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Review Article

CONTROL MECHANISM FOR INDUCED SPAWNING IN FISH REPRODUCTION

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ABSTRACT – In fish reproduction is predominantly a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanism. The act of breeding occur under optimal environmental conditions that are favourable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to brain-that triggers endogenous mechanism into action. Endogenous mechanism is mediated through cascade of various neurotransmitters and hormones secreted by tissues of brain-hypothalamus-pituitary-gonadal axis. The secretions of above axis is regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors. The most important reproductive neuro-hormones are hypothalamic gonadotropin-releasing hormones (GnRH) and gonadotropin-release-inhibiting factors (=hormones) (GRIF or GnRIH) that regulate secretions of pituitary gonadotropin hormones (GtH) which in turn, regulate the synthesis of gonadal steroids responsible for final maturation of gametes. An appropriate environmental stimulus may signal the arrival of optimal conditions for the fry, triggering spawning i.e. spermiation and ovulation. Fish in captivity may not always reproduce at the most favourable time. In this situation, hormones play a critical role in the reproductive processes. Hormone-induced spawning techniques influence this sequential mechanism at several steps, either by promoting or inhibiting the process. Induced reproduction in fish includes two main strategies. The first is the manipulation of culture environment to mimic important characteristics of natural spawning environment of that particular fish. The second strategy is the administration of one or more naturally occurring reproductive hormone or their synthetic analogs in brood fish through injection or dietary methods. Both these strategies are commonly used, sometimes in conjunction with one another. Numerous hormones have been used to induce reproduction. However, recent researches and commercial aquaculture practices suggest the emergence of two lines of hormone-induced spawning as the best for successful breeding at the least expense. These are i) injection or oral administration of GnRH analog (LHRH analog) with dopamine antagonist, and ii) injection of purified gonadotropin (e.g. human chorionic gonadotropin-HCG) either alone or mixed with common carp pituitary extract to improve its potency. In spite of these, application of steroids, pheromones and prostaglandins were also discussed, as a new emerging and less studied field and required more research before its commercial application to achieve captive spawning in majority of cultured fishes.

Key words **:** Induced spawning, environmental factors, exogenous induction endogenous induction, gonadotropin, pituitary extracts, human chorionic gonadotropin, luteinizing hormone releasing hormone (LHRH), LHRH analogs, dopamine , dopamine antagonists, steroids, pheromones, prostaglandins, spawning, ovulation, spermiation, teleost fish.

INTRODUCTION

In teleosts, reproduction is controlled by a cascade of environmental, endocrine and social factors (Dabrowski *et al*, 1996). Most species of teleost fishes are seasonal breeders that exhibit circannual variation in time of breeding. Fish integrate their reproductive activities with seasonal environmental cycles and signals from environmental cues (such as photoperiod, temperature and rain fall etc.) and endogenous physiological cycles input to the neuroendocrine system, which in turn, regulate pituitary and gonadal functions (Lin and Peter, 1996). Thus, reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action resulting spawning (Fig.1). Long before spawning, seasonal cues begin the process of gametes (eggs and sperms) maturation which depend on the fish species and strain size, culture systems and interrelated other environmental conditions. Spawning can be controlled by either placing the fish in an appropriate environment or by changing its internal regulating factors with injected hormones or other chemicals (Rottmann *et al*, 1991a; Thomas and Arnold, 1993; Carrillo *et al,* 1993; Patino, 1997). The internal mechanisms that regulate spawning are similar for most of the fishes while external environmental factors vary considerably among different species according to their habitat and distribution. So, more is known about internal regulatory mechanism of fish reproduction than species specific environmental requirements for spawning. Seasonal spawning limits seed-stock availability to only 2-3 months each year and

represents an important constraint to commercial aquaculture where supplies of fry and fingerlings are required throughout the year (Barnabe, 1994; Watanabe *et al*, 2006). It is impossible to create natural spawning environment in a hatchery for a particular species. In hatchery technology, hormone-induced spawning is the only reliable method for controlled breeding in fish like sturgeon, paddlefish, Indian major corps, Chinese carps, endangered mahseer, catfishes, salmon, seabass, redfish, snook and mullet. (Rottmann *et al*, 1991a; Lin and Peter 1996; Singh and Pandey, 2009; Mittelmark and Kapuscinski, 2010). In the present work an efforts have been made to review the environmental (exogenous) as well as endogenous (hormonal) manipulations in induction of spawning in teleost fishes.

Environmental (Exogenous) Induction of Spawning: It is now well established that variety of environmental factors that play a significant role in reproduction are photoperiod, temperature, water quality, flooding and water current, tides, lunar cycles, rainfall, spawning substrate, nutritional status, stress, disease and parasites, presence of other fish, pesticides/insecticides in the environment (Lam, 1983; Rottmann *et al*, 1991a; Carrillo *et al*, 1993; Dabrowski *et al*, 1996; Patino 1997; Davies *et al*,1999; Pottinger 1999; Izquierdo *et al*,2001; Hotta *et al*, 2001; Srivastava *et al*, 2004; Watanabe *et al*, 2006). Suitable environmental conditions stimulate the reproductive activities while unsuitable ones neglect spawning attempts. Therefore, such influences have attracted the attention of a number of fish culturists to modify the speed of maturation and spawning time of commercially important species in particular for the production of eggs, fry and fingerlings on demand throughout the year (Barnabe, 1994; Carrillo *et al,* 1995; Bromage, 1995; Kolkovski and Dabrowski, 1998; Hotta *et al*, 2001; Watanabe *et al*, 2001, 2006). Current evidence indicates that seasonal photoperiod is primary determinant of seasonal spawning in intensively farmed fish species (Lam, 1983; Bromage *et al*, 1993; Nagahama *et al*., 1993; Davies *et al*., 1999; Bromage *et al*., 2001) while water temperature plays a secondary role in controlling specific timing of final oocyte maturation and ovulation (Bye, 1990; Carrillo *et al*, 1991, 1993; Davies and Bromage, 1991; Tveiten and Johnsen, 1999). El-Sayed and Kawanna (2007) concluded that in Nile tilapia 12 L: 12D photoperiod (a near – natural day length photoperiod) should be adopted for maximum fecundity, seed production and spawning frequencies. However, highest seed production and spawning performance were noticed at longer day length (18L:6D) in Nile tilapia (Ridha and Cruz, 2000; Campos-Mendoza *et al*, 2004). Artificial manipulation of photothermal induction of spawning is indispensable to commercial production of seed-stock of a number of fish species (Bye, 1990; Bromage *et al*, 1993, 2001; Barnabe, 1994; Kolkovski and Dabrowski, 1998; Watanabe and Carroll, 2001; Watanabe *et al*, 2006). Photothermal manipulations have also been used by author to accelerate gonadal recrudescence and to prolong spawning in fresh water murrel *Channa punctatus* (Srivastava and Singh 1991, 1992a). In cultured common carp (*Cyprinus carpio*) adding males and floating vegetation as a spawning substrate (causes 10 fold GtH-II increase) to the holding pond in the evening usually results in spontaneous ovulation and spawning following morning (Lin and Peter, 1996). Combinations of environmental phase shifts and hormone treatment have been successfully used to accelerate recrudescence and spawning or to stagger spawning activity throughout the year in some species such as *Heteropneustes fossilis* (Alok *et al*, 1994, 1998) and gilthead seabream (Zohar *et al*, 1995). Techniques have been developed in recent years for hormone induced spawning (Lin and Peter, 1990, 1996; Peter *et al*, 1991, 1993; Carrillo *et al*, 1993; Thomas and Arnold, 1993; Alok *et al*, 1994, 1998; Berlinsky *et al*., 1996; Smith *et al*, 1999; Patino 1997; Zohar and Mylonas, 2001; Mittelmark and Kapuscinski, 2010) and natural spawning (Lam, 1983; Bye, 1990; Bromage, 1995; Dabrowski *et al*, 1996; Ciereszko *et al*, 1997a; Patino, 1997; Davies *et al*, 1999; Bromage *et al*, 2001; Watanabe *et al*, 2001, 2006). The results of studies in southern flounder demonstrated that photothermal and hormone-induced manipulations enabled year-round (including off-season) spawning of viable eggs to improve the availability of seed-stock for commercial aquaculturists (Watanabe *et al*, 2006). Further, stimulated photothermal conditions induced gonadal maturity and spawning in southern flounder under winter conditions without hormone induction (Watanabe *et al*, 2001). In rainbow trout, there was no difference in quality of gametes under advanced or delayed spawning from that achieved under ambient conditions (Bromage *et al*, 1992).

Internal (endogenous) Induction of Spawning: Environmental cues influence the internal mechanisms of reproduction through the brain-hypothalamus-pituitary-gonadal axis (Rottmann *et al*, 1991a; Peter *et al*, 1991; Singh, 1992; Srivastava and Singh, 1992b, 1993; Lin and Peter, 1996; Davies *et al*,1999; Chaturvedi, 2008; Singh and Pandey, 2009). Endogenous mechanism of reproduction is a complex phenomenon of hormonal cascade involving tissues of hypothalamus-pituitary-gonadal axis (Fig.1). However, details of its regulatory mechanisms are yet obscure and incomplete (Goos *et*

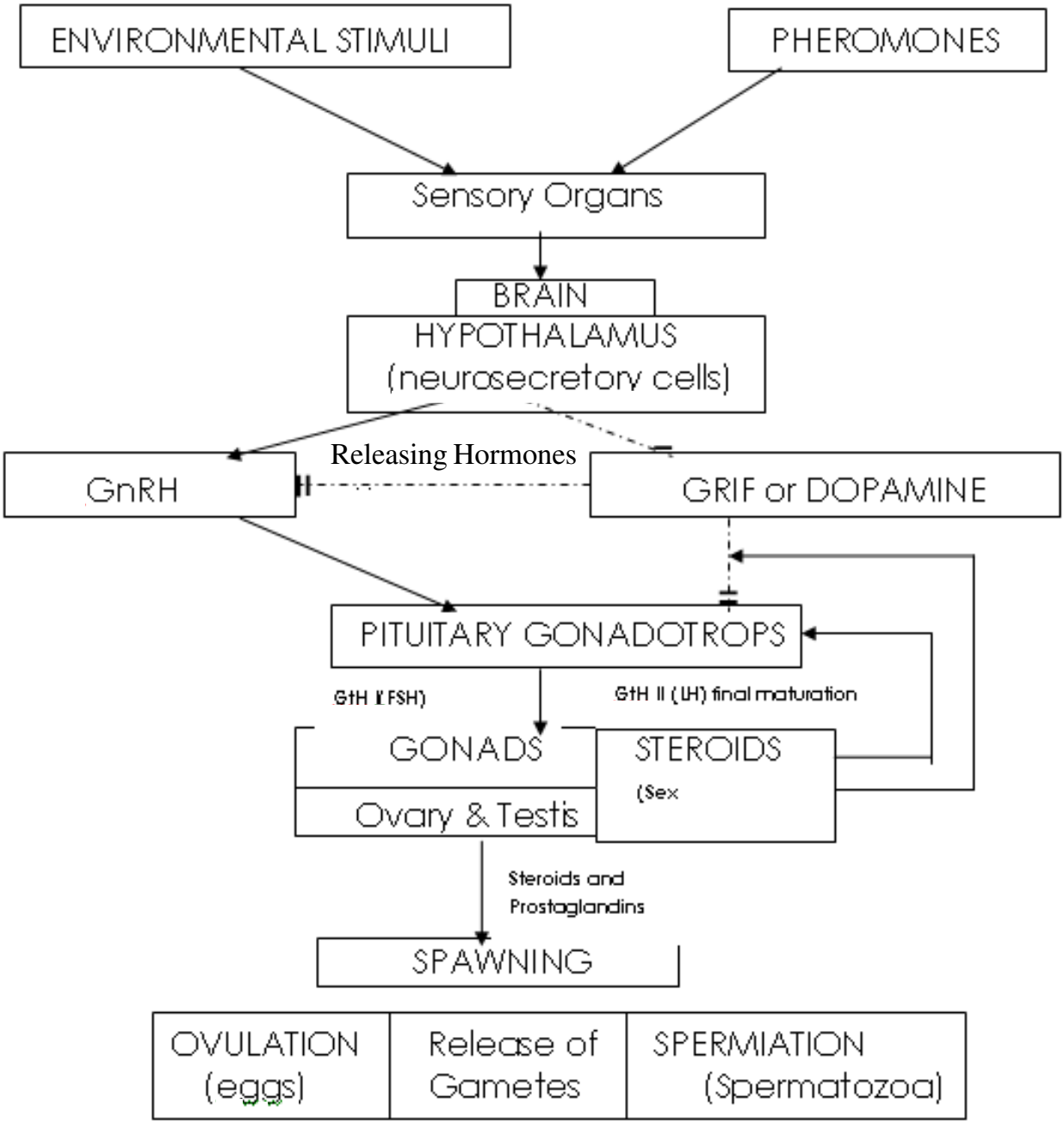

Fig. 1: Natural reproductive mechanism in fishes: (modified from Lin and Peter, 1996). A continuous line with arrow indicates a stimulatory effect; a discrete line with a bar indicates an inhibitory effect; Abbreviations: GnRH- Gonadotropin releasing hormone; GRIF - Gonadotropin release inhibitory factor; GtH-I– Gonadotropin-I; GtH-II – Gonadotropin-II; FSH – Follicle Stimulating Hormone; LH – Luteinizing Hormone.

al,1999; Melamed and Sherwood, 2005). Environmental stimuli are detected by variety of sensory receptors including eye, pineal gland, olfactory organs, taste buds and thermoreceptors etc. and translated by brain and stimuli of reproductive importance are routed to hypothalamus of the brain (Fig.1). Hypothalamic neurosecretory cells (NPO, NLT) and several fibers produce gonadotropin releasing hormone (GnRH) and also gonadotropin release-inhibiting factors (GRIF) (Kah *et al*, 1993; Sherwood and Hew, 1994; Peter and Yu 1997; Subhedar *et al*, 1999). Hypothalamus also contains specific hormone receptors which regulate hormonal activity through feedback mechanism (Peter *et al,* 1991; Goos *et al,* 1999; Melamed and Sherwood, 2005). Experimental evidences suggest that in some teleosts dopamine acts as GRIF by inhibiting GnRH-induced GtH(LH) release (Peter *et al*, 1991; Trudeau and Peter, 1995). GnRH stimulates the secretion of gonadotropin hormones (GtH) release from the teleostean pituitary and GRIF antagonizes GnRH effect by inhibiting the release of GtH

(Barnabe, 1994; Trudeau and Peter, 1995; Prasada Rao, 1999; Goos *et al*, 1999). GtH are a central component of the brain-pituitary-gonadal axis. GtH act on the ovaries and testes which synthesize steroids responsible for final maturation of gametes (Peter and Yu 1997; Davies *et al,* 1999; Goos *et al*, 1999; Singh and Pandey, 2009; Mittelmark and Kapuscinski, 2010). Most of fishes have two types of gonadotropins *i.e.* GtH I and GtH II also referred as follicle stimulating hormone (FSH) and luteinizing hormone (LH) respectively (Querat, 1994; Schulz *et al*,1995. Davies *et al*, 1999). GtH I is associated mainly with early maturation and vitellogenesis while GtH II has a principal role in final maturation and ovulation/ spawning (Swanson, 1991; Schulz *et al,* 1995; Lin and Peter, 1996; Breton *et al*, 1998; Singh and Pandey, 2009; Mittelmark and Kapuscinski, 2010). Steroids and prostaglandins appear to be local ovarian mediators of GtH action causing release of ripe eggs (Goetz *et al*, 1989; Goos *et al*, 1999). Generally, a preovulatory surge of GtH-II is responsible for ovulation and spawning (Lin and Peter, 1996). Elevated blood level of GtH II (LH) trigger i) final maturation of eggs stimulated by progesterone and ii) rupture of follicle layer (ovulation) which is evidently stimulated by prostaglandins (Rottmann *et al*, 1991a). Steroids also appear to induce spermiation in male (Stacey, 1984; Jones, 1987; Rottmann, *et al*, 1991a). Different steroid hormones regulate gonadotropin synthesis, storage and release through feedback mechanism via hypothalamo-hypophysial axis but it is difficult to explain gonadotropic function of pituitary at every stage of sexual cycle (Goos *et al*, 1999).

Substances used for Hormone Induced Spawning: Hormone induced spawning influence reproductive mechanism at several levels, either by promoting or inhibiting the process. The primary substances used for hormone induced spawning are reviewed by many workers (Donaldson and Hunter, 1983; Sherwood, 1987; Jones, 1987; Marte, 1989; Lin and Peter, 1990, 1996; Rottmann *et al*, 1991a; Redding and Patino, 1993; Kime, 1993; Patino, 1997; Francis *et al*, 2000; Naruepon *et al*, 2000; Singh and Pandey, 2009; Mittelmark and Kapuscinski, 2010).

i) Pituitary extracts, human chorionic gonadotropin (HCG) and pituitary gonadotropins regulate gonadal activities.

ii) GnRH (LHRH), LHRH analogs (LHRHa) alone or in combination with dopamine blockers (antagonists) which enhance the potency of LHRHa to stimulate the pituitary secretions.

iii) Steroids to stimulate the gametes directly.

iv) Reproductive pheromones and prostaglandins evidently play a biological role in fish ovulation/ spawning (Jones, 1987).

There are so many variables which impact the ability of injected hormones to induce spawning like readiness of brood fish, age and physiological condition of the brood fish, seasonal variations, environmental factors, source, age and maturity of donor fish, size of fish, previous spawning history, water temperature, spawning substrates etc. (Rottmann *et al*, 1991a; Bhuiyan *et al*, 2006).

Hormone-Induced Spawning: Intensive fish culture systems usually require more reliable methods of induced spawning than original hypophysation technique. This generated considerable research to identify alternative hormonal (chemical) treatments. Traditional methods of induced spawning (injection of GtH-II) include crude extract of carp pituitary, partially purified fish GtH-II and mammalian GtH, especially human chorionic gonadotropin – HCG (Donaldson and Hunter 1983; Peter *et al*, 1988). More recently, GnRH or LHRH and LHRHa are among the preparations that have been tested for induced spawning (Patino, 1997). However, Lin and Peter (1996) suggested a number of problems in traditional technique; i) high species specificity of GtH-II; ii) use of crude pituitary extracts along with a mixture of hormones have a side effect on gametogenesis or other functions; iii) partially purified GtH-II and HCG is highly expensive; iv) use of exogenous GtH-II express immune response which in turn, causes refractoriness to the hormones; v) carp pituitary extracts and HCG are highly variable in potency and have a short storage life.

Pituitary Extract : It contains Gonadotropin hormones which play a major role in spawning (ovulation and spermiation). Injected pituitary material omit the hypothalamus-pituitary link and stimulate ovary and testis directly, resulting spawning (Rottmann *et al*, 1991a; Rothbard and Yaron, 1995). Mode of action of injected pituitary extract, purified gonadotropin (HCG) and LHRHa is shown in Fig. (2). For fresh pituitary extract gravid fish of same species which is to be spawned, is sacrificed due to more hormone content in brood fish, but it is unfortunate and uneconomical. Fresh pituitary glands should be used immediately or preserved either by freezing or acetone-drying for next 5 to 8 years use (Rottmann *et al*; 1991a). Now common carp pituitary or salmon pituitary extracts are available commercially. They are widely used for induced spawning (Zohar, 1989; Marte 1989; Rottmann *et al,* 1991a; Haque and Ahmed 1991; Bromage 1995; Rothbard and Yaron, 1995; Kohinoor *et al,* 1995; Basu *et al.*\, 2000; Szabo *et al,* 2002; Dorafshan *et al,* 2003, Mahmood, 2003; Bhuiyan *et al,* 2006; Rokade *et al,* 2006;

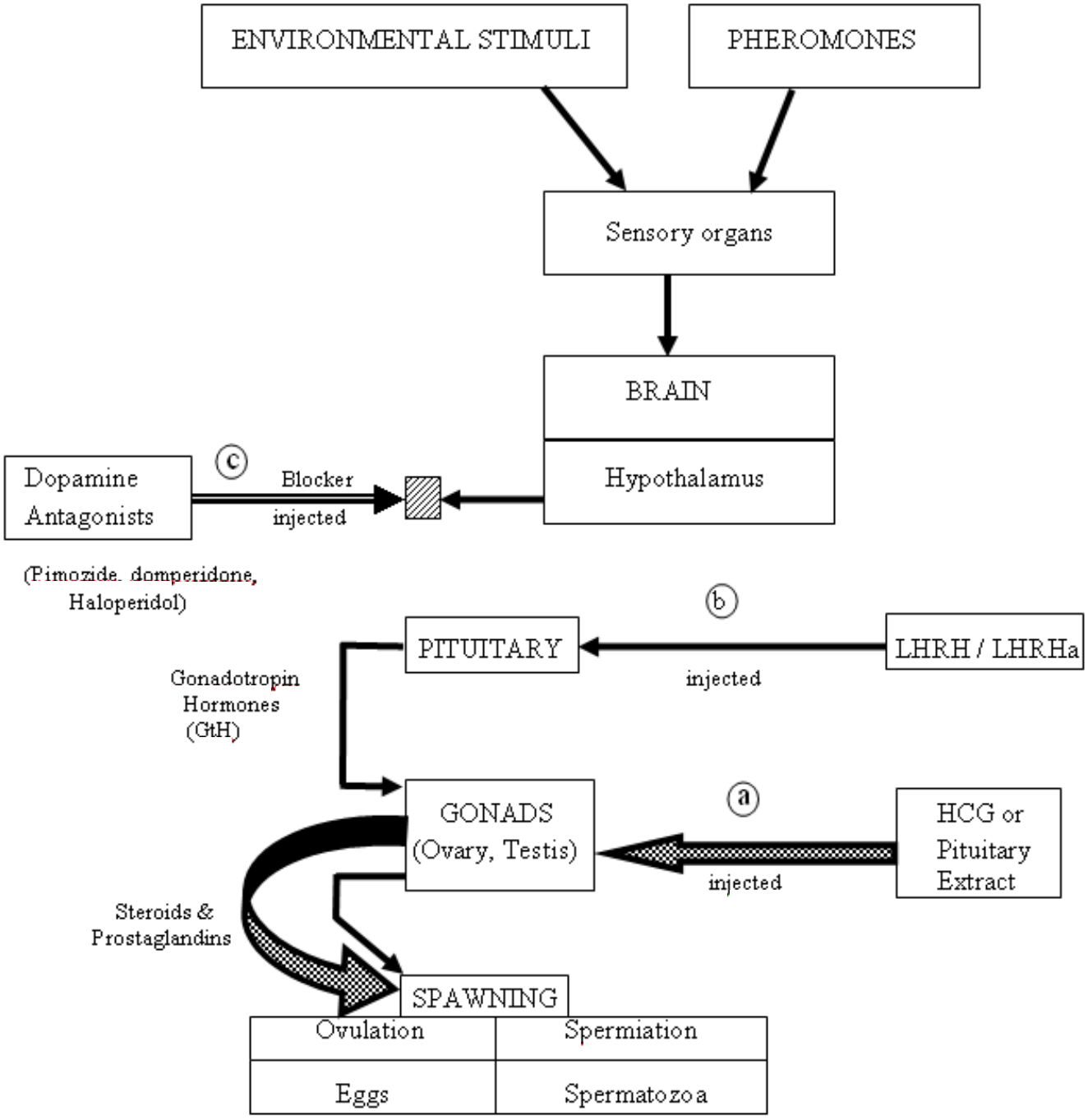

Fig. 2 : Hormone-induced spawning cascade in fishes: a Pituitary extract and HCG (Human Chorionic purified Gonadotropin); b. Synthetic LHRH (Leutinizing Hormone Releasing Hormone) & LHRH analogs (LHRHa); c. Dopamine Antagonists (blockers). (modified from Rottmann *et al*., 1991)

Musa and Bhuiyan, 2008). There is greater chance of successful induced spawning, if donor species is closely related to the recipient fish. Therefore, pituitary extracts from carp is used for successful induced spawning in carp, Thai silver carp, goldfish, Chinese carp, catfish etc. Likewise, salmon pituitary extracts is used in case of salmon and trout. However, both are effective for a wide variety of fishes (Mittelmark and Kapuscinski, 2010). Recently, pituitary gland extract of carp, *Labeo rohita* were used for induced breeding of Gulsha tengra, *Mystus bleekeri* (Day) brooders to determine optimum dose for 100% ovulation and that was 1.1 mg PGE/100 gm. body weight, also responding for maximum fertilization rate (76 ± 5.32%) and hatching rate of egg (79 ± 4.68%) at an ambient water temperature of 250C to 280C (Musa and Bhuiyan, 2008). In fishes, just prior to spawning greatest

hormone content occur and lowest in immature fish and mature fish after spawning (Goos *et al,* 1999). Decrease in potency of commercial pituitary material were noticed by improper collection, processing or storage (Rottmann *et al*, 1991a).

***Human Chorionic Gonadotropin* (HCG) :** It is the most common purified gonadotropin hormone used for effective induced spawning and it mimics the natural pituitary GtH. HCG is widely used for induced spawning in catfish, Chinese carps, Indian major carps, mullet, white bass, red drum, striped bass, other cyprinids, groupers, milk fish, rabbit fish, sea boss etc (Marte 1989; Patino 1997; Basu *et al*, 2000; Haniffa and Sridhar 2002). HCG bypasses the brain-pituitary link, act directly on gonads (Rottmann *et al*, 1991a; Patino, 1997). HCG can cause immunoreactions in the recipient fish and is not effective for all species and seems to be fairly species-specific (see reviews, Marte, 1989; Patino 1997). To avoid this problem, HCG is used in combination with common carp pituitary that exhibit improved potency for spawning (Fig. 2). The solution of two hormones either injected separately or by mixing (Rottmann *et al*, 1991a). HCG offers three major advantages over the pituitary extract: i) it is less expensive ii) it is more stable and thus has a longer shelf life, and iii) it is in purified form (Mittelmark and Kapuscinski, 2010).

***GnRH or LHRH and LHRHa* :** Injections of mammalian Luteinizing hormone releasing hormone (LHRH) have been used experimentally to mimic the GnRH of fish. GnRH (a decapeptide) does not seem to elicit immune response in recipient fish and also has broader species spectrum of effectiveness than GtH. Therefore, GnRH treatment is rapidly becoming a favourite chemical method to induce spawning in fishes (Zohar, 1989;Halder *et al*, 1991; Carrillo *et al,* 1993, 1995; Bromage, 1995; Patino, 1997; Ghosh *et al*, 1999; Eenennaam *et al*, 2008; Singh and Pandey, 2009). However, frequent injections and a comparatively large dose were required (Rottmann *et al*, 1991a). Use of murrel GnRH for successful maturation and spawning have been reported in *Anabas* (Halder *et al*, 1991; Ghosh *et al*, 1999). All brood fish ovulated or spermiated, the best treatment was reported when GnRHa injected alone and Domperidone (a dopamine blocker) was not required for successful ovulation in green sturgeon (Eenennaam *et al*, 2008). Recently, synthetic LHRH analogs (LHRHa or GnRHa) have been manufactured. LHRHa was successfully used to induce spawning in many teleost fish (Crim, *et al*, 1987; Marte 1989; Redding and Patino, 1993). The success rate of spawning by using GnRH analogs have been reported 100% in carps & Groupers, 99.7% in mullet, 99% in milkfish but less than 50% in catfishes. (See review Marte 1989). LHRHa induced ovulation in rainbow trout at 12°C by 1st injection occurred 4 weeks in advance of controls & delayed ovulation at 18°C occurred by 2nd injection after 25 days (Pankhurst *et al,* 1996; Pankhurst and Thomas, 1998). Ovulation in other salmonids were also advanced by 10-30 days following LHRHa administration (Sower *et al,* 1984; Crim and Glebe, 1984; Van Der Kraak *et al,* 1985, 1988; Crim *et al,* 1986; Slater *et al,* 1995). It is highly effective in inducing ovulation in yellow perch (Rinchard *et al*, 2002). These hormones last longer in the system of fish and have potent stimulatory effects on ovulation and spermiation (Fig. 2). Therefore, only one or two small doses are needed to induce spawning (Rottmann *et al,* 1991a; Lin and Peter, 1996). A major drawback of multiple hormone injections or high dose is that some species are unable to cope with stress (handling and severity of treatment) causing gonadal regression and in some cases, even death of broodstock (Rinchard *et al*, 2002; Bhattacharya and Homechaudhuri, 2009). So, dietary administration of GnRHa has been used successfully to induce spawning in spotted sea trout, common carp, rainbow trout, African catfish (Thomas and Boyd, 1989; Breton *et al*, 1995., Lin and Peter, 1996; Patino, 1997). LHRHa stimulates the fish own pituitary to produce and release GtH necessary for spawning. LHRHa has its wide spectrum uses in fishes (Crim *et al*, 1987; Redding and Patino, 1993; Dabrowski *et al*, 1994; Ciereszko *et al*, 1997a; Rinchard *et al*, 2002; Dorafshan *et al*, 2002; Szabo *et al*, 2002). One of the synthetic analogs that has been used successfully is Des-GLY10, [D-Ala6] – LH-RH Ethylamide (Rottmann *et al*, 1991a; Lin and Peter, 1996; Patino, 1997).

LHRHa and Dopamine Blockers : LHRHa (GnRHa) is not species specific but it alone is unable to induce their full spawning potential in cyprinids and some other fishes (rainbow shark, goldfish, red-tailed black shark, yellow perch) due to endogenous GRIF (dopaminergic substances) that inhibit GtH release from pituitary. In these species GnRHa normally applied with combination of dopamine antagonists, like domperidone (DOM), metoclopramide, pimozide (PIM) and haloperidol (HAL), reserpine (Manickam and Joy, 1989; Marte 1989; Rottmann *et al*, 1991a; Peter *et al*, 1993; Naruepon *et al*, 2000; Arabaci *et al*, 2001; Szabo *et al*, 2002; Dorafshan *et al*, 2003; Paykan Heyrati *et al*, 2007; Eenennaam *et al*, 2008; Marimuthu *et al*, 2009). Several commercially available synthetic ovulating agents in readymade form containing GnRHa and Dopamine antagonist like Ovaprim, Ovopel, Dagin, Aquaspawn and Ovatide are becoming

very popular and found to be efficient and successful spawning agents in different fish species (Peter *et al*, 1988; Horvath *et al*, 1997; Cheah and Lee, 2000; Marimuthu *et al*, 2000, 2007, 2009; Brzuska 2001, 2003, 2006; Haniffa and Sridhar, 2002; Das 2004; Sahoo *et al*, 2005; Rokade *et al*, 2006; Bhattacharya and Homechaudhuri 2009; Chowdhury *et al*, 2010). Ovatide (GnRHa + Dopamine antagonist), a new ovulating agent has successfully been tested by Central Institute of Fisheries Education (ICAR), Mumbai including other part of India since 1997. By using Ovatide and its analogs successful complete spawning has been reported in several fish species like several cyprinids (Horvath *et al*, 1997; Thakur and Reddy, 1997) catfishes (Cheah and Lee 2000; Marimuthu *et al*, 2000; Mukherjee and Das 2001; Sahoo *et al*, 2005), murrels (Haniffa *et al*, 1996; Marimuthu *et al*, 2007, 2009). Low and higher doses of drugs are reported to affect the egg quality, lead to partial spawning or reduced fertilization and hatching rate (Sahoo *et al*, 2005; Marimuthu *et al*, 2009; Bhattacharya and Homechaudhuri, 2009). About 91% fertilization and hatching rate were reported in *Channa punctatus* by using Ovatide at the dose of 0.4ml/Kg BW (Marimuthu *et al*, 2009). In those fishes where dopamine inhibitory tone is strong, higher dose of LHRHa/ GnRHa + antagonist was required (Nandeesha *et al*, 1990; Szabo *et al*, 2002; Paykan Heyrati *et al*, 2007; Bhattacharya and Homechaudhuri 2009). Ovatide is less expensive, easy to store, simple to use and has lower viscosity as compared to Ovaprim and other hormones used for commercial fish breeding in aquaculture farms by hatchery operators and seed producers (Marimuthu *et al*, 2009). Further, the synthetic hormones like Ovaprim and Ovatide act at the pituitary level leading to the secretion of fish's own endogenous gonadotropins, while in case of hypophysation technique and administration of HCG exogenous gonadotropins, they are directly delivered into the body (Mittelmark and Kapuscinski 2010).

The combination of a GnRHa and a dopamine antagonist for induced ovulation and spawning in cultured fish is a highly effective procedure called the 'Linpe Method' (Peter *et al,* 1988). Dopamine blocks spontaneous release of GtH and also blocks or modulates the actions of GnRH (Peter, 1982) as supported by *in vitro* experiments (Chang *et al*, 1984). The inhibitory action of dopamine on GtH secretion can vary in potency between species (Lin and Peter 1996; Szabo *et al*, 2002). In goldfish, carps and catfish dopamine inhibition is very strong, so injection of a dopamine blocker (antagonists) potentiate the action of LHRHa, leading to a large release of GtH II (LH) and ovulation results. However, in bream, sciaenid and loach, dopamine inhibition is weak, so injection of a high dose of LHRHa (superactive GnRH- analogs) alone is effective in stimulation of GtH II release and ovulation (Lin and Peter, 1996; Patino, 1997). Superactive GnRHa are far more effective because they are purer, resistant to enzymatic degradation by the pituitary, kidney and liver and have prolonged biological half-lives in circulation (Zohar *et al,* 1995; Lin and Peter, 1996; Patino, 1997; Prasada Rao,1999; Mittelmark and Kapuscinski 2010). When LHRHa and dopamine antagonist are used in conjunction, reproductive success dramatically increases (Mittelmark and Kapuscinski, 2010). However, in green sturgeon Domperidone was not required for successful ovulation and the best treatment was GnRHa injection alone (Goswami and Sharma 1997; Eenennaam *et al*, 2008).

Experimental results indicate that the use of dopamine blockers prevent this negative feedback, enhancing the effectiveness of LHRHa for those species which respond strong dopamine inhibition (Rottmann *et al*, 1991a; Peter *et al*, 1993; Lin and Peter, 1996; Paykan Heyrati *et al*, 2007; Mittelmark and Kapuscinski, 2010). The 'Linpe Method' is rapidly gaining wide acceptance for induced breeding in fish farms in China and several other countries including India (Halder *et al*, 1991; Lin and Peter, 1996). The use of murrel GnRH in the 'Linpe Method' with Ca++ is satisfactory for induced breeding and final maturation of *Anabas* (Halder *et al*, 1991. The dopamine antagonists (trade name Ovaprim, Syndel Laboratories Ltd., Canada) spawning kit is especially formulated for use with salmonids, cyprinids and other cultured fresh water fish like catla, rohu, mrigal, fringe-lipped carp, silver carp, bighead carp and grass carp in various fish farms located in different agro-climatic regions of the world. According to several reports Ovaprim was the most potent in induced breeding of fish (Nandeesha *et al*, 1993; Francis *et al*, 2000; Haniffa and Sridhar 2002; Bhattacharya and Homechaudhuri 2009) but not quite successful in catfishes (Basu *et al*, 2000; Chowdhuri *et al*, 2010). Use of LHRHa/GnRHa coupled with dopamine antagonist and gonadotropin offer best chance for success at least expense. The use of ovaprim is advantageous (Nandeesha *et al*, 1991; Mittelmark and Kapuscinski 2010). In trials on fish farms, the percentage of spawning success, the number of eggs obtained per Kg. body weight of brooders, the fertilization rate and hatching percentage remain consistently higher with Ovaprim or other hormonal drugs as compared to carp pituitary extracts or HCG treatment in almost all instances (Manickam and Joy, 1989; Lin and Peter, 1990, 1996; Francis *et al*, 2000; Basu *et al*, 2000; Mittelmark and Kapuscinski, 2010). More recently,

several studies demonstrated the feasibility of using the oral or dietary route to deliver hormone and catecholaminergic drugs to fish for inducing GtH-II release, ovulation and spawning. Oral administration of superactive analogs of GnRHa as well as dopamine antagonists in diet or by intubation, have potential value in aquaculture as a reliable method of inducing spawning in stress-susceptible, or in small ornamental fishes which cannot be injected easily (See reviews, Breton *et al*, 1995; Lin and Peter, 1996; Patino, 1997). Although higher doses of GnRH analog or drugs may be required to induce spawning by oral administration than by injection, but its increased costs could be offset by labour savings (Lin and Peter, 1996). Thus oral/dietary administration of drugs is economical, reliable, less stress causing and check mortality in brood fishes. Reducing stress and injury to brood fish following hormone treatment can increase the success of hormone-induced spawning (Rottmann *et al*, 1991 b).

Steroids, Reproductive Pheromones and Prostaglandins : Fish commonly use reproductive hormones (steroids and prostaglandins) both as endogenous signals between reproductive tract and brain and as exogenous signals (hormonal pheromones) that sychronize gamete maturation and/or spawning interactions between and among conspecies (stacey, 2003).

Steroids : Steroids like progesterone, estradiol and testosterone have been tested experimentally for inducing maturation and spawning (ovulation, spermiation) in various fish species (Goetz 1979, 1983; Fostier *et al,* 1983; Kime, 1993; Borg, 1994; Singh and Pandey 2009). However, steroid treatments is little popular for hormone-induced spawning and less studied (See reviews Goetz, 1983; Jones, 1987; Rottmann *et al*, 1991a; Lin and Peter, 1996; Prasada Rao, 1999). Stress associated high mortality was observed in fish injected with LHRHa + 17,20βP steroid for induced spawning in yellow perch (Rinchard *et al.,* 2002) indicate that this treatment was severe. Hormonal treatment studies indicated that 17, 20βP is conjugated in yellow perch and the sulphated steroids may act as a spawning pheromones as reported in goldfish (Sorensen *et al*, 1995). In spotted sea trout identification of two receptors in ovary suggested that both steroids may play distinct role in final maturation and ovulation processes in this species (Patino and Thomas, 1990; Pinter and Thomas, 1995). In male teleost fish both testosterone and 11-ketotestosterone regulate spermatogenesis and spermiation (Ciereszko *et al*, 1997 b;Rinchard *et al,* 2002).

Pheromones : Pheromone is another interesting but largely untested means for chemical control of maturation and spawning in fishes (See review, Patino, 1997; Stacey 2003; Singh and Pandey 2009; Singh and Nautiyal 2009). It is well documented that certain gonadal hormones and/or their metabolites (prostaglandins and C_{18}, C_{19}, C_{21} steroids) act as sex pheromones in some freshwater teleosts (Sorensen and Goetz 1993; Scott and Vermeirssen 1994; Stacey *et al*, 1994; Stacey and Cardwell, 1995) Teleost gonads produce conjugated steroids that play a significant role as pheromones (Scott and Vermeirssen 1994). An increased sensitivity to 15K-PGF sex pheromones during breeding phase promote spawning success in male *Barilius bendelisis* (Bhatt *et al*, 2002) and also in other teleosts (Sorensen and Goetz 1993). However, olfactory sensitivity of steroidal pheromones is confined to the prespawning period Bhatt *et al*, 2002) The steroid DHP (17α, 20β- dihydroxy-4-pregnen-3-one) produced by female goldfish, controls not only the onset of ovarian maturation in females but also spermiation in males. By adding in water, this steroid could be used to control the timing or amount of sperm production. Indeed, this procedure has been tested to confirm the feasibility to increase male fertility in common carp and goldfish (Zheng *et al*, 1995). However, more research is necessary before its commercial application in aquaculture practices.

Prostaglandins : It evidently play a critical biological role in stimulating fish ovulation, rupture of follicle and expulsion of mature oocytes (Goetz, 1983; Stacey, 1984; Jones, 1987; Goetz *et al*, 1989, 1991; Mittelmark and Kapuscinski 2010). Pheromonal and reproductive function of F prostaglandins and their metabolites have been reported in teleost fish (Sorensen and Goetz, 1993). *In vitro* studies 17, 20βP steroid induces ovulation through production of prostaglandin in yellow perch (Goetz, 1979, 1997; Berndtson *et al*, 1989; Goetz and Garezynski, 1997). Prostaglandins and free conjugated steroids are considered to act as pheromones in teleost fish (see reviews: Sorensen and Goetz, 1993; Scott and Vermeirssen, 1994; Sorensen *et al*, 1995; Stacey and Cardwell, 1995; Dabrowski *et al*, 1996). However, more research is required before its commercial application to achieve captive spawning in vast majority of cultured fishes (Patino, 1997).

Conclusions : Reproduction in fishes is predominantly a periodic phenomenon regulated by interplay of a number of exogenous (photoperiod, temperature and rainfall etc.) and endogenous (hormonal) factors. Environmental stimuli received by sensory organs are directed to brain-hypothalamus that triggers internal mechanisms into action. Internal mechanisms control the reproductive activities including spawning through secretions of hypothalamo-hypophysial (pituitary)-gonadal axis. The

secretions of neurotransmitters and hormones of the above axis is regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors. Hormone-induced spawning techniques influence this sequential mechanism at several steps, either by promoting or inhibiting the concerning processes. Pituitary extracts, purified gonadotropins (e.g. HCG) induce gonads. Further, injection(s) or oral administration of LHRH analogs (LHRHa or GnRHa) alone or in combination with dopamine antagonists enhance the potency of LHRHa to stimulate the pituitary gonadotropins release hence, the spawning (ovulation and spermiation). Dietary feeding of LHRHa and dopamine antagonists save fish from stress and mortality and it is preferred, reliable and economical practice. Physiological and biochemical mechanisms of gonadal maturation and spawning through hormonal induction and its side effects in food chain are still poorly recognized and needs extensive and diversified research in vast varieties of aquaculture fishes. Further, available practical environmental methods (e.g. putting milk cans, presence of vegetation, changing photoperiod, increase in temperature in hatchery, rainfall, running water etc.) of induced spawning should be used together with chemical / hormonal (injection of goadotropin & LHRHa + dopamine antagonist) methods to achieve successful captive spawning in majority of fishes, so that reliable and continuous supply of fry and fingerlings could be obtained throughout the year. Induced spawning methods vary from species to species and situation to situation, so needs thorough evaluation through researches and practices and select those which best suit the circumstances. Further, after spawning the techniques of incubation and care for eggs, and care for hatched fry must be thoroughly evaluated through research and experimentation. However, adequate techniques of reproductive and broodstock management exist only for limited species and it needs extensive research and field trials in various aquaculture fishes for commercial and economical fish farming.

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