; Peter and Yu, 1997; Zohar and Mylones, 2001). It is the most commonly used due to wider availability, higher purity and standardized activity. Also, piscine pituitary extracts and purified LH obtained through chromatographic separation are used (Lam, 1982; Donaldson and Hunter, 1983; Zohar, 1989b). It has been tested with variable success in many commercially important fish, often in combination with GnRHa. It has been suggested that due to the large molecular size and heterologous nature of GtH preparations, fish develop an immune response and attempts to use it for more than one reproductive season either fail or require an increasingly higher dose (Zohar, 1989b; Peter and Yu, 1997; Zohar and Mylonas, 2001). GnRH analogues are used in a group of fish species successfully. In carp by the use of GnRH analogues, together with antidopaminergic drugs (pimozide, domperidone, etc.), was successful. Antidopaminergic drugs (are well-known neuroleptic compounds with an extremely long half-life, exhibiting a wide range of physiological effects. The presence of dopaminergic inhibition has been decisively demonstrated in many cyprinid fish and the African catfish (*Clarias gariepinus*, Clariidae), but appears to be absent or weak in most commercially important marine fish (Zohar et al., 1995b). As a result, spawning induction protocols using a combined GnRHa and DA antagonist treatment are used extensively only for cyprinid fish (Peter et al., 1993; Yaron, 1995; Zohar and Mylonas, 2001).

The action of GnRHa takes place at a higher level of the animal endocrine pathway, stimulating the reproductive events in a more balanced and integrated way, with the other physiological functions correlated to the reproduction cycle (Mylonas et al., 1996). Therefore, GnRH seems stimulates also the release of other pituitary hormones involved in the reproductive cycle, like growth hormone (GH), thyroid stimulating hormone (TSH) and Somatolactine (Mylonas et al., 1998b; Zohar and Mylonas, 2001). In the use of gonadotropin hormone, at the beginning, the effective doses of GnRH or its analogues used to induce ovulation in cultured fish ranged from 1 to 15 mg/kg bw (Donaldson and Hunter, 1983; Zohar and Mylonas, 2001). In the majority of scientific reports concerning fish reproduction published in the 1980s, the doses of GnRH analogues ranged from 50 to 100 µg/kg bw. Presently, recommended doses of GnRH analogues for aquaculture vary between 10 and 50 µg/kg bw depending on the fish species, analogue, and mode of its administration (Yu et al., 1997; Zohar and Mylonas, 2001; Zohar et al., 2010). However, injection of GnRHa does not always result in 100% ovulation (Zohar et al., 1995b). In most reports concerning the effect of GnRH analogues, it was shown that GnRH alone is unable to induce ovulation or that the percentage of ovulated females is extremely low at 10 to 20% (Weil et al., 1980; Kouril et al., 1983; Sokolowska et al., 1984; Podhorec and Kouril, 2009). Due to the strong dopaminergic inhibition of LH secretion, typical for the family Cyprinidae, a majority of trials with ovulation induction using only GnRHa failed (Weil et al., 1980; Sokolowska et al., 1984).

According to the results of other authors (Peter et al., 1988; Yaron, 1995; Heyrati et al., 2007; Hamackova et al., 2001; Kouril et al., 1986) the only exceptions to the strong dopaminergic activity in Cyprinidae are tench (Tinca tinca), and rudd (Scardinius erythrophthalmus), in which even a dose of 1 µg/kg mGnRHa was able to stimulate ovulation in a small number of females. As a consequence of the identification of DA's role in LH

inhibition in Cyprinidae, Peter et al. (1988) developed the so-called LinPe method using the simultaneous administration of GnRHa and effective dopamine D2receptor antagonist. DI disinhibitis dopaminergic effect and strengthens the gonadotropin cell stimulation critical for induction of the reovulatory surge of LH (Podhorec and Kouril, 2009).

In studies of the effect of dopamine in fish, the goldfish were the first to demonstrate the effects of blockage of dopamine actions on GtH-II release and ovulation by determining the response to (D-Ala6, Pro9-Net)-mammalian GnRH (mGnRH-A) when given on combination with the dopamine receptor antagonist, pimozide. The presence of dopamine inhibition of GtH-II release has been extended to a number of other teleost species, including:

African catfish, coho salmon, Japanese eel, European eel, rainbow and brown trout and tilapia (Lin and Peter, 1996).

Based on the evidence that the failure of cultured fishes to undergo full spermiation and OM is the result of diminished LH release from the pituitary, manipulations of reproductive function have focused first on the use of exogenous LH preparations that act directly at the level of the gonad, and more recently on GnRHa-with or without DA that releases the endogenous LH stores from pituitary. Endogenous LH, in turn, acts at the level of the gonad to induce steroidogenesis and the process of OM and spermiation.

The results indicate that for the induction of a sustained increase in milt production, especially in individuals that are stripped spawned during the reproductive season, a longterm hormonal therapy is necessary, either through the use of multiple injections or controlled release delivery systems. Many different GnRHa delivery systems have been used to enhance spermiation in cultured fishes, such as Atlantic salmon, rainbow trout, coho salmon and Chinook salmon (Mylonas and Zohar, 2001b). In the European seabass, treatment with GnRHa-delivery systems resu lted in increased milt production for 28 to 35 days, compared to 7 days only when a single injection of GnRHa was given (Ma♂anos et al., 2002; Sorbera et al., 1996; Mylonas et al., 2010). Also in the striped bass, GnRHa delivery systems induced increases in milt production for 14 to 20 days (Mylonas et al., 1997, 1998a; Mylonas et al., 2010). To increase the bioavailability and efficiency of GnRHa, several sustained delivery systems have been used successfully in spawning induction of various species of fish; these include cholesterol pellets, cholesterol, cellulose pellets, ethylenevinyl acetate (EVAC), polylactide-glycolide (PLGA) and fatty acid dimer sebasic acid (Fad-Sa) microspheres (Zohar and Mylones, 2001; Mylonas and Zohar, 2001b). GnRHa implants have also been used in Atlantic halibut to enhance the quality of the sperm, which is extremely viscous and exhibits very little spermatozoa motility towards the end of the spawning season (Vermeirssen et al., 2003).

The used of different hormone such as CPE, GnRH an LRH in spawning induction therapies in synchronous different fish species are presented in Table 1. Studying the last two decade due to development of aquaculture, the use of hormones to induce OM and ovulation have been increased. Based on the table, most popular hormone for induction is GnRH. Early application of GnRHa was the synchronization of Om and ovulation in salmonids. Treatment with GnRHa is used 1 to 2 week before spawning. A double injection of 10 to 100µg/kg B.W. spaced 3 days apart. GnRHa is given in a priming dose (5 to 10%) and a resolving dose (95 to 90%). The same results single or multiple injection of GnRHa has also been used extensively in other fish species such as common carp, sturgeon fishes, and mullet to synchronize ovulation. In sturgeon priming and resolving injection spaced 10 to 24 h apart, and ovulation is accomplished 24 to 50 h afterwards. A double treatment with CPE was used in sturgeon fish farm to induce OM and ovulation (Yousefian et al., 2010a). In propagation of sturgeon first we should be confirmed maturity of fish. Gonad maturity is detected by taking sample, using a plastic cylindrical probe. High degree of migration of germinal vesicle (G.V.) expressed by polarization Index (PI) indicated spawnable females. PI is the ratio of distance between germinal vesicle and the animal pole to the diameter of the oocyte. PI within the range of 5 to 6 was more advanced in maturity than higher or lower in the study of Yousefian et al., 2010a. In order to take place of OM and ovulation, spawner was stimulated by usage of suspension of powdered acetone dried sturgeon pituitaries at a rate of 45 to 50 mg per female of *Acipenser pesicus* (Yousefian et al., 2010a).

A single treatment with CPE has also been reported to be effective (Williot et al., 2005).

In several fin fishes, DA antagonist is also used, administered together with priming dose. In Tench (Tinca tinca) a single injection of 20µg/kg GnRHa induced ovulation while in common carp the same amount of the hormone with 5 mg/kg of DA analogonis pimozide induce ovulotion. In the gray mullet (*Mugil cephalus*), two injections of 20µg/kg GnRHa together with 15 mg/kg1of the DA antagonist metoclopramide were very effective in inducing spawning within 24 h (Aizen et al., 2005). Induce oocyte vitelogenesis and ovulation of Gray mullet (*M. cephalus*), are described in detail by Yousefian et al. (2009), by using of mammalian gonadotrophins and synthetic analog of luteinizing hormone releasing hormone (LRH-A2). For successful spawning, a priming injection for females should be when an average egg diameters is 600 µm or more. A ripe ova have a thin layer cytoplasm covering the yolk and have a single oil droplet. In this case, the egg diameter was 960 µm and oil globule 360 µm. Hydration was observed about 10 h after the

injection of effective dose and spawning within 4 to 6 h after beginning of hydration at 24 ± 1°C and water salinity 33 to 35 ppt. During this time GSI increased from 17 to 28%. The percent of fertilization was up to 80. A combination of hormone in most cases showed beter effectiveness compairing to one single hoemoes. The study was performed in order to try to provoke the gonadotropin wave and ovulation in Chalcarburnus chacoides using GnRH analogue, pituitary extract and dopamine antagonist (Yousefian et al., 2008).

Different doses and injection protocols were applied using a combination of LRH-Aa, metoclopramide and carp pituitary extract. *C. chalcoides* show wide individual variation and much slower response to different hormonal stimulation. Saline (0.9% Nacl) injected fish were used as a control group and no ovulation occurred in this group. Based on the spawning ratio, fertilization rate and hatching rate, the combination of LRH-Aa 5 µg/kg, carp pituitary extraction 2.7 mg/kg and metoclopramide 2 mg/kg B.W. doses were found to be more efficient than carp pituitary (4mg/kg). No females ovulated in the treatment group receiving metoclopramide at 10 mg/kg and LRH-Aa alone at 100 µg/kg (Yousefian et al., 2008).

In the chum salmon (*Oncorhynchus keta*), a single injection of 70µg/kg GnRHa together with 700µg/kg the DA antagonist pimozide were effective in inducing OM and synchronizations ovulation within one week (Park et al., 2007). In rainbow trout (Oncorhynchus mykiss) two injections of 25µg/kg GnRHa spaced 1 day apart induce synchronizations of ovulation in 100% of the population 11 days after treatment without affecting broodstock survival and egg quality (Vazirzadeh et al., 2008).

Our recent work on brown trout showed, two injections of 20µg/kg GnRHa in combination with 10mg/kg DA antagonist domperidone or metoclopramide spaced 4 days apart, have been synchronized ovulation successfully in the Caspian brown trout (Salmo trutta caspius) within 1 to 2 weeks after treatment. Another hormone which is wildly used is hCG and are preferred over GnRHa by several scientices. The advantage of hCG is that it acts directly at the level of the gonad and does not require the activation of the pituitary gonadotropes or existence of LH stores. Occasionally, GnRHa is not effective or requires a long time to elicit a response. Therefore, hGC may be more appropriate because it acts much faster, via direct gonadal stimulation, in inducing FOM, spermiation and spawning (Hodson and Sullivan, 1993; Zohar and Mylonas, 2001).

A single injection of hCG at 1000 or 2000 IU/kg was effective in inducing OM and ovulation in the spotted sea bass (*Lateolabrax maculatus*) (Lee and Yang, 2002) and in Japanese catfish (*Silurus asotus*), a single injection of 10,000 IU/kg hCG induced OM and ovulation (Kumakura et al., 2003), in pike perch (Sander Luciperca) single or multiple injections of 200 IU/kg hCG (Zakes and Szczepkowski, 2004), and in catfish (*Pseudoplatystoma fasciatum*), treatment with CPE and hCG were both effective in inducing OM and ovulation (Leonardo et al., 2004). In fishes which migrate to the river for spawning, actually are at final stage of naturatiom, therefore no necessary any hormonal treatment. An example are *Caspoan Kutum* (*Rutilus frisii kutum*) which will migrate to the river and are artificially spawn just by gentile forcing in the belly of male and female (Yousefian and Mousavi, 2008).

CONCLUSION

The number of fish species currently under domestication efforts is rising up, due to the development of commercial aquaculture. For domestication and establish a sustainable aquaculture it is necessary to stimulate the fish and control reproduction processes of fish in captivity to acquire high quality seed. Different species of fish have their own physiological and characteristics in reproduction. Therefore it is suggested for different fish useing separate protocol of hormines and for new species to have pre-experiment to illustrate the exact dosage and hormonal combination.

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