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Review Article

Hormone Application for Artificial Breeding Towards Sustainable Aquaculture – A Review

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ABSTRACT

Aquaculture has been the fastest-growing area of worldwide food production and is becoming a vital component of the global economy to feed the rising world population. Hence, directed toward continuing the current level of per head consumption, comprehensive aquaculture production needs to attain eighty million tonnes by 2050. However, some cultured marine fish species, such as salmonids, striped bass, and gilthead seabream, as well as freshwater fish, such as captive Mediterranean amberjack populations (*Seriola dumerili*) and Mekong River giant catfish (*Pangasianodon gigas*), exhibit reproductive dysfunction, especially in female brood stock when reared in captivity. Captive females face complications with unsynchronised ovulation, fail to undergo final oocyte maturation (FOM), and no longer spawn due to a lack of luteinising hormone (LH). Thus, artificial breeding has been widely used in aquaculture practices to increase cultured fish production. Farmer has extensively applied commercial hormones such as human chorionic gonadotropin (hCG), Ovaprim, Ovatide, and Ovaplant, through injection and implantation of hormones to stimulate breeding in many farmed fish species. However, artificial breeding is still in its development phase, and some methods are still unable to induce spawning in certain fish species. Different methods, doses, and delivery systems of artificial hormones could improve the efficiency and effectiveness of artificial breeding. This paper discusses the current research on artificial breeding in various fish species as well as new

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approaches or techniques to be applied in the area to regulate the reproductive process in captive fish for sustainable aquaculture.

Keywords: Artificial breeding, artificial hormones, fish reproduction, synthetic hormones, sustainable aquaculture

INTRODUCTION

For the past three decades, aquaculture has been the world's fastest-growing food production subsector, producing more than half of all the fish consumed directly by humans (Filipski & Belton, 2018). This industry contributes to human nutrition in ways that are essential for human health through its essential proteins and micronutrients, including calcium, vitamin A, vitamin B12, and omega-3 fatty acids (Carbone & Faggio, 2016; Jayasankar, 2018). With the continuous growth of the world's population, food and feed supply should rise by 70% by 2050 (de Bruijn et al., 2017). Consequently, the aquaculture supply is expanding due to the rising global demand for fish (Karim et al., 2020). Asian countries contribute approximately 89% to the worldwide aquaculture output, with China dominating (61.5% of the global aquaculture output), followed by India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, Chile, Myanmar, and Thailand (Ahmed & Thompson, 2019).

Fish culture is a significant industry, cultivating various types of marine and freshwater fish worldwide. Fish are often raised in confined areas, such as ponds or net cages, with efforts to maximise the production per unit area (Mehana et al., 2015). Domestication is the capacity of fish to regulate reproductive processes in captivity and obtain good quality seeds for commercialisation (Mylonas et al., 2010; Passini et al., 2019). Most captivity-raised fish experienced some form of reproductive failure. These dysfunctions are most likely due to a mix of captivity-induced stress and differences in social and environmental factors between wild and farmed fish. This combination of adaptations initiates the endocrine mechanisms that lead to final oocyte maturation (FOM) and ovulation (Zohar & Mylonas, 2001). Previous research linked impaired hormonal regulation at the hypothalamus-pituitary-gonadal (HPG) axis to poor reproductive success due to the lack of reproductive control, low sex hormone levels, slow or delayed gametogenesis, and small egg size (Milla et al., 2020). Females in captivity frequently failed to complete FOM, ovulation, and spawning, while males experienced decreases in sperm quantity or quality (Mylonas et al., 2007; Zohar & Mylonas, 2001). These problems have slowed aquaculture production, resulting in a shortage of seeds. Thus, artificial breeding is an excellent method for restocking wild populations and endangered species.

The HPG axis in fish regulates gametogenesis and final maturation (Mylonas et al., 2010). It begins with environmental stimuli (water rise, temperature, feeding, rainfall, and photoperiod) that induce the brain's release of gonadotropin-releasing hormones (GnRHs) (Figure 1) (Fakriadis et al., 2020). GnRHs stimulate gonadotropin

hormones (GTHs) in the anterior pituitary gland, namely, luteinising hormone (LH) and follicle-stimulating hormone (FSH). FSH regulates vitellogenesis and spermatogenesis in endocrine systems, while LH regulates FOM and spermiation (Mosha, 2018). Fish in captivity often lack environmental cues. Therefore, various hormones that promote fish reproduction should be applied to reduce reproductive failure in captivity (Mosha, 2018; Mylonas et al., 2010). In addition, this approach guarantees the availability of fish seeds throughout the year (Ochokwu et al., 2015). Given that many tropical fish species only reproduce once a year (Ochokwu et al., 2015), artificial breeding may also be one of the best solutions to meet the demand and could reduce the fish seeds that rely on the wild.

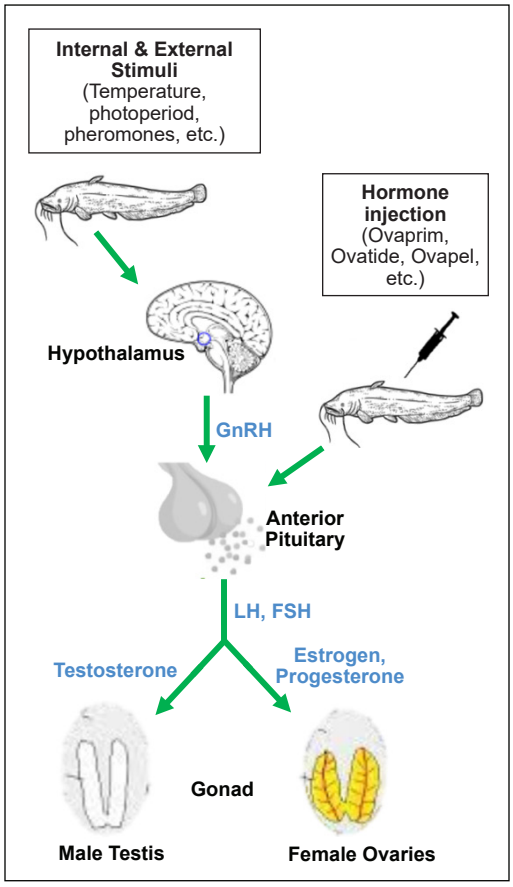

Figure 1. Schematic diagram of hypothalamus-pituitary-gonad (HPG) axis
Note. GnRH = Gonadotropin-hormone releasing hormone; LH = Luteinising hormone; FSH = Follicle stimulating hormone

The Development of Hormones for Artificial Breeding

The advancement of induced spawning techniques has enabled farmers to commercially breed valuable fish in captivity (Marimuthu, 2019). The administration of exogenous hormones, such as carp pituitary extract (CPE), human chorionic gonadotropin (hCG), luteinising hormone-releasing hormone analogue (LHRHa), and salmon gonadotropin-releasing hormone analogue (sGnRHa), is an alternative method of imitating environmental and hormonal factors to promote ovulation and egg maturation in fish for efficient seed production (Su et al., 2013). Several commercial ready-to-use synthetic hormones contained gonadotropin-releasing hormone analogue (GnRHa) and dopamine antagonists (domperidone, DOM), such as Ovaprim, Ovopel, Ovatide, Ovupin-L, Dagin, Aquaspawn, and Ovulin, are gaining popularity and being effectively used to stimulate reproduction (Kutwal et al., 2017; Marimuthu, 2019). However, it has been suggested that synthetic hormones alone or in combination are more cost-efficient and effective.

Carp Pituitary Extract (CPE). Carp pituitary extract (CPE) is the most frequently utilised hormone for inducing spawning in fish worldwide and is used by culturists, especially the common carp, major Indian carp, and Chinese carp (Kahkesh et al., 2010). However, obtaining CPE is difficult, time-consuming, and inefficient, as it requires sacrificing many brood stocks to collect many pituitaries with a low success rate (Kutwal et al., 2017; Zohar & Mylonas, 2001). Furthermore, CPE is unpredictable in terms of quality, has a low activity level in the HPG axis, and could transmit diseases from donor to recipient fish. These problems have contributed to the increasing usage of human chorionic gonadotropin (hCG) to induce fish breeding (Dunham et al., 2000).

Human Chorionic Gonadotropin (hCG). Human chorionic gonadotropin (hCG) is a gonadotropin hormone discovered in the blood and urine of pregnant women in 1927 by Aschheim and Zondex (Elakkanai et al., 2015). This hormone is distinguished by wide market availability and high chemical purity that ensures its effectiveness (El-Hawarry et al., 2016). Human chorionic gonadotropin has been used to promote FOM and successfully induced spawning in numerous catfish species, including *Clarias gariepinus*, *Clarias batrachus*, and *Heteropneustes fossilis* (Kahkesh et al., 2010; Kather Haniffa & Sridhar, 2002; Inyang & Hettiarachchi, 1994; Sahoo et al., 2007).

Luteinizing Hormone Releasing Hormone Analogue (LHRHa) or Salmon Gonadotropin-Releasing Hormone Analogue (sGnRHa). Luteinising hormone-releasing hormone (LHRH) is a hypothalamic decapeptide, a synthetic analogue that activates the gonadotrophs of the pituitary to secrete gonadotropin (GTH) in teleost fish (Roy, 2016). The Linpe technique is an induced breeding technique that combines LHRHa or salmon gonadotropin-releasing hormone analogue (sGnRHa) with dopamine antagonists such as pimozide or domperidone (DOM). This technique is effective for silver carp, common carp, mud carp, black carp, grass carp, and loach (Peter et al., 1988). The Linpe method is efficient and reduces stress on brood stock as it only requires a single injection. Previous research revealed that a single injection of LHRHa or pellet implant successfully induces the spawning of marine species, such as milkfish, rabbitfish, sea bass, and mullet. However, this approach is ineffective for several freshwater species, including cyprinids (Roy, 2016). Other benefits of LHRHa include repeatability without loss of efficacy and the ability to produce LHRHa in pure form (Su et al., 2013). Thus, synthetic analogues of LHRHa are increasingly used to induce ovulation and spawning in teleost fish as the hormone is highly stable with precise dosage.

Ovaprim. Ovaprim is a synthetic hormone composed of a combination of the sGnRHa and DOM. Ovaprim is administered intramuscularly or intracoelomically and acts directly on the pituitary gland, boosting the production of gonadotropic hormones (GTH) while inhibiting the dopaminergic

inhibition of gonadotropin secretion. Ovaprim has been widely used in Asia and the United States to induce spawning in marine and freshwater fish (Abbas et al., 2019; Nuraini et al., 2017). Ovaprim has been reported to stimulate spawning in seurukan fish successfully (*Osteochilus vittatus*), selais (*Ompok hypophthalmus*), common carp (*Cyprinus carpio*), mali-mali (*Labiobarbus festivus*), lelan fish (*Osteochilus pleurotaenia*), yellowfin porgy (*Acanthopagrus latus*), stinging catfish (*Heteropneustes fossilis*), John's snapper (*Lutjanus johnii*), and mrigal carp (*Cirrhinus mrigala*) (Abbas et al., 2019; Mosha, 2018; Nuraini et al., 2017; Surnar et al., 2015; Watson et al., 2009). A study led by Watson et al. (2009) found that after injecting several species with Ovaprim, the average success rates increased to 50% (ovulation) and 54% (spermiation), and only 1.3% deaths were recorded, showing the efficiency of Ovaprim as hormonal therapy for artificial breeding.

Comparative Study: Different Approaches of Artificial Hormones in Fish

Study 1: Comparison of Luteinising Hormone Releasing Hormone Analogue (LHRHa) and Carp Pituitary Extract (CPE). A study of the efficacy of using LHRHa to breed hybrid fry between channel female catfish (*Ictalurus punctatus*) and blue male catfish (*Ictalurus furcatus*) through LHRHa implant or injection and CPE injection (Su et al., 2013). In this experiment, LHRHa was diluted in 0.85% physiological saline and administered intraperitoneally at a starting dose of 20–30 g with 85% active ingredient per kg, followed by a resolving dose of 100–150 g/kg 12 h later. Meanwhile, LHRHa implants were administered into the muscle in a single dose of 75–100 g/kg body weight into the posterior and ventral to the dorsal, and the CPE was dissolved in 0.85% saline for 30–45 min before injection at a starting dose of 2 mg/kg or a dissolution dose of 8 mg/kg.

The result of this experiment was in line with the study by Hutson (2006) that evaluated the implant and injection of LHRHa in producing hybrid catfish from channel female catfish (*I. punctatus*) and blue male catfish (*I. furcatus*). According to the findings, LHRHa injection or implantation successfully induced breeding in channel female catfish and produced hybrid catfish embryos. High doses of injection and implants increased fecundity and the number of egg stripes in females. In this study, LHRHa implants produced significantly more ($P < 0.001$) fry/kg of broodstock than CPE. The implant method is more efficient as it requires only a single administration, which can reduce the stress during handling, compared to the LHRHa injection method, which requires two injections. Therefore, LHRHa is an excellent synthetic hormone for use in the ovulation of female catfish and the expansion of commercial hybrid catfish production.

Tan-Fermin and Emrata (1993) reported a significant variation in egg yield after female Asian catfish (*Clarias macrocephalus*) were induced with different

LHRHa administered with pimozide. A current study found that injection of 0.05-1.10 µg LHRHa in combination with 1 µg pimozide/g successfully promotes 100% ovulation in all *C. macrocephalus* females 16 h after treatment (El-Hawarry et al., 2016; Roy, 2016). Therefore, the appropriate inducing agent dose is critical for obtaining an optimal spawning egg number in catfish (Dhara & Das, 2018). A study by Kahkesh et al. (2010) showed that the use of LHRHa with CPE in the induced breeding of Benni (*Barbus sharpeyi*) achieves an excellent spawning success rate (87.5%), with high fertilisation (94.57%) and hatching (78.42%) rates.

Study 2: Comparison of Ovaprim and Carp Pituitary Extract (CPE). A study was performed with different doses of carp pituitary extract (CPE) and Ovaprim to observe the effects of inducing agents at varied temperatures and latency periods on the fertilisation and hatching rate of Asian catfish (*Clarias batrachus*) (Dhara & Saha, 2013). This experiment used gravid fish. For the CPE, the pituitary glands were obtained from newly deceased adult carp fish and used as a natural inducer. The procedures were carried out at three temperatures (26 °C, 28 °C, and 30 °C). Carp pituitary extract was administered at dosages of 40 and 120 mg/kg of body weight for females, while the males received 25 and 50 mg/kg of body weight. Meanwhile, the other group of females received Ovaprim at 0.8 and 2.0 ml/kg of body weight, and the males received 0.4 and 1.0 ml/kg of body weight through injection.

After 15 h of injection (latency period), the female fish were stripped to release eggs, the male testes were carefully removed, and eggs and sperms were collected. The results show that at 28 °C, the highest percentages of fertilisation (80%) and hatching (71%) were observed under high CPE treatment, and at latency periods of 14 and 15 h, perfectly smooth stripping and eggs without clusters were recorded. These results are similar to Srivastava et al. (2012), who used Ovaprim at 1.0–2.0 ml/kg body weight and a 14–18-hour latency period in *C. batrachus*. The inappropriate combination of dosage and latency period may lead to breeding failure in species, as shown in a study by Zonneveld et al. (1998). This study reveals that high dosages of Ovaprim and CPE result in significantly increased ($P < 0.05$) egg release, fertilisation, and hatching rates at all temperatures compared with those at low stimulant doses.

Study 3: Sustain Release of Human Chorionic Gonadotropin (hCG) by Osmotic Pump. In an *in vivo* study by Murugananthkumar et al. (2017) on *C. batrachus* and *C. gariepinus*, the osmotic pumps approach was used for the sustained release of the hCG inserted alongside the gonads in the peritoneal cavity and sutured with a 30-mm sterile catgut to prevent the backflow of the body's osmotic pump. The osmotic pump was loaded with 5,000 IU hCG or saline (as a control) and intraperitoneally implanted in catfish for 21 days during the pre-spawning phase (May–June). According to the manufacturer, the

osmotic pump used in this experiment can emit approximately 5 µl of solution each day when the fish are kept at constant ambient temperature. Previous studies proved that using an osmotic pump for the sustained release of hCG successfully promotes vitellogenesis and spermatogenesis in fish (Kagawa et al., 2009). Furthermore, the mean gonadosomatic index (GSI) and motility of the sperm for the group of males treated with hCG (*C. batrachus* and *C. gariepinus*) revealed a significant increase of 5% in the GSI ($P < 0.05$) compared with the control.

Meanwhile, the GSI in females receiving hCG implantation exhibited a significant increase of 41% ($P < 0.01$) compared with the control group. On the one hand, compared with the control group, the histology of hCG treatment in catfish testes demonstrated a normal development of spermatogenesis in germ cells towards the spawning phase in which the lumen was partly filled with spermatocytes and spermatids. On the other hand, the control group of female fish exhibited few development stages of oocytes. At the same time, the hCG treatment increased the quantity of fully developed immature oocytes and spawning-like growth in the fish ovary. This approach has been previously proven effective in sexually immature fish (Kagawa et al., 2009; Murugananthkumar, 2017).

Similar effectiveness was observed when using hCG to induce spawning in spotted murrels (*Channa punctatus*) and stinging catfish (*H. fossilis*). The effectiveness increased with the combination of Ovaprim hormone through the intermuscular injection method (El-Hawarry et al., 2016; Kather Haniffa & Sridhar, 2002). A natural hormone (i.e., hCG) and a synthetic hormone (i.e., Ovaprim) were injected into snakehead fish (*Channa marulius*) and successfully induced spawning in brood fish; the combination of Ovaprim and hCG effectively and successfully achieved up to 100% spawning rate (Hafeez-ur-Rehman et al., 2015). A significant enhancement in the fecundity of hCG implanted fish was reported through the experiment.

Study 4: Comparison of Ovaprim and Combination of LHRH with hCG. Dhas et al. (2017) assessed the effects of synthetic hormones on the reproductive performance of green chromide (*Etroplus suratensis*). This experiment used spawners that exhibited the second stage of gonadal development (contained spherical and opaque ovules) due to the onset deposition of the egg yolk. The ovary size ranged from 35 mm to 52 mm, the diameter was from 0.5 mm to 1.75 mm, and the ovaries were divided into three treatment groups: the hCG + LHRH, Ovaprim, and control (physiological saline) groups. The results showed that the hormone influenced the change in egg size in the oocytes, and the previtellogenic and mature phases depended on the type of hormone administered. The combination of hCG and LHRH produced the largest eggs, followed by Ovaprim and the control group (0.85% saline). The hCG + LHRH was the best treatment, with the highest fecundity and striping responses

(1.23 ml), highest fertilisation rate of 82.54%, and the highest hatching rate of 80.83%. Ovaprim followed with fecundity and stripping of 0.84 ml, fertilisation rate of 75.42%, and hatching rate of 73.69%.

A similar result trend was also observed in the males' treatment. The hCG + LHRH treatment enhanced the number of sperm cells (1,887 sperms/µl), followed by Ovaprim (860 sperms/µl) and the control (1,858 sperms/µl). The sperm motility improved by 89.78% when hCG + LHRH was administered, followed by Ovaprim (82.34%) and the control group (70.23%). This study revealed that the parameters were at the highest value under hCG + LHRH treatment, followed by Ovaprim treatment. Nwokoye et al. (2007) discovered that treating African giant catfish (*Heterobranchus bidorsalis*) with Ovaprim at 0.5 ml/kg body weight increased the fertilisation rate (98.31%). However, similar dosages were administered to induce spawning in spotted murrel (*C. punctatus*) and catfish (*Heteropneutes fossilis*), only producing low fertilisation rates of 75.0%, respectively (Kather Haniffa & Sridhar, 2002). The results indicate that the Ovaprim dosage should be varied depending on the species and the weight of the spawner (Dhas et al., 2017).

Study 5: Ovarian Lavage with Sperm and Hormone Mixture (Sperm + Pituitary Gland Extract). Ovarian lavage is an *in vitro* fertilisation that uses hormones to stimulate ovulation and spermiation. Ovarian lavage is a novel method in which hormone is administered intramuscularly or intraperitoneally while sperm is delivered into the ovarian lobes via a catheter (Müller et al., 2019). By using this method, female fish from group 1 were administrated with 5.0 mg/kg CPE, homogenised saline (0.7% NaCl) was injected intraperitoneally, and the eggs were fertilised using a traditional dry method. Fish from group 2 were injected similarly, but the sperms for the fertilisation process were injected into the ovarian lobe of the female. Females in group 3 were given homogenised 5 mg/kg of CPE with 2.0 ml/kg of sperm injected into each lobe of the ovarian cavity. The eggs in treatment group 1 were striped and fertilised with 2.0 ml/kg sperm. The eggs in treatment groups 2 and 3 were stripped, and fertilisation was activated by adding water.

The result showed that the ovulation ratio was 100% in group 1 with a latency time of 9.5 h, followed by group 2 with 85.7% and group 3 with 71.4% and a latency time of 10 h. This study's findings indicate that using sperm at a dose of 2.0 ml/kg in combination with 5 mg of a 2.0 ml dose of CPE resulted in successful ovulation and a high rate of fertilisation in silver catfish, *Rhamdia quelen* (Ittzés et al., 2020). The administration of hormones in combination with the injection of sperm from many males can be used in breeding programs to minimise bottleneck effects, synchronise egg production, avoid inbreeding, and reduce mortality. Modified gametes, such as polyploid or cryopreserved sperm, can also be used *in vitro* fertilisation (Müller et al., 2018). In captivity, a similar approach has been applied to spawning green-spotted puffer fish *(Dichotomyctere nigroviridis*). A

catheter was introduced into the oviduct at a 3 µl/g body weight rate as an alternative method of delivering hCG hormone to the fish (Watson et al., 2009).

Study 6: Commercial Hormone in Combination with Hormonal Antagonist. A study was conducted on African catfish (*Clarias gariepinus*) using homogenised carp pituitary in a physiological saline solution of 0.9% NaCl (4 mg of carp pituitary in one ml saline solution), hCG, synthetic LHRHa, and GnRHa with 10-mg benzyl alcohol. The hormone and hormone analogue were administered with 1 ml of dimethyl sulfoxide in combination with a DOM to measure the effects of using CPE, HCG, and LHRHa or GnRHa in combination with DOM on breeding performance. Seventy-two (72) females were randomly assigned to 10 treatment groups (i.e., T1, T2, T3, ... T10) in a complete randomised experimental design. Each experiment was performed in duplicate, with n = 4 fish in each experiment. Specific hormones were injected intramuscularly into the dorsal muscle of the female and male fish, and after 10 h of treatment, eggs and sperm were collected. A 2.5 ml of sperm was introduced to 100 g of eggs to fertilise the eggs for each treatment. Water was added to the active sperm, and the mixture was incubated at room temperature for 2 min before the eggs were rinsed with a 0.9% NaCl solution to complete the fertilisation process.

The fertilised eggs were washed with water and incubated in ventilated tanks at room temperature (28 °C) until hatching. The results revealed that the combination of hormones and a DOM effectively triggered ovulation in each *C. gariepinus*, with an ovulation rate of 100%. The highest ovulation rate was recorded from T4 (CPE + DOM: 100%), T6 (HCG + DOM: 100%), T8 (LHRHa + DOM: 100%), and T10 (GnRHa + DOM: 100%) followed by the ovulation rate in T3 (CPE: 87.5%) and T5 (HCG: 75%). The lowest ovulation rate was observed in T9 (GnRHa: 25%), followed by T7 (LHRHa: 12.5%). A study suggested that dopaminergic inhibition may contribute to the efficacy of hormone administration (Zohar et al., 1995). Mehdi and Seyed (2011) explained that due to the dopamine hormone suppressing pituitary secretion of GTH, the administration of DOM increases the effects of the GnRH analogue, resulting in a significant secretion of LH and ovulation. The combination of GnRHa and DOM can also successfully induce complete ovulation in female Malaysian mahseer (*Tor tambroides*) (Azuadi et al., 2011). Furthermore, a study showed that using DOM alone is ineffective in inducing complete ovulation in species, resulting in an insufficient response to stripping and showing that DOM without GnRHa is incapable of promoting maturation and ovulation (El-Hawarry et al., 2016).

DISCUSSION

Various artificial hormones have been developed to address the reproductive system's failure and the dysfunction of gonadal development in captive fish. A previous study showed that using dopamine

antagonists (e.g., DOM) with other hormones could increase the fertilisation rate and produce good-quality seeds. In addition, domperidone does not cross the blood-brain barrier in goldfish and other teleosts fish by lowering the risk of negative side effects in treated fish (Peter et al., 1988). Another study states that in goldfish, common carp, and loach, sGnRHa is more efficient than LHRHa (Lin et al., 1988). However, other findings revealed that using LHRHa with pimozide or other dopamine antagonists was proven effective in promoting ovulation in female fish (Roy, 2016).

The use of hCG, CPE, GnRHa, and LHRHa with or without DOM effectively induces spawning (Table 1); past studies showed that the use of hormones with DOM could increase the success of fish farming (El-Hawarry et al., 2016). Apart from that, some studies suggested that combining GnRHa with DOM could boost GTH production, leading to increased sex steroids and the development of ovarian lobules (Sharaf, 2012). In addition, the use of GnRHa reportedly promotes maturation and spawning in various species, including African catfish (*C. gariepinus*), stinging catfish (*H. fossilis*), Siberian sturgeon (*Acipenser baeri*), brown trout (*Salmo trutta*), red seabream (*Pagrus major*), and starry flounder (*Platichthys stellatus*) (Alavi et al., 2012; Marimuthu, 2019; Okuzawa et al., 2016).

Aside from the dosage and type of hormone used, the delivery technique is also crucial in determining the success rate of artificial breeding. The implant method has potential because of the minimum handling of hormone insertion into fish, which could reduce handling stress and labour (Su et al., 2013). Furthermore, ovarian lavage is also preferred because it is a simple method for administering spawning hormone and eliminates injections for small species, such as spotted green pufferfish (*Tetraodon nigroviridis*) (Watson et al., 2009). Furthermore, the ovarian lavage approach is less time-dependent than the established *in vitro* fertilisation, and it could utilise sperm samples from chosen males depending on any factor to introduce a new generation (Ittzés et al., 2020).

The development of aquaculture technology, such as artificial breeding, has led to the application of technologies from low to high levels of development to increase the production of cultivated species. The use of breeding technology helps control reproduction in captivity, especially for wild and fish species that fail to reproduce and spawn naturally in captivity. Moreover, artificial breeding is essential for novel candidate species that are undomesticated and have a limited brood stock harvested from the wild to achieve sustainability in commercial aquaculture production. Some cultured fish may not spawn in captivity due to seasonal availability, lack of environmental cues, dysfunction, stress culture environments, or physio-physical factors, such as salinity and temperature. Therefore, well-established artificial breeding will help support the population of endangered species of commercial interest and those in the wild and captivity.

Table 1
Comparative studies of different approaches to artificial hormones in fish

Comparative study	Fish species	Aim of study	Dosage	Results	References
Comparison of luteinizing hormone-releasing hormone analogue (LHRHa) and carp pituitary extract (CPE)	Channel female catfish, *Ictalurus punctatus*	LHRHa implant and injection and CPE injection	LHRHa injection: 20–30 g/kg and 100–150 g/kg bw LHRHa implant: 75–100 g/kg bw CPE injection: 2mg/kg bw	LHRHa implants produced significantly more ($P < 0.001$) fry/kg than CPE, and there are significant differences LHRHa injection and CPE injection ($P < 0.05$)	Su et al. (2013)
Comparison of Ovaprim and carp pituitary gland extract (CPE)	Asian female catfish, *Clarias batrachus*	Observe different doses of CPE and Ovaprim at varied temperatures and latency periods on the fertilization and hatching rate	CPE: 40 and 120 mg/kg of bw Ovaprim: 0.8 and 2.0 ml/kg of bw	At 28°C, the greatest percentages of fertilisation (80%) and hatching (71%) were observed on high CPE treatment and Ovaprim at all temperature	Dhara and Saha (2013)
Sustain release of human chorionic gonadotropin (hCG) by osmotic pump	Adult catfishes, *Clarias batrachus* and *C. gariepinus*	*In vivo* induction of hCG by using an osmotic pump implanted intraperitoneally during the pre-spawning phase for 21 days LHRHa injection, 20–30 g/kg and 100–150 g/kg body weight	Osmotic pump: 5,000 IU hCG and saline (as a control)	GSI in females receiving hCG implantation revealed a significant increase of 41% ($P < 0.01$) compared to the control. Males also showed a positive outcome with a significant increase of 5% in the GSI ($P < 0.05$) when compared to the control	Murugananthkumar et al. (2017)
Comparison of Ovaprim and combination hormones of LHRH with hCG	Cichlid, *Etroplus suratensis*	To assess the effect of Ovaprim and combination hormone of hCG with LHRH on *E. suratensis*	hCG + LHRH: 1,000 IU +1 ml/kg fish Ovaprim: 1 ml/kg fish	The use of the combination hormone hCG + LHRH resulted in the highest fecundity and striping responses (1.23 ml), highest fertilization rate of 82.54%, and the highest hatching rate of 80.83%, followed by Ovaprim with fecundity and stripping (0.84 ml), fertilization rate (75.42%), hatching rate (73.69%), and the lowest in control	Dhas et al. (2017)

Table 1 *(continue)*

Comparative study	Fish species	Aim of study	Dosage	Results	References
Ovarian lavage with sperm and hormone mixture (sperm + CPE)	Silver catfish, *Rhamdia quelen*	To study the effectiveness of the ovarian sperm injection method compared to the traditional method	CPE: 5 mg/kg bw Sperm: 2.0 ml/kg bw	The ovulation ratio was 100% in group one, followed by group 2 with 85.7% and group 3 with 71.4%. The use of sperm at a dose of 2.0 ml/kg in combination with 5 mg of a 2.0 ml dose of CPE resulted in successful ovulation and a high rate of fertilization	Ittzés et al. (2020)
Commercial hormone in combination with a hormonal antagonist	African catfish, *Clarias gariepinus*	To study the effects of using CPE, hCG, LHRHa, and GnRHa with or without domperidone (DOM)	0.9% NaCl: 4 mg carp pituitary in one ml saline solution hCG: 4,000 IU/ kg bw LHRHa: 50 µg/ kg bw GnRHa: 40 µg/kg bw DOM: 10 mg/ kg bw	A combination of hormones with a DOM effectively triggered ovulation in each *C. gariepinus*, with an ovulation rate of 100%. The highest ovulation rate was recorded from T4 (CPE + DOM: 100%), T6 (HCG + DOM: 100%), T8 (LHRHa + DOM: 100%), and T10 (GnRHa + DOM: 100%) followed by the ovulation rate in T3 (CPE: 87.5%) and T5 (HCG: 75%). While the lowest ovulation rate was observed in T9 (GnRHa: 25%), followed by T7 (LHRHa: 12.5%). The use of CPE, HCG, LHRHa or GnRHa together with dopamine antagonist (DOM) successfully induced ovulation in 100% of the experimental *C. gariepinus* broodfish	El-Hawarry et al. (2016)

FUTURE PROSPECT AND CONCLUSION

Artificial hormones could offer a promising technique to breed fish in captivity, ensuring seed availability, improving genetic loss, and reducing the dependency on wild-caught fingerlings. This review highlighted a comprehensive discussion and various types of artificial hormones, their delivery systems, and dosages used in artificial breeding within different fish species. In the future, more research should be focused on studying the latency period, thermodynamics, and metabolic pathways to ensure the developed hormones are safe, efficient, and effective for fish breeding.

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