Int Aquat Res (2024) 16:113–142
https://doi.org/10.22034/IAR.2024.2008470.1624

REVIEW

The potential of aromatase inhibitors in fish masculinization: a comprehensive review of applications, mechanisms and future perspectives

Jham Lal . Anand Vaishnav . Soibam Khogen Singh . Pradyut Biswas . Naresh Kumar Mehta . Dharmendra Kumar Meena . Gusheinzed Waikhom

Received: 02 March 2024 / Accepted: 16 June 2024 / Published online: 30 June 2024

Abstract A class of drugs known as aromatase inhibitors can effectively inhibit aromatase, an enzyme that transforms androgens (male hormones) into estrogens (female hormones). These inhibitors are commonly used in medical treatments for hormone-sensitive conditions such as breast cancer in humans. In the context of fish, there has been some research on the use of aromatase inhibitors to alter the sexual development and secondary sexual characteristics of certain species. Aromatase inhibitors in fish may be used to manipulate sex ratios in aquaculture. In some fish species, the sexes of individuals are influenced by environmental variables, including temperature. By administering aromatase inhibitors, it is possible to suppress the production of estrogens and bias the sex ratio towards males. This can be desirable in certain aquaculture operations where males are preferred, such as for increased growth rates or reduced aggression. It is important to highlight that the use of hormone manipulation techniques in fish farming is subject to regulations and restrictions, as the potential environmental impacts and welfare considerations need to be carefully evaluated. The use of any pharmaceutical product in aquaculture requires rigorous testing and approval processes to ensure its safety and efficacy. This review discusses the multifaceted use of aromatase inhibitors in the manipulation of sex ratios in aquaculture, while emphasizing the imperative need for the systematic evaluation of environmental impacts and ethical considerations associated with this practice, and compliance with regulatory frameworks governing their implementation.

Keywords Fish . Anastrozole . Letrozole . Fadrozole . Exemestane . Masculinization

Introduction

Aromatase inhibitors are a class of pharmaceutical compounds primarily used in the treatment of hormone receptor-positive breast cancer and other conditions associated with excess estrogen production. These drugs work by inhibiting the activity of the enzyme aromatase, which plays a crucial role in the synthesis of estrogen. Aromatase inhibitors, which are used in the treat of ovarian and breast cancer among post-menopausal women (Howell et al. 2005), have a notable impact on masculinization in fish by impeding the process of estrogen-induced ovary ifferentiation. In the context of fish reproductive biology, the cytochrome P450 aromatase enzyme plays a crucial role in the conversion of androgens into estrogens, making it an important factor in sex determination processes. The conversion process holds utmost significance in the use of aromatase inhibitors, as it triggers masculinization by inhibiting the induction of estrogens from

Jham Lal . Anand Vaishnav . Soibam Khogen Singh (✉) . Pradyut Biswas . Naresh Kumar Mehta . Gusheinzed Waikhom
College of Fisheries, Central Agricultural University, Lembucherra, Tripura-799210, India
e-mail: gengang@gmail.com

Dharmendra Kumar Meena (✉)
ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata, West Bengal-700120, India
e-mail: dkmeenacifri@gmail.com

androgens. Estrogens play a more active role in the typical female sexually distinct process than do spontaneous androgenic substances, which have no involvement in this process that allows for sexual distinction. In order to establish all-male fish populations, synthetic aromatase inhibitors that are readily accessible on the market include letrozole, exemestane, anastrozole, fadrozole, as well as others. Aromatase inhibitors exert their masculinizing effects in fish by inhibiting estrogen-induced ovarian differentiation (Komastu et al. 2006).

In other vertebrates, aromatase inhibitor (AI) therapy has been used to study the key functions of estrogens and aromatase in female sex differentiation. Piferrer et al. (1994) studied a brief 2-hour treatment of newly hatched female embryos with the nonsteroidal inhibitor fadrozole, which led to complete sex reversal in Chinook salmon (*Oncorhynchus tshawytscha*), a gonochoristic fish species. Similar to this, 4-hydroxyandrostenedione, a steroidal AI, caused the change of ovaries towards testes in the frog organism *Rana catesbeiana* (Yu et al. 1993). In the case of *Alligator mississippiensis*, fadrozole had a relatively mild impact on ovarian development (Lance and Bogat 1992). Masculinization was also observed in turtles when treated with nonsteroidal AIs like fadrozole or letrozole, as well as in newts of the species *Pleurodeles waltl* (Chandard and Dournon 1999). While these findings provide insight into how AIs affect sexual distinction, there is a paucity of information regarding the functional significance of aromatase in the sexual orientation transition that occurs naturally in organisms with various sexes. The mechanisms underlying natural sex changes in hermaphroditic species also remain poorly understood.

Aromatase inhibitors constitute advancements in the field of medicine, particularly in the management of hormone-sensitive conditions. These drugs have greatly changed the way we treat diseases by particularly focusing on a key component in the intricate system of human hormone control (Brueggemeier 2001). Aromatase is a vital enzyme that orchestrates the conversion of androgens, typically associated with male physiology, into estrogens, the primary female sex hormones. This enzymatic transformation has far-reaching implications for various aspects of human health, ranging from the development of secondary sexual characteristics to the maintenance of bone density and overall hormonal equilibrium (Carreau et al. 2003). The fascinating world of aromatase inhibitors explores their crucial role in medical therapies, with a particular focus on their application in the treatment of hormone receptor-positive breast cancer. By dissecting the mechanism of action and their broader implications, we aim to shed light on how these inhibitors have become indispensable tools in the figureht against hormone-related diseases, offering hope and improved outcomes for countless individuals worldwide (Choueiri et al. 2004). The aim of this comprehensive review is to summarize the potential of aromatase inhibitors (AIs) in fish masculinization, covering their applications, mechanisms, and future perspectives. AIs are commonly used to treat postmenopausal women with ovarian and breast cancer, and they have been found to induce male characteristics in fish. The review highlights the potential of AIs in the commercial production of male fish, particularly in the ornamental fish sector, and discusses the need for further research to optimize the duration and dosage of oral treatments for complete masculinization.

Fish masculinization

Fish masculinization is a phenomenon in aquatic biology that involves the development of male secondary sexual characteristics in female fish or the alteration of the normal sexual differentiation process. This condition is often linked to exposure to endocrine-disrupting compounds or environmental factors that interfere with the hormonal system of fish, particularly during the critical period of sexual development. There are several methods for fish masculinization:

1. Hormonal treatment: Hormonal treatment is one of the most common methods used to induce masculinization in female fish. Typically, synthetic hormones such as 17-alpha methyltestosterone or 17-beta hydroxy-5-alpha-androstan-3-one are administered to female fish either through their diet or by immersion in a hormone solution. These hormones promote the development of male secondary sexual characteristics, including the growth of testes and the production of sperm (Sarker et al. 2022).

2. Genetic manipulation: Genetic manipulation techniques can also be used to produce genetically male fish from genetically female individuals. This is often done through genetic modification or selective breeding to produce individuals with male sex chromosomes (Hussain 1998).

3. Temperature control: In some fish species, the temperature at which eggs are incubated can influence

the sex of the offspring. This process, known as temperature-dependent sex determination (TSD), can be manipulated to produce more males by exposing the eggs to specific temperature ranges during incubation (Verma et al. 2020).

4. Natural variation: In certain fish species, there is natural variation in sex determination mechanisms. Some individuals may have the ability to change their sex in response to environmental or social factors. This natural plasticity can sometimes be exploited to induce masculinization when needed (Tenugu and Senthilkumaran 2022).

Fish masculinization is often used in aquaculture to produce all-male populations, as male fish typically grow faster and larger than females in many species. This can improve the efficiency of fish farming operations. Additionally, it may be used in fisheries management to control the sex ratio of wild fish populations or to conserve threatened or endangered species. The use of methyltestosterone may have a beneficial role in fish farming, but its use should be carefully managed to minimise any potential negative impacts on fish conservation and the environment. It's essential to balance the economic benefits of aquaculture with the need to protect and conserve wild fish populations and their ecosystems.

Sex reversal

In the animal kingdom, sexual identity is often formed throughout the initial phases of growth and development and, once fixed, typically doesn't change throughout the course of an individual's lifespan. Nevertheless, in certain teleost fish species, adults have the remarkable ability to change their sex in response to shifts in social dynamics (Devlin and Nagahama 2002). Several studies examining the endocrine aspects of sex change in fish have highlighted the pivotal role of estrogens, particularly estradiol-17β (E2), in orchestrating the transformation of gonadal sex (Frisch 2004). Estrogens are predominantly manufactured within the gonads through enzymatic processes that utilise testosterone as their substrate. Within the steroidogenic pathway, the terminal enzyme responsible for this conversion is cytochrome P450 aromatase (Simpson et al. 1994).

Sex reversal through hormonal treatment is a technique employed through the method of sexual distinguishing. This practice originated as a solution to address issues arising from the stocked fish ponds becoming overpopulated and the resulting challenges related to overcrowding, which led to the stunted growth of fish. In the context of commercial fish farming, the presence of mixed-sex fish became undesirable for producers due to the substantial variation in fish sizes at the time of harvest. This variation occurred because males tended to grow faster than females, resulting in a wide range of sizes. Consequently, producers en-

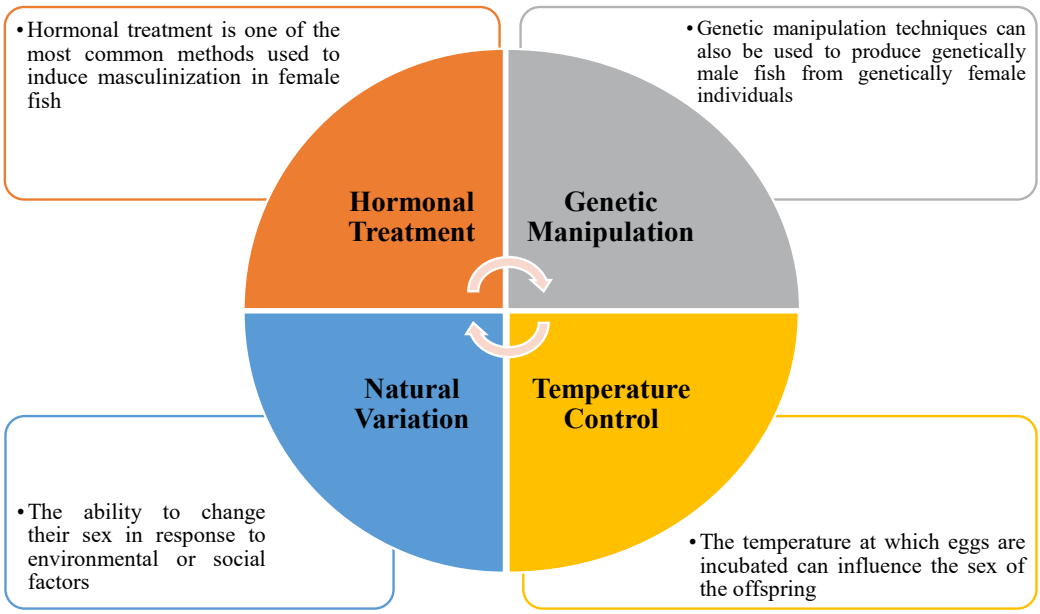


Fig. 1 Different methods of fish masculinization

countered difficulties in maintaining the uniformity of their fish populations. To overcome these challenges and optimise yields within the rearing period, the preference shifted towards male fry due to their rapid growth and larger size. This strategic choice aimed to achieve a more consistent and profitable fish production process. The generation of mono-sex fish populations can be achieved through a variety of techniques, including manual differentiation between males and females, hybridization, chromosomal manipulation, and hormonal treatment. Among these methods, hormonal treatment, sometimes known as sex reversal, stands out as the simplest and most efficient approach. Hormonal treatment can be precisely controlled through the administration of androgen and estrogen hormones (Megbowon and Mojekwn 2014). In practical terms, hormonal treatment proves to be highly productive as it promotes increased weight gain and accelerated growth rates. Additionally, it ensures uniformity within the population and effectively regulates unwanted breeding (Singh 2013; Taranger et al. 2010). This method is widely favoured for its practicality and effectiveness in enhancing fish production, achieving consistent results, and managing breeding outcomes.

This practice originated as a solution to address issues arising from the stocked fish ponds becoming overpopulated and the resulting challenges related to overcrowding, which led to the stunted growth of fish. In the context of commercial fish farming, the presence of mixed-sex fish became undesirable for producers due to the substantial variation in fish sizes at the time of harvest. This variation occurred because males tended to grow faster than females, resulting in a wide range of sizes. Consequently, producers encountered difficulties in maintaining the uniformity of their fish populations. To overcome these challenges and optimise yields within the rearing period, the preference shifted towards male fry due to their rapid growth and larger size. This strategic choice aimed to achieve a more consistent and profitable fish production process. The generation of mono-sex fish populations can be achieved through a variety of techniques, including manual differentiation between males and females, hybridization, chromosomal manipulation, and hormonal treatment. Among these methods, hormonal treatment, sometimes known as sex reversal, stands out as the simplest and most efficient approach. Hormonal treatment can be precisely controlled through the administration of androgen and estrogen hormones (Megbowon and Mojekwn 2014). In practical terms, hormonal treatment proves to be highly productive as it promotes increased weight gain and accelerated growth rates. Additionally, it ensures uniformity within the population and effectively regulates unwanted breeding (Singh 2013; Taranger et al. 2010). This method is widely favoured for its practicality and effectiveness in enhancing fish production, achieving consistent results, and managing breeding outcomes. Despite their clinical success, AIs are not without side effects. Common adverse events include joint pain, hot flashes, and bone loss, necessitating vigilant monitoring and the use of adjuvant therapies, such as bisphosphonates, to counteract these effects.

Aromatase inhibitors

Aromatase inhibitors (AIs) are a class of pharmaceutical compounds that play a pivotal role in the management of hormone receptor-positive breast cancer, a prevalent form of the disease. These inhibitors are designed to target the aromatase enzyme, which is responsible for converting androgens into estrogens in postmenopausal women. Estrogen fuels the growth of hormone receptor-positive breast cancer cells, making the suppression of estrogen synthesis a cornerstone of therapy. Aromatase inhibitors have emerged as powerful tools in this endeavor (Arora and Potter 2004).

Three main AIs are commonly employed in clinical practice: anastrozole, letrozole, and exemestane. These drugs work by inhibiting aromatase, thus reducing estrogen levels in the body. They differ in their mechanisms of action and pharmacokinetics, allowing for treatment strategies based on patient characteristics and individual needs. AIs have proven to be highly effective in postmenopausal women with hormone receptor-positive breast cancer, either as adjuvant therapy following surgery or as a first-line treatment for advanced disease. Their use has led to improved disease-free survival rates and a reduced risk of recurrence when compared to the previous standard, tamoxifen, which functions as a selective estrogen receptor modulator (Gonnelli and Petrioli 2008).

Sex reversal through hormonal treatment is a technique employed through the method of sexual distinguishing. Furthermore, AIs are not suitable for premenopausal women, highlighting the importance of patient-specific treatment selection (Johnston and Dowsett 2003). Aromatase inhibitors represent a significant

advancement in the treatment of hormone receptor-positive breast cancer. Their ability to suppress estrogen production has proven to be a valuable strategy in reducing the risk of recurrence and improving survival rates in postmenopausal women. However, their side effects and limited applicability to premenopausal patients underscore the importance of careful consideration and personalized treatment plans in the figureht against breast cancer (Gonnelli and Petrioli 2008).

Aromatase inhibitors have found widespread application in the manipulation of sexual differentiation in fish. These inhibitors can be categorized into three generations: first-generation (such as aminoglutethimide), second-generation (including Formestane and Fadrozole), and third-generation (comprising Anastrozole, Letrozole, and Exemestane). Furthermore, within the third generation, these inhibitors can be further classified into two types: Type I and Type II, based on their distinct characteristics. Type I inhibitors possess a steroidal structure that closely resembles androgens. They exert their action by permanently obstructing the substrate-binding site of the enzyme, leading to irreversible inactivation. In contrast, Type II inhibitors are non-steroidal and act in a reversible manner (Mokbel 2002). This classification system offers valuable insights into the diversity of aromatase inhibitors and their specific modes of action when employed to regulate sexual differentiation in fish.

Aromatase inhibitors are available in two distinct types: non-steroidal compounds, which exert a reversible effect, and steroidal compounds, which cause irreversible inhibition (Geisler 2011). Aromatase inhibitors come in two categories: natural and synthetic. Natural inhibitors encompass androstenedione and testosterone, while synthetic inhibitors include Fadrozole, Letrozole, Anastrozole, and Aminoglutethimide. Natural aromatase inhibitors operate through hydroxylation, leading to the formation of an indissoluble covalent bond that permanently obstructs the aromatase enzyme complex. In contrast, synthetic inhibitors engage in a reversible binding process with the enzyme's active site. Letrozole, a synthetic drug, stands out as a selective and potent inhibitor, as it exhibits a strong affinity for a specific group of aromatase enzymes (Brodie 1991).

1. Steroidal aromatase inhibitors: Steroidal AIs, represented by exemestane, possess a steroidal core structure. These compounds work by irreversibly binding to the aromatase enzyme, rendering it inactive. Exemestane, for example, forms a covalent bond with the enzyme, permanently inhibiting its ability to convert androgens into estrogens. Due to their irreversible action, steroidal AIs are particularly effective in postmenopausal women with breast cancer (Miller et al. 2008).

2. Non-steroidal aromatase inhibitors: Some examples of non-steroidal AIs are anastrozole and letrozole. These drugs have a chemical structure that is not steroidal. They act by reversibly competing with the aromatase enzyme for its active site, temporarily blocking estrogen synthesis. While non-steroidal AIs are generally considered less potent than their steroidal counterparts, they are still highly effective and are commonly used in the treatment of hormone receptor-positive breast cancer (Geisler et al. 1996).

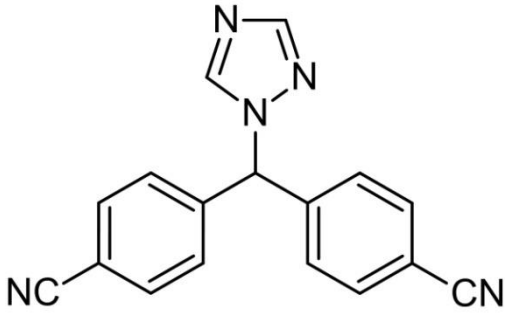
Additionally, aminoglutethimide promotes the production of various mixed-function oxidases (Murray et al. 1993), which enhances the clearance of oestrone sulphate (Lnning 1999). By speeding up the metabolism of other substances, such as tamoxifen, aminoglutethimide can also lead to medication interactions (Lien et al. 1990). Tamoxifen's metabolism is unaffected by letrozole or anastrozole, but tamoxifen slightly speeds up the metabolism of letrozole (Itoh et al. 2005). Additionally, it seems to have some impact on how anastrozole is metabolised. Contrary to nonsteroidal drugs, steroidal aromatase inhibitors covalently bind to the exact same enzyme location as the endogenous steroid substrate present, resulting in a selective as well as irreversible (or "suicide") suppression of the activity of the enzyme (di Salle et al. 1990).

Letrozole

Letrozole falls into the category of medications known as aromatase inhibitors, and it finds common application in the therapy of hormonal receptor-positive breast cancer, especially among postmenopausal women. Its mechanism of action revolves around the reduction of estrogen production within the body, a crucial function since estrogen can stimulate the proliferation and metastasis of specific breast cancer types, as highlighted by Bhatnagar (2007). Letrozole's mode of action is highly specific, targeting the enzyme aromatase responsible for converting androgens (male hormones) to estrogens (female hormones) in several tissues, such as adipose tissue and the breast. Through the inhibition of aromatase, letrozole effectively lowers estrogen levels in the body, thus retarding or obstructing the growth of estrogen-sensitive cancer

cells, as elucidated (De Ronde and de Jong 2011).

Letrozole is a member of a group of medications that are called nonsteroidal aromatase inhibitors, which act as inhibitors of estrogen synthesis with antineoplastic properties. Its mechanism of action involves reducing the body's estrogen production, thereby impeding the growth of certain breast cancer cells that rely on estrogen for their growth. Letrozole, a third-generation aromatase inhibitor, specifically and reversibly blocks aromatic enzymes, leading to the inhibition of estrogen-dependent breast cancer cell growth. Aromatase, a cytochrome P-450 enzyme present in a variety of organs, which include the premenopausal ovary, liver, and breast, catalyses the transformation of androstenedione and testosterone through estrone and estradiol, indicating the penultimate stage in oestrogen biosynthesis (Bhatnagar 2007).
Molecular formula: C17H11N5
Molecular weight: 285.30
US brand name: Femara



Code name: CGS 20267
Chemical structure: 4, 4-(1H-1, 2, 4 triazol-1-yl, ethylene) dibenzonitrile

Letrozole, known by its INN name and trade name Femara®, is an orally administered non-steroidal aromatase inhibitor primarily used as adjuvant therapy for hormonally-responsive breast cancer. Estrogens are generated through the conversion of androgens, facilitated by the aromatase enzyme. Letrozole functions by competitively and reversibly binding to the heme of its cytochrome P450 unit, effectively halting the production of estrogens (Bhatnagar 2007). This action is highly specific, with letrozole not affecting the production of mineraloids or corticosteroids. In contrast, the antiestrogenic effect of tamoxifen, a prominent treatment option prior to the advent of aromatase inhibitors, is attributed to its interference with the estrogen receptor rather than inhibiting estrogen production (Nunez et al. 2004). The United States Food and Drug Administration (FDA) has granted approval for Letrozole in the treatment of hormone receptor-positive or receptor status-unknown local or metastatic breast cancer in postmenopausal women. However, its use can result in side effects indicative of hypoestrogenism. Long-term usage raises concerns about potential osteoporosis development, which is why Letrozole prescriptions often coincide with prescriptions for osteoporosis-preventing medications like Fosamax (Cohen et al. 2002). Letrozole has demonstrated its ability to significantly reduce estrogen levels by approximately 98 percent while concurrently increasing testosterone levels. Athletes and bodybuilders often favour Letrozole for its anti-estrogen properties during steroid cycles, as it helps reduce bloating caused by excessive water retention and prevents the development of gynecomastia, a side effect associated with certain anabolic steroids (Arroyo et al. 2021). However, it's important to note that doses exceeding 2.5 mg per day can potentially lead to temporary loss of libido, and doses exceeding 5 mg per day over extended periods may pose risks to kidney health. Additionally, letrozole has demonstrated the ability to delay the fusion of growth plates in adolescents. This delay can enhance the effectiveness of growth hormone treatment, making letrozole a valuable option for addressing short stature in adolescents and children (Garcia-Velasco et al. 2005).

Letrozole used in fish as a masculinization

Femara, a widely recognised brand name for Letrozole (CGS 20267), belongs to the category of non-steroidal triazole derivatives and stands as one of the most potent aromatase inhibitors known to date (Smith

1999). It has been approved as the initial course of treatment for postmenopausal women with metastatic breast cancer and hormone-receptor positivity (Haynes et al. 2003). Letrozole has the potential to prevent the transformation of an androgenic steroid into oestrogen as well as lessen the negative effects brought on by the overuse of testosterone-producing steroids, in addition to its main indication (Haynes et al. 2003). Impressively, letrozole can inhibit aromatase to an extent of 98–99%, resulting in a substantial reduction of serum estrone and E2 levels, often to undetectable limits in patients (Smith 1999).

The effects of the aromatase inhibitor (AI) letrozole on the growth of gonadal tissue, blood hormone levels, and aromatase functioning in a 2-year-old female red-spotted grouper (*Epinephelus akaara*) during the breeding season are explored by Li et al. (2005). Following implantation with the aromatase inhibitor, there was a significant reduction in aromatase activities within the gonads. Notably, it was found that the red-spotted grouper's sex reversal caused by the aromatase inhibitor may be significantly influenced by the change in serum levels of 11-KT as well as 17-beta-estradiol (E2) that occurred. Letrozole, a synthetic aromatase inhibitor, interferes with an essential stage in the production of endogenous estrogens from androgens. Additionally, the research conducted by Sun et al. in 2007 demonstrated that letrozole, especially at concentrations exceeding 5 µg/L, led to a dose-dependent reduction in plasma vitellogenin levels in females.

Letrozole may enter the water bodies through various pathways, including manufacturing processes and ingestion or excretion. Approximately 6% of the supplied doses of letrozole are unaltered after administration, even though nearly all of it can be converted via the cytochrome P450 isozymes towards the pharmacologically ineffective carbinol metabolites (Haynes et al. 2003). Letrozole was found in samples of wastewater at levels as high as 2.4 ng/L. Only one time prior, letrozole was found in wastewater effluent at a lower value of 0.28 ng/L. Both of the samples of wastewater as well as samples of clean water exhibited modest levels of letrozole decomposition following the UV treatment. Despite the greatest UV irradiance, the content of wastewater dropped by 24%. Only around 5% of letrozole was destroyed at the fuente level usual for ultraviolet (UV) disinfection at waste water treatment facilities (Alitalo et al. 2022).

Anastrozole

Anastrozole falls under the category of aromatase inhibitors and is frequently prescribed to manage hormone receptor-positive breast cancer, particularly in postmenopausal women. Similar to other aromatase inhibitors, anastrozole functions by reducing estrogen levels within the body. This is particularly important because estrogen can promote the development and metastasis of certain forms of breast cancer (Milani et al. 2009). Anastrozole's primary mechanism of action revolves around the inhibition of the enzyme aromatase. Aromatase plays a pivotal role in converting androgens (male hormones) into estrogens (female hormones) within various tissues, including adipose tissue and the breast. By impeding aromatase activity, anastrozole effectively curtails the synthesis of estrogen, thereby slowing or even halting the growth of cancer cells driven by estrogen. Typically administered in the form of tablets, anastrozole is generally taken once daily. This treatment is primarily prescribed to postmenopausal women because, in their case, the

Table 1 Letrozole used as a aromatae inhibitor on fish masculinization

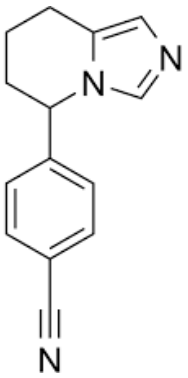
Fish species	Methods	Doses of AI	Masculinization (%)	References
Rosy barb (*Pethia conchonius)*	Oral administration	100 mg/Kg	60.18%	Lal et al. (2023a)
Rainbow trout (*Oncorhynchus mykiss*)	Oral administration	150 mg kg^{-1} diet	84.63%	Hajibeglou and Machanlou (2023)
Rock bream (*Oplegnathus fasciatus*)	Oral administration	Letrozole	100%	Liu et al. (2023)
Male sheep (Ovis aries)	Oral administration	0.5mg/Kg	ovine reproduction.	Lisboa Neto et al. (2023)
Rainbow trout (*Oncorhynchus mykiss)*	Oral administration	1.5 mg/kg LET-CS	76.66%	Alijani et al. (2022)
Rainbow trout (*Oncorhynchus mykiss*)	Oral administration	50 mg/kg diet	Letrozole successfully masculinization of gonads	Xu et al. (2021)
Greasy grouper (*Epinephelus tauvina)*	Oral administration	LET (0.2) + MT (5.0 mg/kg)	100%	Ranjan et al. (2015)
Yellow catfish (*Pelteobagrus fulvidraco)*	Oral ingestion	50 mg kg-1 diets	83.3%	Shen et al. (2015)
Siamese fighting fish (*Betta splendens*)	Immerssion method	500 and 1000 µg/L	100%	Katare et al. (2015)
Tilapia (*Oreochromis mossambicus*)	oral administration	100-200 mg/kg feed	100%	Das et al. (2012)
Guppy (*Poecilia reticulata)*	oral administration	75	97%	Jeevamani et al. (2012)
Guppy (*Poecilia reticulata)*	Immersion	100 ppm	93%	Jeevamani et al. (2012)
Bluegill sunfish (*Lepomis macrochirus)*	Oral administration	500 mg/Kg	70%	Gao et al. (2010)
Bluegill sunfish (*Lepomis macrochirus*	Oral administration	50 mg/Kg	59%	Gao et al. (2010)
Nile tilapia (*Oreochromis mossambicus*)	dietary administration	17α-MT (100 ppm+25 ppm	100%	Das (2007)
Zebrafish (*Danio rerio)*	Oral administration	500g/g of food	100%	Fenske and Segner (2004)
Young zebrafish (*Danio rerio*)	Oral administration	100, 1000 mg/Kg	100%	Uchida et al. (2004)

ovaries are no longer the primary source of estrogen production. Instead, estrogen is generated through the conversion of androgens in different tissues (Krásenská 2016).

Anastrozole belongs to the class of nonsteroidal aromatase inhibitors, specifically those characterised as reversible Type II inhibitors. Its mode of action involves binding reversibly to the aromatase enzyme and competitively inhibiting it, thereby preventing the conversion of androgens into estrogens in peripheral (extragonadal) tissues (Simpson 2003). Studies have shown that this medication can achieve substantial inhibition of aromatase activity. At a dosage of 1 mg per day, it has been found to achieve inhibition rates ranging from 96.7% to 97.3%. When administered at a higher dosage of 10 mg per day, anastrozole demonstrates an even more significant inhibition of aromatase, reaching up to 98.1% (Lønning et al. 2003; Lønning 2003). Hence, a daily dosage of 1 mg is regarded as the minimum necessary to achieve maximal aromatase suppression using anastrozole. This reduction in aromatase activity leads to a substantial decrease of at least 85% in estradiol levels among postmenopausal women. Notably, anastrozole has no impact on the concentrations of corticosteroids or other adrenal hormones (Lonning et al. 2003). The lack of cross-resistance among nonsteroidal aromatase inhibitors, including anastrozole as well as letrozole and steroids such as exemestane, appears to be due to variations in the mechanisms of action. These drugs' very selective suppression of aromatase does not interfere with the generation of various other steroids, such as aldosterone, thyroid stimulating hormone, or adrenal corticosteroids. Anastrozole, specifically, does not possess progestogenic, androgenic, or estrogenic activity (Yates et al. 1996; Kvinnsland et al. 2000; Astrazeneca 2000).

Fadrozole

Fadrozole, also known by its trade name Afema®, is an aromatase inhibitor medication that was initially developed for the treatment of hormone receptor-positive breast cancer, similar to other drugs like anastrozole and letrozole. However, Fadrozole has been less commonly used compared to other aromatase inhibitors due to its limited availability and the development of alternative medications with better efficacy and safety profiles (Michaud and Buzdar 1999). Fadrozole works by inhibiting the activity of the enzyme aromatase, that is accountable for turning androgens into estrogens. By reducing estrogen production in the body, Fadrozole helps to slow down or inhibit the growth of hormone receptor-positive breast cancer cells that rely on estrogen for their growth (Chumsri et al. 2011). Fadrozole is generally used in postmenopausal women with hormone receptor-positive breast cancer, as these women have reduced ovarian estrogen production and rely more on peripheral tissue aromatization for estrogen synthesis (Raats et al. 1992).



Initially developed as an aromatase inhibitor for addressing estrogen-dependent breast cancer (Dutta and Pant 2008), Fadrozole was later discovered to exhibit significant potency in inhibiting aldosterone synthesis, both in laboratory settings (Schumacher et al. 2013) and clinical applications (Demers et al. 1990; Trunet et al. 1992). Therefore, Fadrozole has proven to be an effective second-line therapeutic choice for

Table 2 Anastrozole in fish masculinization

Fish species	Methods	Doses of AI	Masculinization (%)	References
Dwarf gourami (*Trichogaster lalius*)	Oral administration of anastrozole	200 ppm	90.32%	Katare et al. (2021)
Dwarf gourami (*T. lalius*)	Immersion treatment of anastrozole	1000 µg/L	100%	Katare et al. (2021)
Dwarf gourami (*T. lalius*)	Oral administration	60 AI+140 MT ppm.	89.70%	Katare et al. (2018)
Cyprinus carpio	Oral administration	(200 mg/kg	98.1%	Singh and Singh (2013)

postmenopausal women having metastatic breast cancer. Fadrozole is classified as a nonsteroidal, competitive aromatase inhibitor, as documented by Steele et al. (1987). An alternative approach to achieve sex reversal is by reducing estrogen production through the inhibition of aromatase activity. Aromatase is responsible for converting androgens into estrogens, and nonsteroidal inhibitors like Fadrozole (CGS 16949A; Steele et al. 1987) are used for this purpose. Fadrozole's mechanism of action has been described as that of a reversible competitive inhibitor. In this mode, both the substrate and the inhibitor for the same binding site on the aromatase enzyme (Brodie 1991).

Mechanisms of action

Fadrozole, an aromatase inhibitor, has gained attention in aquaculture and fisheries research for its potential to induce masculinization in fish populations. This process involves the development of male characteristics in female fish, and it has practical applications in the production of monosex fish populations, which can enhance growth and reproduction in aquaculture. The use of fadrozole in fish masculinization, the underlying mechanisms, and provides references to relevant studies (Venkatasubramanian et al. 2019). Fadrozole exerts its effects by inhibiting the enzyme aromatase, which plays a crucial role in the conversion of androgens into estrogens. By blocking aromatase activity, fadrozole reduces estrogen levels in fish, leading to a shift toward masculinization. This hormonal alteration results in the development of male secondary sexual characteristics, such as testes development and the production of sperm (Schroeder et al. 2017).

Fadrozole used as a fish masculinization

Tzchori et al. (2004) performed an investigation that demonstrated the ability of an aromatase inhibitor, fadrozole, to promote sex reversal in genetically female fry of the common carp at the key time of sexual determination. In vivo and in vitro tests on mammals (Schieweck et al. 1988) and chickens (Elbrecht and Smith 1992) have been used to demonstrate the effectiveness of this substance in lowering oestrogen production. In the area of fish research, Fadrozole's potency has been demonstrated in sexual differentiation studies involving the chinook salmon, *Oncorhynchus tshawytscha* (Piferrer et al. 1994), and Nile tilapia (Kwon et al. 2000). In vivo tests on developing female coho salmon, *Oncorhynchus kisutch*, revealed that fadrozole inhibits the production of oestrogen (Afonso et al. 1999).

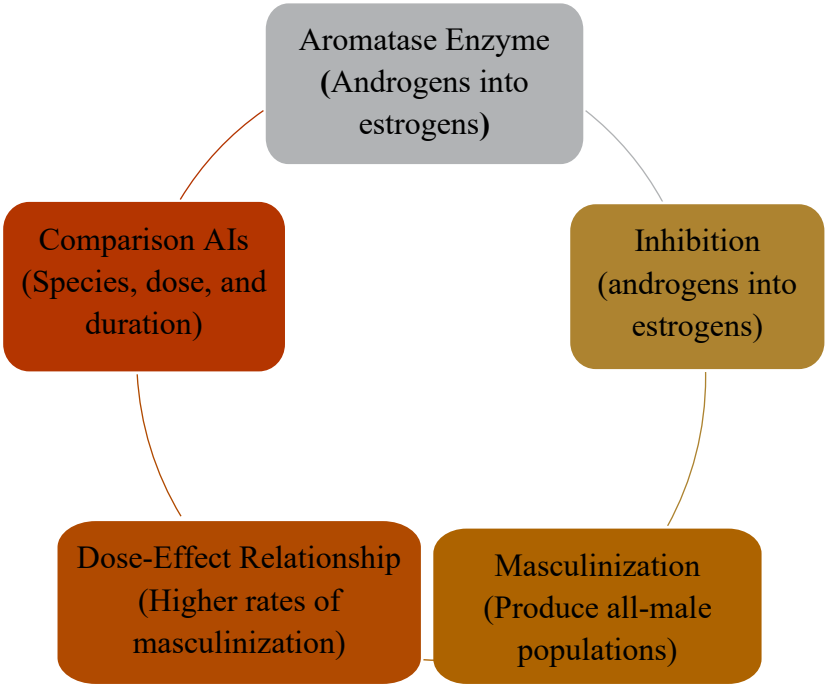


Fig. 2 The mechanism of action of fedrozole on fish masculinization

Sex reversal involves the reduction of estrogen production by inhibiting aromatase activity, which is responsible for converting androgens into estrogens. This inhibition is achieved using nonsteroidal inhibitors like Fadrozole (Steele et al. 1987). Fadrozole's mechanism of action has been characterized as a reversible competitive inhibition, where both the substrate and the inhibitor compete for the same binding site on the aromatase enzyme (Brodie 1991). In fish research, Fadrozole's effectiveness has been demonstrated in studies related to sexual differentiation in species such as the chinook salmon, *Oncorhynchus tshawytscha* (Piferrer et al. 1994), and Nile tilapia (Kwon et al. 2000). Fadrozole effectively suppressed estrogen synthesis in maturing female coho salmon, *Oncorhynchus kisutch*, as demonstrated (Afonso et al. 1999). However, the effects of fadrozole appear to be strongly influenced by the hormonal status of the fish, the stage of gonad development, and the specific fish population under investigation. Currently, it remains a challenge to provide a definitive explanation for the variation observed in fadrozole's impact, where in some instances it stimulated LH secretion while producing the opposite effect in other cases (Mikolajczyk et al. 2006).

Exemestane

Exemestane is an oral drug categorised as a substance that inhibits aromatase that is frequently offered under the commercial name Aromasin®. It is frequently given for the treatment of hormonal receptor-positive breast cancer, mainly in postmenopausal women. This enzyme is crucial for the generation of oestrogen from androgens (male hormones) in a variety of tissues, includes adipose tissue as well as the breast (Deeks and Scott 2009). By reducing estrogen levels in the body, Exemestane helps to slow down or inhibit the growth of estrogen-sensitive cancer cells that rely on estrogen for their growth. Unlike some other aromatase inhibitors, Exemestane is classified as a steroidal aromatase inhibitor. This means that it irreversibly binds to the aromatase enzyme, effectively deactivating it and preventing the conversion of androgens into estrogens (Chumsri et al. 2011). Exemestane is typically taken as an oral tablet on a daily basis. It is generally prescribed for postmenopausal women with hormone receptor-positive breast cancer, as these women have reduced ovarian estrogen production and rely more on peripheral tissue aromatization for estrogen synthesis (Zucchini et al. 2015).

Mechanism of action

Exemestane, a powerful aromatase inhibitor (AI), holds a critical role in the treatment of hormone receptor-positive breast cancer. Specifically designed for postmenopausal women, Exemestane aids in inhibiting estrogen production, thereby arresting the growth of hormone-sensitive breast cancer cells. This write-up explores the mechanism of action, clinical applications, and key considerations associated with Exemestane. Exemestane achieves its therapeutic efficacy by targeting aromatase, an enzyme predominantly found in adipose tissue. Aromatase plays a pivotal role in converting androgens, like testosterone, into estrogen. Exemestane acts as a selective and irreversible inhibitor of aromatase, effectively blocking this conversion. As a result, circulating estrogen levels substantially decrease, thwarting the hormonal stimulation of breast cancer cells and subsequently slowing their growth and proliferation (Glück 2010).

Table 3 Fedrozole in fish Masculinization

Fish species	Methods	Doses of AI	Masculinization (%)	References
Zebrafish (*Danio rerio),* (*Betta splendens*)	Fadrozole	50 µg/L	91%	Venkatasubramanian et al. (2019)
Nile tilapia (*Oreochromis niloticus*)	Oral administration	500 ppm of fadrozole-loaded PLGA	97.76% for 15 days	Joshi et al. (2019)
Nile tilapia (*O. niloticus)*	Oral administration	250 mg/kg feed	100%	Golan and Levavi-Sivan (2014)
Common carp (Cyprinus carpio)	Oral administration	100 mg/kg	97.1%	Singh and Singh (2013)
Fugu (*Takifugurub ripes*)	Oral administration	500 and 1000 µg/g feed	Prevented ovarian chamber development and induced testicular differentiate	Rashid et al. (2007)
Common carp and goldfish	Oral administration		sGnRHa-stimulated LH secretion	Mikolajczyk et al. (2006)
Golden rabbitfish (*Siganus guttatus)*	Administered Fadrozole	500 mg/Kg	87 and 100%	Komatsu et al. (2006)
Golden rabbitfish (*Siganus guttatus)*	Dietary administration	10 and 100 mg/Kg	38 and 60%	Komatsu et al. (2006)
Zebrafish (*Danio rerio*)	Oral administration	1000 µg g^{-1} $diet^{-1}$	100% gonadal masculinization	Uchida et al. (2004)
Common carp (*Cyprinus carpio L.)*	Dietary administration	200 and 400 mg/kg	97%	Tzchori et al. (2004)
Nile tilapia (*Oreochromis niloticus)*	Dietary administration	75 and 100 mg/Kg	100% all-male	Afonso et al. (2001)
Nile tilapia (*Oreochromis niloticus)*	Dietary administration	200 and 500 mg/Kg	92.5 and 96%	Kwon et al. (2000)
Japanese flounder (*Paralichthys olivaceus)*	Oral administration of Fadrozole	100 mg/Kg	100% allmale populations	Kitano et al. (2000)

Exemestane on fish masculinization

Exemestane is a steroidal aromatase inhibitor that can be taken orally and works irreversible by chemically approaching the androstenedione biological substrates (di Salle et al. 1992). It is known as an aromatase "inactivator" due to its "suicidal" suppression of aromatase, which is comparable to formestane. Exemestane demonstrates robust inhibition of periphery aromatase action, which is characterised by the dependent on time inactivation of the enzyme. In fact, it exhibits an impressive 40 to 156-fold higher efficacy against aromatase when compared to aminoglutethimide (di Salle et al. 1994; 1990; Giudici et al. 1988). Exemestane works as a steroids irreversible inactivator of aromatase, a key enzyme involved in the conversion of androstenedione and other steroids into oestrogen. In the case of fish, the transformation from female to male during sex change is correlated with a decline in estrogen levels (Bhandari et al. 2003). Aromatase inhibitors (AIs) have been created as pharmaceuticals to suppress oestrogen production, especially for cancer treatment (Steele et al. 1987). Among the third-generation AIs, exemestane stands out as an oral steroidal-type AI renowned for its remarkable efficacy in treating metastatic breast cancer (Dank 2002). Exemestane achieves this by inhibiting aromatase, utilizing a chemical structure that closely resembles the natural substrate androstenedione.

17α-methyltestosterone (MT)

17-alpha-methyltestosterone is a synthetic derivative of testosterone, which is a male sex hormone (androgen). This compound has been used medically in the past to treat various conditions, but its use has significantly decreased due to safety concerns and the availability of alternative treatments (Rivero-Wendt et al. 2020). 17-alpha-methyltestosterone has androgenic properties, meaning it can promote the development of male secondary sexual characteristics, such as facial hair growth and deepening of the voice (Lindqvist 2004). In addition to its androgenic effects, 17-alpha-methyltestosterone also has anabolic properties, meaning it can promote muscle growth and protein synthesis. This led to its use in bodybuilding and athletic circles, although

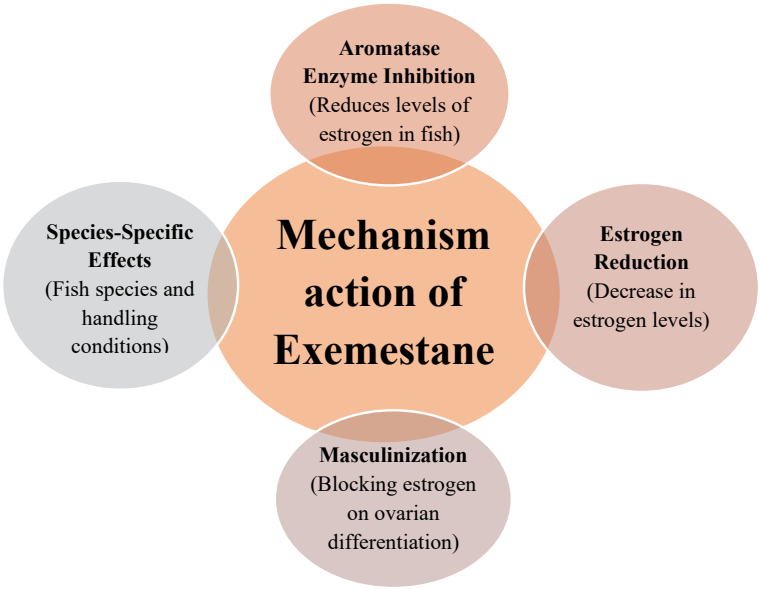


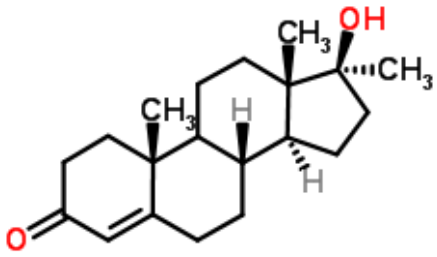
Fig. 3 Mechanism action of exemestane on fish masculinization

Table 4 Exemestane in fish masculinization

Fish species	Methods	Doses of AI	Masculinization (%)	References
Olive flounder (*Paralichthys olivaceous*)	Exemestane and tamoxifen	-	Expression of estrogen receptor mRNA and vitellogenin mRNA	Kim et al. (2023)
Zebrafish (*Danio rerio*)	Injection of exemestane	35 lL	Retraction of the ovary	Rahaman et al. (2020)
Red tilapia (*Oreochromis* spp.	Oral administration of Exemestane	100 mg/Kg	90.6 ± 1.21%	Betancur López et al. (2014)
Nile tilapia (*Oreochromis niloticus)*	Oral administration of Exemestane	1000 and 2000 mg/Kg	100% masculinization	Ruksana et al. (2010)
Nile tilapia (*Oreochromis niloticus*)	Oral administration of Exemestane	100 and 500 mg/ Kg	8.3 and 83.3%	Ruksana et al. (2010)
Nile tilapia (*Oreochromis niloticus)*	Oral administration		75.3% males	Guiguen et al. (1999)

its use for this purpose is not recommended due to safety concerns (McCullough et al. 2021).

Structure



Molecular Formula:-$C_{20}H_{30}O_2$
Molecular Weight:-302.5
Brand name: Android
MT is a synthetic androgenic steroid with a rich history in both medicine and sports. It is a modified derivative of testosterone, the primary male sex hormone. This comprehensive write-up explores its chemical structure, medical applications, and mechanisms of action, side effects, and legal status, with references to credible sources. The chemical structure of MT includes a methyl group added at the 17th carbon position of the testosterone molecule. This alteration is crucial as it enhances its oral bioavailability, allowing it to resist rapid liver metabolism, a characteristic central to its pharmaceutical use (Saartok et al. 1984).

Mechanism of action

MT operates through a mechanism similar to testosterone. It binds to androgen receptors in various tissues, including muscle and bone cells, promoting the activation of specific genes responsible for the development of male secondary sexual characteristics (Heinlein and Chang 2002). Androgen receptors are proteins located within the cells that are specifically designed to bind with androgens, including testosterone and its synthetic derivatives like MT. This includes enhanced muscle growth, increased bone density, and the development of facial and body hair. When MT binds to an androgen receptor, it forms a hormone-receptor complex. This complex then translocates to the cell nucleus (Gao et al. 2005).

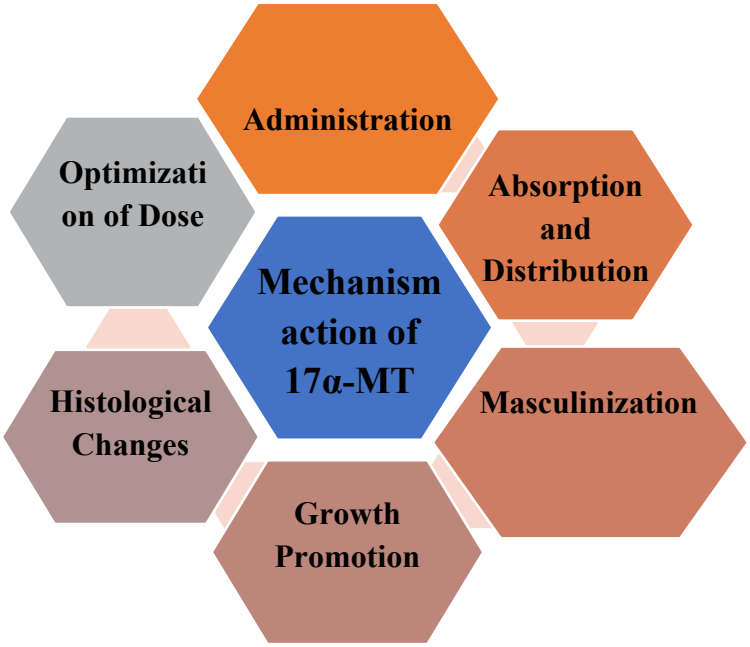


Fig. 4 The mechanism of action of MT on fish masculinization

17α-methyltestosterone used in fish as a masculinization

In broad terms, estrogen treatment tends to induce feminization in genetic males, while androgen treatment promotes masculinization in genetic females. In order to create male monosex populations of Nile tilapia, farmers often use hormonal treatments, with MT being a popular option. The popularity of MT is primarily due to its simplicity, high efficiency, reliability, and cost-effectiveness (Baroiller and Cotta 2018). This method is expected to remain the predominant means of obtaining all-male offspring for a significant duration in key tilapia-producing nations. The androgen MT has undergone testing across more than 25 species within various fish families, including Salmonidae, Cichlidae, Cyprinidae, Anabantida, Poecilidae, and Cyprinodontidae. Notably, Cichlidae typically require lower androgen doses compared to other fish families (Beardmore et al. 2001).

Kitano et al. (2000) reported the sex of Japanese flounder can be altered by water temperature or sex steroid hormone treatment. High water temperature can suppress P450 aromatase gene expression and cause phenotypic sex-reversal to males. Treatment with fadrozole or MT induces sex-reversal and suppression of P450arom gene expression. This effect is counteracted by co-administration of estradiol-17β. RT-PCR does not detect P450arom mRNA in males. MT a man-made male sex hormone, is utilized in the treatment of hormone deficiencies. It controls or suppresses the expression of aromatase, which could potentially impact the production of estrogen. This finding has significance in comprehending the impacts of 17α-methyl testosterone in hormone replacement therapy and could perhaps provide unique opportunities for the prevention or treatment of hormone-sensitive cancer (Mor et al. 2001).

Aromatase inhibitor on fish masculinization

Aromatase inhibitors are drugs that block the action of the enzyme aromatase, which is responsible for converting androgens (male sex hormones) into estrogens (female sex hormones). These inhibitors are often used in medical contexts to treat conditions like breast cancer and hormonal imbalances. They can also have unintended effects when they come into contact with aquatic environments (Miller 2003). In aquatic biology, the term “fish masculinization” is often used in the context of endocrine disruption caused by exposure to certain chemicals, including aromatase inhibitors. Endocrine disruptors can interfere with the normal hormonal signaling in fish and other aquatic organisms, leading to various physiological and reproductive abnormalities. In the case of aromatase inhibitors, they can affect fish by reducing the conversion of androgens into estrogens, which can lead to a skewed hormonal balance in male and female fish (Delbès et al. 2022).

1. Exposure: Aromatase inhibitors may enter aquatic environments through various sources, such as wastewater discharge or runoff from agricultural areas where these compounds are used.

2. Hormonal disruption: When fish are exposed to aromatase inhibitors, their endocrine system can be disrupted. In males, this can lead to increased levels of androgens, resulting in the development of male secondary sexual characteristics (muscularization) even in genetic females. In females, it can lead to reduced estrogen levels, affecting their reproductive health (De Ronde and de Jong 2011).

3. Effects: The effects of masculinization in females and altered hormonal balance in males can include changes in behavior, reproduction, and overall health. For example, female fish may exhibit masculinized physical traits, reduced fertility, and altered behaviors associated with mating and parenting (Hines 2011).

Mechanism of aromatase inhibitors

The mechanism underlying the action of aromatase inhibitors in fish masculinization entails the suppression of the aromatase enzyme, responsible for converting androgens into estrogens. Through preventing aromatase activity, the synthesis of endogenous estrogens is disrupted, leading to higher levels of androgens and a shift towards a male phenotype in fish.

1. Inhibition of aromatase enzyme: Aromatase inhibitors, such as letrozole or fadrozole, work by binding to the active site of the aromatase enzyme, preventing its activity. Aromatase is responsible for the conversion of androgens, such as testosterone, into estrogens, including estradiol. By inhibiting aromatase, the transformation of androgens into estrogens is blocked, leading to an increase in androgen levels (De

Table 5 17α-methyltestosterone in fish Masculinization

Fish species	Methods	Doses of AI	Masculinization (%)	References
Siamese fighting fish (*Betta splendens*)	Supplemented diet	60 and 100 mg kg $^{-1}$ of 17α-MT	Successfully produced all-male stocks	Kumkhong et al. (2024)
Nile tilapia (*O. niloticus*)	Oral administration	30 mg MT kg^{-1} feed	99.8%	do Carmo Ota et al. (2024)
Rainbow trout (*Oncorhynchus mykiss*)	Oral administration	70 mg/kg feed	86%	Hajibeglou and Machanlou, 2023)
Red tilapia (*Oreochromis spp.*).	Immersion technique	1000 µg/L of 17 MT for 6 h	95.33%	Karaket et al. (2023)
Mandarin fish (*Siniperca chuatsi*)	Oral administration	500 mg/kg	14.7%	Huang et al. (2023)
Red Tilapia (*Oreochromis spp.)*	Oral absorption		50%	Yostawonkul et al. (2023)
Yellowtail tetra (*Astyanax lacustris*)	Orally administered	20 and 60 mg MT/kg	76.7%	Farias et al. (2023)
Nile Tilapia (*Oreochromis niloticus*)	Oral administration	70mg/kg	95%	Asad et al. (2023)
Rainbow trout (*Oncorhynchus mykiss*)	Commercial trout diet		76.67%	Irani and Noori, (2023)
Tilapia (*Oreochromis niloticus*)	Through feed	MT at 25 days	undifferentiated gonads	Silva et al. (2023)
Orange-Spotted Grouper (*Epinephelus coioides*)	Oral administration	50 mg/kg	*Sex-change impermanent*	Carman et al. (2023)
Rosy barb (*Pethia conchonius*)	Oral administration	25 mg/kg MT through gel-based feed	84.72%	Lal et al. (2023a); Lal et al. (2023b)
Oriental river prawn (*Macrobrachium nipponense*)	Dietary supplementation	200 mg/kg MT	Neo-males (sex-reversed female prawns)	Cai et al. (2023)
Rock bream (*Oplegnathus fasciatus*)	Oral administration	MT	100%	Liu et al. (2023)
Nile tilapia (*Oreochromis niloticus)*	Oral administration	60 mg/kg	94% masculinization	e Silva et al. (2022)
Mandarin fish (*Siniperca chuatsi*)	Orally administered	100 mg/kg	100%	Liu et al. (2021)
Red Tilapia Hybrid	Oral administration	60 mg/kg	90% masculinization	M Zaki et al. (2021)
Blue Peacock Cichlid (*Aulonocara nyassae*)	Oral administration	100 mg/kg diet	100% masculinization	Karsli, (2021)
Nile tilapia (*O. niloticus*)	Oral administration	10 mg/kg	95.65%	Ghosal et al. (2021)
Rainbow trout (*Oncorhynchus mykiss*)	Immersion methods	400 µg/L	100%	Weber et al. (2020)
Nile tilapia (*O. niloticus*)	Orally administered			Chávez-García et al. (2020)
Ussuri catfish, (*Pelteobagrus ussuriensis*)	Oral administration	MT (Methyltestosterone) and AI (Artificial Insemination)	Preferred approach for masculinizing	Wang et al. (2019)
Walking catfish (*Clarias magur*)	Oral administration	100mg/kg	Better result	Wisdom et al. (2018)
Red tilapia (*Oreochromis niloticus*)	Oral administration	60 mg/kg diet	The maximum sex reversal	Singh et al. (2018)
Bluegill sunfish, (*Lepomis macrochirus*)	Oral administration	500mg kg-1 diet	Highest male	Gao et al. (2010)
Nile Tilapia (*O. niloticus*)	Oral administration	75 and 100 mg/kg	100% male population	Afonso et al. (2001)

Ronde and de Jong 2011).

2. Increase in androgen levels**:** With the inhibition of aromatase, the levels of androgens, particularly testosterone, rise in the fish's body. Androgens are male sex hormones liable for the emergence of male characteristics, including muscle growth and secondary sexual traits.

3. Masculinization of phenotype**:** Elevated androgen levels, resulting from aromatase inhibition, drive the masculinization process in fish. This includes the growth of secondary masculine sexual traits in men, which include more muscle, altered fin morphology, and coloration patterns associated with males (Hayman et al. 2021).

It is important to note that the specific effects and extent of masculinization may vary among fish species and individuals. Factors such as species-specific endocrine regulation and genetic backgrounds can influence the response to aromatase inhibitors. It is worth mentioning that the masculinization process using aromatase inhibitors is temporary, as the effects are reversible when the inhibitor is no longer administered. Once the inhibition of aromatase is lifted, endogenous estrogen synthesis resumes, and the fish can potentially revert to their original female phenotype. While the use of aromatase inhibitors for fish masculinization has shown promise, further research is needed to better understand the molecular and physiological mechanisms underlying the process. Additionally, the long-term effects and potential impacts on fish reproduction, behavior, and ecological interactions require thorough investigation to ensure the responsible and sustainable use of these inhibitors in fish populations (Chen et al. 2020).

Oral masculinization methods

Oral administration of the synthetic androgen MT is used in the rainbow trout business to induce sex reversal in hereditary females, converting them into homogametic XX males, commonly known as neomales. All-female lines can be made with the help of this method. Because of the difficulties related to earlier sexual maturity in males, it is essential to produce all-female lines in order to reduce production losses and maintain product quality (Bye and Lincoln 1986). Furthermore, the creation of all-female lines becomes essential when triploidy is implemented, as it helps circumvent the adverse effects of gonadal development. In triploid salmonids, ovarian development is significantly impaired, while testicular development remains less affected, as previously elucidated by Lincoln and Scott in 1984 and more recently confirmed by Han et al. (2010).

Present approaches for masculinizing fish populations involve the incorporation of androgens into feed. This process typically entails newly hatched larvae being reared in specialized enclosures known as hapas or ponds, where they are directly fed on the pond surface. While this method serves its purpose, it does carry the risk of environmental contamination. An inherent drawback is the uneven distribution of hormones as social feeding hierarchies develop among the fish. Moreover, hormone loss occurs due to uneaten food and dispersion into the water column. It's worth noting that hormones dissolved in water have been observed to degrade within approximately a week in the water column. However, there is evidence of long-term persistence in pond sediments, as demonstrated by Fitzpatrick et al. 1999. As an alternative to feeding, another technique involves immersing fish in a solution containing hormones. This approach has been employed successfully in masculinizing salmonids and tilapia, as documented in studies conducted by Piferrer and Donaldson (1989), Feist et al. (1995), and Contreras-Sanchez et al. (1999).

Fish masculinization techniques through immersion

A novel protocol for the immersion-based administration of the steroid has been devised (Weber et al. 2020). In this method, female rainbow trout are immersed in a 400 g/L concentration of MT in a static bath. The immersion duration spans 2 hours, and the initial immersion takes place one week after hatching. Subsequently, at the start of first feeding, a series of 6 immersions once a week begins. This meticulously crafted protocol consistently yields a nearly 100% male population, and approximately half of these males exhibit functional gonads. An advantage of this procedure is that it produces only a little amount of steroid-tainted water, making cleanup and disposal less of a hassle. It's noteworthy that this protocol was developed through refinement of a treatment proposed by Feist et al. (1995) that also entailed immersing rainbow trout in a 400 g/L MT solution for two hours in a static bath, beginning a week after hatching.

In the updated protocol, a significant modification involved the introduction of weekly treatments, commencing at the onset of first feeding. This adjustment was inspired by the proven efficacy of oral administration when initiated at this critical developmental stage. Typically, first feeding occurs approximately three weeks post-hatching, a timeframe that aligns with our hatchery facilities' water temperatures, which usually range between 12 and 14°C. It's important to note that during the protocol's development, the impact of shorter immersion durations or starting the immersions at a later developmental stage was not assessed. However, Weber and Leeds (2022) conducted a study to investigate the impact of immersion length and the interval between the first and second immersions. Their findings indicated that a 1-hour immersion proved to be more effective compared to the 2-hour immersion duration. The current gold standard for MT immersion protocol, as established by Weber et al. (2020), is widely employed in a comprehensive pedigree-based selective breeding program for rainbow trout. The ongoing effort is aimed at enhancing this immersion protocol by conducting a thorough evaluation of two critical factors: the number of weeks between immersions and when the second immersion should be performed, all with the goal of optimizing sex reversal outcomes. In contrast, Gale et al. (1999) achieved more favorable results, ranging from 73% to 92%, by employing a 3-hour immersion treatment on both the 10th and 13th days post-fertilization. The relatively inconsistent and ineffective outcomes seen in sex reversal, as reported in various studies, may be attributed, at least in part, to challenges related to inadequate and non-uniform diffusion of hormones into tilapia larvae. For instance, Bart et al. 2003 observed that fish immersed in only 20–25% of males were produced using MT solutions for 2 and 48 hours.

Ultrasound-based immersion techniques for inducing fish masculinization

A novel approach utilizing low-frequency ultrasound with controlled cavitation levels has been applied to improve the transport of substances into both human and fish tissues (Mitragotri et al. 1995). According to the study by Bart et al. (2001), they used the cavitation effect to improve the release of calcein through the skin and gills of fish. Mitragotri et al. (1995), on the other hand, used a changed ultrasonic bath with lower voltage to create cavitation, which made it easier for insulin-like proteins to pass through human skin tissue. While it is well known how ultrasound-enhanced transport works with controlled cavitation levels in human skin (Mitragotri et al. 1995), it is still not completely clear how and at what rate substances enter fish models. We thought that treating tilapia fry with an immersion procedure and ultrasound would improve the movement of hormones, which would make the rate of masculinization more consistent and high. Using two different hormones, 17α-methyldihydrotestosterone (MDHT) and trenbolone acetate (TBA), each at two different concentrations (100 and 250 pg/L), in immersion protocols, the main goal of this study was to find out how ultrasound-induced cavitation levels affected sex inversion. Additionally, out of the three samples that were treated with TBA at 250 pg/L along with ultrasound, two had an amazing result: all three sets of fry turned into males, with the highest mean percentage of males being 98%.

An alternative way of fish masculinization

Climate variability has a significant effect on fish reproduction trends, profitability, availability of food, as well as recruitment (Walther et al. 2002). Many fish and reptile species exhibit temperature-sensitive gender determination (TSD), a phenomenon in which environmental temperature has a long-lasting effect on the sex assignment of developing living things (Ospina-Alvarez and Piferrer 2008; Conover 1984; McNair et al. 2015). It is called environmental gender determination (ESD) when an organism's sex is affected by its surroundings, including temperature (Charnov and Bull 1977). The sexuality of teleost fishes is highly flexible, and their sex is determined by a number of different systems, some of which are sensitive to environmental and social cues (Devlin and Nagahama 2002; Capel 2017).

Gender-skewed sex ratios occur when genetic females are exposed to cooler (18°C) or warmer (28°C) water temperatures or a blue tank background colour throughout a crucial early stage of development (approximately 35 to 65 mm total length, TL) in fish culture. This means that a maximum of 50% females is possible, and that male-biased sex ratios may result from high temperatures or other circumstances that cause XX females to become more masculinized. The influence of the surrounding environment on gender ratios in juveniles in southern founder populations or in other Paralichthids species in their native envi-

ronments has not been studied. If these fish encounter adverse conditions during the critical phase of sex determination, there exists a potential risk of masculinization, which could have detrimental consequences for an already declining fishery that relies on females. The presence of annual or geographical variations in sex ratios among wild juvenile southern founder remains unknown. In particular, when temperatures are held constant in lab conditions, southern founder show a tendency towards sex reversal, with temperatures of 18 and 28°C inducing male development and 23°C emerging as the ideal temperature for stimulating female development (Luckenbach et al. 2003).

Honeycutt et al. (2019) observed that the highest proportion of female founder fish (approximately 35%) was obtained when the temperature profile of the habitat was replicated in a recirculating system, encouraging fluctuations in temperature that on average around 23°C throughout the critical stage of sex determination. This was much higher than the percentage of females present in the 19°C and 27°C treatments, respectively. Despite having the highest percentage of females, the group exposed to varying temperatures of 23°C also showed a modest male bias, with about 65% males instead of the expected 1:1 sex ratio. In natural settings, the temperature of the water varies on a daily and yearly basis. Because of this, it has been argued that research with steady temperatures may not adequately depict the complicated nature of natural environmental influences (Kingsolver et al. 2015).

Synergistic effects of aromatase inhibitor

The synergistic effects of aromatase inhibitors in fish masculinization have been demonstrated in various species, including Nile tilapia and rosy barb. The combined use of AIs with other sex steroids can enhance masculinization, leading to improved growth rates and coloration in fish. A study on the synergistic effects of anastrozole-17a and methyltestosterone in the fish *T. lalius* found that the combined treatment enhanced masculinization compared to individual treatments. The findings indicated that the highest level of masculinization was observed at a concentration of 60 parts per million (ppm) of AI combined with 140 ppm of MT. Additionally, the treated groups exhibited a prevalence of female offspring with dominating characteristics, as well as a suppression of ovarian function and a hindrance in the production of estradiol, a form of estrogen (Katare et al. 2018). Letrozole and MT are two examples of non-steroidal aromatase inhibitors that have shown a dramatic uptick in use during the past decade for changing the sex ratio and altering gonadal development in Mozambique tilapia. Letrozole, a nonsteroidal aromatase inhibitor, was shown to efficiently modify the sex ratio of *O. mossambicus* in a seminal work by Das et al. (2012). For instance, a diet containing 100 or 200 mg/kg of letrozole alone produced 97-100% male offspring. Furthermore, when letrozole was combined with MT, it also led to a male-dominant population of 92-100%. This represents a significant milestone, possibly marking the first documented instance of complete masculinization of *O. mossambicus* through the use of letrozole.

This implies that the aromatase inhibitor likely impeded the conversion of MT or natural androgens into estrogens, a notion supported by the findings of Piferrer et al. (1994). They observed that when a combination of MT treatment and fadrozole was employed, it resulted in an overwhelmingly high proportion of males, reaching approximately 98-100% (at MT 10 mg/kg + fadrozole 10 mg/kg). Additionally, Piferrer et al. (1994) demonstrated that the combination of LET 100 + 17α-MT 25 mg consistently produced a substantial male population, implying that letrozole effectively blocked the aromatization of aromatizable MT, thereby restoring its complete androgenic potential.

Gonadal masculinization in all-female triploid rainbow trout (RBT) populations mediated by exogenous testosterone and aromatase inhibitors was investigated by Xu et al. (2020), who revealed the complex biochemical pathways underlying this process. In particular, from day 94 post-fertilization (dpf) through day 377 dpf, letrozole (LET) was more successful than MT in promoting morphological masculinization of the gonads. Aromatase inhibitors (AIs) are thought to be helpful for sex reversal in fish by reducing the amount of endogenous estradiol produced, which in turn reduces the amount of 11-ketotestosterone (11-KT) produced from testosterone (T). This perturbation promotes spermatogenesis by triggering the proliferation of spermatogonia. 11-ketotestosterone (11-KT) levels were shown to rise with LET treatment, as was predicted (Garcia et al. 2013; Li et al. 2005; Miura and Miura 2003). This resulted in the commencement of spermatogenesis in physiologically immature fish.

Ranjan et al. (2015) showed that female greasy grouper might undergo a transient sex reversal when

given the hormone MT alone or in combination alongside the female hormone letrozole, whereas male control fish maintained their normal ovarian development. Inhibition of MT to oestrogen via letrozole has been hypothesised to account for this result (Piferrer et al. 1994). Additionally, it is plausible that letrozole prevents the ovaries from producing estrogen (E2). Notably, the combination of 0.2 mg of letrozole and 5 mg of MT per kilogram of body weight (BW) led to the complete transformation of all individuals into M2 stage males from the 60th day onward. In contrast, when 5 mg of MT per kilogram BW was administered alone, it resulted in only 66.67% of individuals reaching the M2 stage after 120 days. This suggests that letrozole plays a crucial role in inhibiting the aromatization of MT, facilitating the early transformation of females into sex-reversed males as early as 60th days.

This notably short treatment period of 60th days stands as one of the quickest recorded instances of successful sex reversal in greasy grouper. Treatment with MT likely elevates androgen levels in the bloodstream. However, it's important to note that MT can also be converted into estrogen by the enzyme P450 aromatase, potentially leading to an increase in estrogen levels (Kwon et al. 2002). Furthermore, alongside the exogenous steroids introduced into the system through implantation, the use of an aromatase inhibitor (AI) serves to boost androgen levels by blocking the conversion of androgen to estrogen, ultimately increasing the androgen-to-estrogen ratio. Consequently, the effectiveness of aromatase inhibitor treatment in combination with exogenous steroids for inducing male differentiation surpasses that of treatment with MT alone.

LET and MT affects on fish gonadosomatic index (GSI)

The Gonadosomatic Index (GSI) serves as a widely employed, general biomarker for assessing potential adverse impacts of environmental pollutants, as noted by Naderi et al. (2014). The purpose of this index is to provide a simple metric for quantifying and documenting the degree of maturity of an exposure group. For example, Noaksson et al. (2005) found that GSI might be used as a proxy for reproductive capacity in some investigations. In a study by Rivero-Wendt et al. (2020), it was observed that the GSI values in female and male zebrafish remained unchanged, indicating that MT did not exert an influence on the reproductive capacity of adult zebrafish. Conversely, a trend towards a decrease in the GSI of male gonads was noted, implying that androgens could potentially lead to atrophy of the male gonad.

In the study conducted by Joshi et al. (2015), the gonadosomatic index (GSI) in female groups treated with LTZ-loaded PLGA nanoparticles exhibited a slight decrease as the dosage increased. Conversely, in males, the GSI increased with higher dosages of LTZ-loaded PLGA nanoparticles. In a separate investigation, Das (2007) found that different dosages of letrozole, alone and in combination with MT, had no appreciable effect on the GSI of *O. mossambicus*. Piferrer et al. (1994) reported in our studied that genetic females given fadrozole had GSI and maturation rates that were indistinguishable from those of conventional males. The GSI value are increased in males with larger dosages of LTZ-loaded PLGA nanoparticles are consistent with those of Ankley et al. (2002), who reported that GSI in males increased with increasing dosages of fadrozole.

Application of aromatase inhibitors for fish masculinization

The application of aromatase inhibitors (AIs) has been studied extensively for the purpose of masculinization in fish. AIs work by blocking the conversion of androgens into estrogens, which is crucial for sexual differentiation in fish. This allows for the development of male characteristics and can be used to create all-male populations, which can be commercially valuable due to the higher market demand for male fish. A study published in the Revista Colombiana de Ciencias Pecuarias found that the oral administration of Letrozole (LET) and Exemestane (EM) at different doses (25 and 100 mg/Kg) was effective in increasing the proportion of male red tilapia *(Oreochromis spp.*) without significant differences between the two inhibitors (Betancur et al. 2014). Another study used dietary aromatase inhibitors to induce masculinization in rosy barb (*Pethia conchonius*). The results showed that the treatment increased the growth rate and coloration of the fish, and the gonado-somatic index (GSI) was significantly reduced in females (Lal et al. 2023a). A study on the rice field eel (*Monopterus albus*) found that the oral administration of Letrozole (300 mg/kg feed) led to the conversion of primary ovaries into mature testes. The fish maintained their male sex

and produced functional sperm after the cessation of treatment (Jiang et al. 2022). A study on the western mosquitofish (*Gambusia affinis*) assessed the effects of aromatase inhibition on fishes with group-synchronous oocyte development. The results showed that exposure to aromatase inhibitors led to reproductive dysfunction and a reduction in plasma vitellogenin (VTG) levels (Doering et al. 2021). The use of aromatase inhibitors has been shown to be an effective method for inducing masculinization in various fish species. The specific inhibitor and dosage used can affect the outcome, but overall, these compounds have been found to be useful in creating all-male populations.

Growth characteristics of fish during masculinization

The potential for enhancing fish growth through the use of natural and synthetic steroid treatments has been explored across various fish species, including *Perca flavescens, Oreochromis niloticus, Cyprinus carpio, Oncorhynchus mykiss,* and *Oreochromis aureus* (Pandian and Sheela 1995). According to Dekimpe and Micha (1974), male African mud catfish tend to achieve larger sizes than females, a phenomenon attributed to the anabolic effects of androgens. Furthermore, the application of exogenous androgen, such as MT), has been found to enhance the growth of *Clarias gariepinus* by stimulating protein biosynthesis, with the extent of growth improvement correlated with the dosage and duration of MT treatments.

The effects of adding tamoxifen to bagrid catfish (*Pseudobagrus fulvidraco*) feed pellets on the fish's growth were studied by Park et al. (2003). The results showed a marked increase in the growth rate compared to the control group, with the best growth rate occurring at a concentration of 50 ppm. Similarly, Turan and Akyurk (2005) found that feeding fingerlings of the African mud catfish (*Clarias gariepinus*) a meal that included 75 mg of red clover, a phytoestrogen, per kilogramme significantly increased their growth rate. When compared to a control group, the experimental group saw significantly more growth. Furthermore, *Tribulus terrestris* extract, a phytoandrogen, has been shown to stimulate growth in both convict cichlid (*Cichlasoma nigrofasciatum*) and guppy (*Poecilia reticulata*) (Cek et al. 2007a, 2007b), demonstrating the potential advantages of phytoandrogens in this regard.

According to Enuekwe and Okonji's (2019) research, *Clarias gariepinus* final body weight and net output significantly increased following a 180th day grow-out phase. In particular, when compared to the placebo group, those who took 60 mg of MT twice daily for 28 and 35 days experienced a significant improvement. This result clearly demonstrates that *C. gariepinus* development rate as well as biomass output is greatly increased after MT treatment, showing the treatment's effective growth-promoting impact.

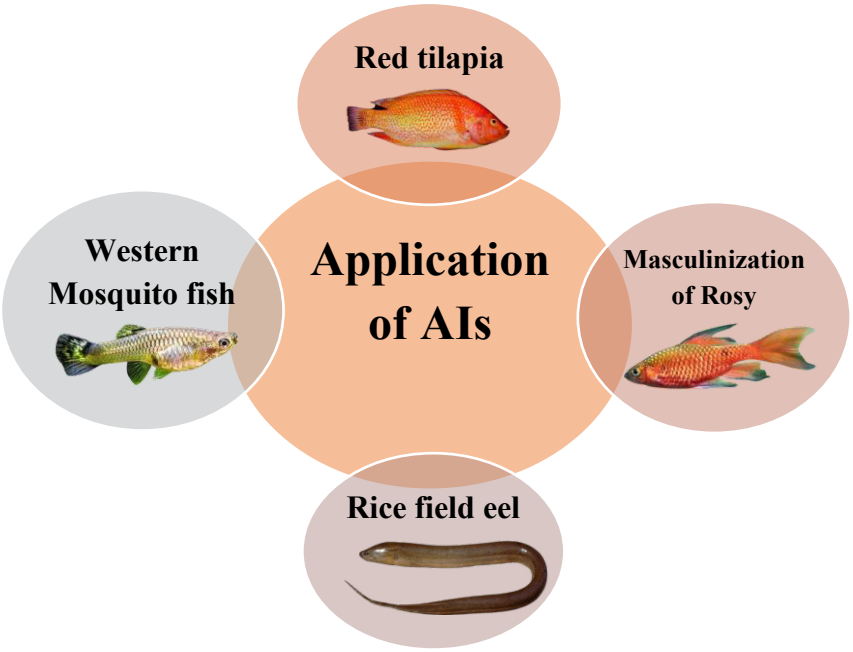


Fig. 5 Application of aromatase inhibitors on fish masculinization

Among the treatment groups, the one administered 60 mg of MT per kilogram of feed for duration of 35 days exhibited the highest total fish production, reaching 13,411g. This level of fish production surpassed the control group, which did not receive any hormone treatment, by 1.4 times. These results align with prior research indicating the anabolic effects of MT in fish (Mateen 2007).

Effects of AIs on environmental sustainability and consumer well-being

Fish have complex systems for determining their sex, which include both heritable and environmental factors, as well as a hybrid form that incorporates both. Fish sex differentiation is similarly complex and sensitive to a wide range of variables, such as raising temperature, external hormones or substances associated with hormone pathways (e.g. aromatase inhibitors, estrogen/androgen receptor antagonists), social variables, as well as epigenetic alterations. It is difficult to produce monosex communities and to fully comprehend the underlying mechanisms due to the complexity and plasticity of the systems as well as processes that contribute to the ultimate determination of an individual's sex (Shen et al. 2015).

The incorrect or illegal dumping of water including residue of these substances, most commonly employed for fish treatment, is another pathway for hormones to enter the environment. Medicated feed that isn't digested by the fish is one potential source of this contaminating thing, along with fish excrement. Several researchers have found that the liver is essential for the metabolization of these hormonal substances into water-soluble molecules (Leonhardt 1997; Specker and Chandlee 2003). The hormones used for sex reversal are almost entirely eliminated from the body via the process of metabolism and elimination in the form of bile as well as urine within a matter of hours or days.

Estrogens are subsequently excreted primarily in the form of inert conjugates composed of sulfuric and glucuronic acids. These conjugates lack direct biological activity but serve as reservoirs for precursor hormones, which can potentially be transformed back into active steroids with estrogenic properties by environmental bacteria. This bacterial activity is particularly evident in environments like raw sewage and sewage treatment plants, as demonstrated by Ying et al. (2002). In simpler terms, the sex reversal technique has the potential to introduce environmental pollution if the water used for fish treatment is not adequately purified and treated. Sewage effluents include azoles and may block aromatase, however there is insufficient data to conclude that they are producing masculinizing effects in fish. This ambiguous effect may be attribuTable, in part, to the relatively high levels of potent estrogenic substances also found in sewage effluent, which may counteract masculinizing influences (although recent research has shown that elevated estradiol levels can reduce CYP19 expression in fish, as shown by Shanthanagouda et al. (2013). The quantities of azoles shown in laboratory experiments to cause masculinization in fish are far higher than those found in these effluents, possibly by as much as three orders of magnitude when additive effects are included. The apical implications of fish, which might be defined as encompass characteristics akin to masculinization, can arise from various other factors, as previously outlined. The potential consequences indicated previously may resemble responses seen in wild fish populations near particular pulp mill and wastewater discharges. Although none of these findings have been disproven, no causal relationship between azole exposure and any of these observations has been established. In addition, aromatase inhibition does not appear to be a common mechanism underlying these effects. Azide-based biocides and medicines have been shown to be present in wastewater discharges, albeit at trace amounts (Matthiessen and Weltje 2015).

Fish health concern with aromatase inhibitors

Aromatase inhibitors have been found to have significant adverse effects on the health of fish. These chemicals can inhibit the activity of cytochrome P450 aromatase (CYP19), leading to decreased plasma 17β-estradiol (E2) and vitellogenin (VTG) levels, which are essential for oocyte development and maturation. This inhibition can cause reproductive dysfunction and decreased egg production in fish, potentially leading to population-level declines (Doering et al. 2021; Liao et al. 2014). Inhibition of aromatase activity can disrupt the reproductive cycle of fish, leading to decreased egg production and viability. This can have significant impacts on fish populations and potentially even lead to population declines (Doering et al. 2021; Liao et al. 2014). Aromatase inhibitors can also cause feminization in male fish, leading to the development of female characteristics. This can be seen in the increased production of VTG, a protein typically produced in female

fish, and the suppression of testicular development (Lal et al. 2023a). Exposure to aromatase inhibitors can disrupt hormone homeostasis in fish, leading to changes in the endocrine system and potentially causing adverse effects on overall health (Liao et al. 2014). Some studies have found that aromatase inhibitors can also affect antioxidant activity in fish, potentially leading to increased oxidative stress and further health issues (Lal et al. 2023a). The use of aromatase inhibitors in fish farming and the environment can have significant negative consequences for fish health and potentially even human health. Therefore, careful handling and monitoring of these chemicals are crucial to ensure environmental and biological safety.

Health and environmental considerations

Aromatase inhibitors are drugs typically prescribed for patients with hormone receptor positive breast cancer. Aromatase is an enzyme that is responsible for the conversion of androgens towards oestrogen. Here are some health and environmental considerations associated with aromatase inhibitors:
Health considerations:

1. Efficacy in the management of breast cancer: Postmenopausal women with hormone receptor-positive breast cancer benefit greatly from aromatase inhibitors. They can help decrease the likelihood of a second cancer developing and increase the likelihood of survival overall (Early Breast Cancer Trialists' Collaborative Group 2015; Hadji et al. 2017).

2. Side effects: Aromatase inhibitors frequently cause unwanted adverse reactions that involve muscular as well as joint discomfort, sweating, and vaginal dryness. Patients' daily lives may be negatively affected by these unwanted consequences.

3. Bone health: A significant concern is the potential impact on bone health. Aromatase inhibitors can lead to bone density reduction and an increased risk of fractures. Patients often require bone-protecting medications like bisphosphonates or denosumab.

4. Cardiovascular health: Some studies suggest a potential association between aromatase inhibitors and cardiovascular issues, although the evidence is not yet definitive (Khosrow-Khavar et al. 2020).

5. Menopausal symptoms: Aromatase inhibitors can exacerbate menopausal symptoms including hot flashes and mood changes in postmenopausal women.

Environmental considerations:

1. Pharmaceutical disposal: Proper disposal of unused or expired medications, including aromatase inhibitors, is essential to prevent environmental contamination. Flushing medications down the toilet or sink can introduce pharmaceutical compounds into water systems.

2. Water contamination: Pharmaceutical residues, including aromatase inhibitors, can persist in wastewater and enter natural water bodies. These residues may have the potential to affect aquatic ecosystems and organisms.

3. Environmental risk assessment: Regulatory agencies are increasingly conducting environmental risk assessments of pharmaceuticals, including aromatase inhibitors, to understand their potential ecological impacts.

4. Effluent treatment: Advances in wastewater treatment processes and the development of more efficient removal methods are being explored to reduce the presence of pharmaceutical residues in wastewater effluents (Bound and Voulvoulis 2005; Larsson and Fick 2009).

Food safety and environmental considerations related to steroid use must be considered during the sex reversal procedure in tilapia. The onus is on manufacturers to guarantee that consumers get fish products made with environmentally friendly processes. The Food and Drug Administration (FDA) is responsible for overseeing the safe and effective use of pharmaceuticals in aquaculture in the United States. Unlike more established forms of livestock husbandry, the aquaculture industry is relatively new, and as a result, only a limited number of drugs have received FDA approval for use in this context. Methyltestosterone is the most commonly utilised steroid, and endeavours are being made to have it approved. This endeavor is supported by a wealth of scientific studies pertaining to methyltestosterone, which provide essential data for the drug approval process. However, the body of knowledge surrounding alternative androgens used for sex reversal is considerably more limited. Consequently, a more extensive investigative effort is necessary to generate the data required to facilitate the drug approval process, as discussed by Phelps and Popma (2000).

Importance of fish masculinization

In the ornamental fish industry, numerous species display distinct sexual dimorphism, with male fish often preferred by hobbyists due to their more vibrant pigmentation and typically more developed fins. These species encompass livebearers like the sailfin molly (*P. velifera*), guppy (*Poecilia reticulata*), sunset platy (*Xiphophorus variatus*) and balloon molly (*P. latipinna*), as well as egg-laying species such as red Australian rainbow (*Glossolepis incisus*), dwarf gourami (*Colisa lalia*), rosy barb (*Barbus conchonius*) and Figurehting fish (*Betta splendens*). The males of established varieties command a significantly higher price due to increased demand, sometimes reaching a price difference of up to four times that of females. Although raising an all-male population would be more expensive overall, the wide price disparity between male and female offspring makes the effort worthwhile (Fernando and Phang 1994). Consequently, the commercial production of all-male populations of these ornamental fish using sex reversal technologies has the possibility to significantly increase the financial advantages of this type of aquaculture enterprise. In the context of the food fish industry, as aquaculture continues to contribute a growing share of the world's seafood supply, tilapia cultivation is set to assume an increasingly crucial role. The efficiency and straightforwardness of this production technique have led to hormone-induced sex reversal becoming the preferred commercial method for generating male tilapia fingerlings, greatly contributing to the rapid expansion of the tilapia industry. An anabolic response is often linked alongside an increase in protein synthesis and muscle mass, but in androgen-treated tilapia, the increased growth is primarily attributable to the higher development of males (Iwalewa and Mojekwu 2014).

Future perspectives of aromatase inhibitor

It is crucial to assess the potential environmental impacts of using aromatase inhibitors in aquaculture. The impacts of aromatase inhibitors on masculinization in fish remain poorly understood, and more study is required to uncover the underlying molecular and physiological mechanisms. Future perspectives of aromatase inhibitor on fish masculinization can include:

1. Genetic and epigenetic approaches: Future research may explore the potential of genetic and epigenetic interventions in conjunction with aromatase inhibitors to enhance masculinization in fish. This could involve genetic selection for individuals with higher masculinization potential or epigenetic modifications to promote desired phenotypic changes.

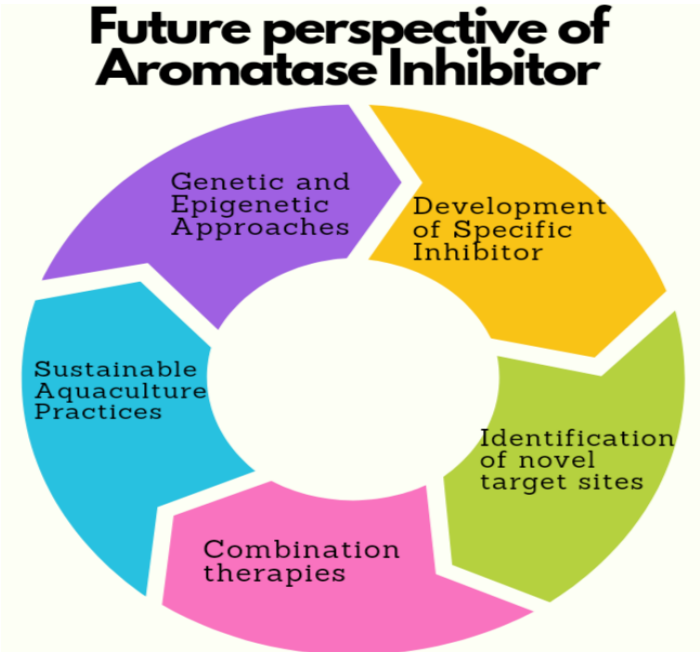


Fig. 6 The future perspectives of aromatase inhibitor

2. Development of specific Inhibitors: Further advancements in pharmaceutical research could lead to the development of more specific and potent aromatase inhibitors tailored to fish species. These inhibitors can have improved efficacy, reduced off-target effects, and better bioavailability, resulting in enhanced masculinization outcomes.

3. Identification of novel target sites: Exploring additional target sites within the estrogen biosynthesis pathway could provide alternative approaches for masculinization. Identifying and targeting enzymes or receptors involved in estrogensignaling pathways may offer new avenues for manipulation and regulation of sexual development.

4. Combination therapies: Future studies may investigate the synergistic effects of combining aromatase inhibitors with other compounds, such as growth promoters or hormone agonists, to achieve more significant masculinization and growth enhancement in fish. This could lead to the development of integrated treatment protocols for optimal results.

5. Sustainable aquaculture practices: With a growing focus on sustainability in aquaculture, future perspectives may involve evaluating the environmental and economic impacts of using aromatase inhibitors for fish masculinization. Research may aim to optimize treatment protocols, minimize environmental contamination risks, and ensure the overall sustainability of the production systems.

Conclusions

In conclusion, aromatase inhibitors may be used to masculinize fish and manage invasive species. However, more research and environmental effect assessments are needed to assure safe and responsible use in aquatic systems.For fish masculinization, aromatase inhibitors may be beneficial. Monosex populations can be used in aquaculture for selective breeding and production efficiency. It could also reduce invading fish species by reducing females. Aromatase inhibitors in fish populations may have environmental and ecological effects. We must evaluate long-term implications, including reproductive and ecological damage. For safe and effective use, accurate dosing methods and off-target effects must be considered. Aromatase inhibitors affecting fish physiology, behavior, and long-term reproductive fitness need further study. We must also assess the transfer of these substances to non-target animals and their persistence in aquatic ecosystems. Regulations and recommendations should also assure appropriate and sustainable aromatase inhibitor use in fish masculinization.

Credit authorship contribution statement Jham Lal: Conceptualization; writing original draft. Anand Vaishnav: writing original draft. Soibam Khogen Singh: Conceptualization; visualization; writing–review and editing. Pradyut Biswas: Conceptualization; writing–review and editing. Naresh Kumar Mehta: Writing–review and editing. Dharmendra Kumar Meena: Writing–review and editing. Gusheinzed Waikhom: Writing–review and editing.

Acknowledgements The first authors acknowledge the support from the Vice Chancellor, Central Agricultural University, Imphal and the Dean, College of Fisheries, Central Agricultural University, Tripura. No external funding support was received for this review.

Conflict of interest The authors declare that they have no conflict of interest.

References

Afonso LOB, Iwama GK, Smith J, Donaldson EM (1999) Effects of the aromatase inhibitor Fadrozole on plasma sex steroid secretion and oocyte maturation in female coho salmon (*Oncorhynchus kisutch*) during vitellogenesis. Fish Physiol Biochem 20(2):231-241. https://doi.org/10.1006/gcen.1998.7198

Afonso LOB, Wassermann GJ, Terezinha de Oliveira R (2001) Sex reversal in Nile tilapia (*Oreochromis niloticus*) using a nonsteroidal aromatase inhibitor. J Exp Zool 290(2):177-181. https://doi.org/10.1002/jez.1047

Alijani H, Amini Chermahini M, Zargham D, Mohiseni M (2022) The effect of letrozole and letrozole-chitosan nanoparticles on masculinization of rainbow trout larvae (*Oncorhynchus mykiss*). Iranian J Fish Sci 21(6):1383-1396. 10.22092/IJFS.2022.128212

Alitalo OS, Rantalainen, AL, Pellinen J (2022) Anticancer drugs gemcitabine, letrozole, and tamoxifen in municipal wastewater and their photodegradation in laboratory-scale UV experiments. Water Air Soil Poll 233(8):292. https://doi.org/10.1007/s11270-022-05763-x

Ankely GT, Kahl MD, Jensen KM, Hornung MW, Korte JJ, Makynen EA, Leino RL (2002) Evaluation of the aromatase inhibitor fadrozole in a short term reproduction assay with the fathead minnow. Toxicol Sci 67:121-130. https://doi.org/10.1093/toxsci/67.1.121

Arora A, Potter JF (2004) Aromatase inhibitors: current indications and future prospects for treatment of postmenopausal breast cancer. J Am Geriatr Soc 52(4):611-6. https://doi.org/10.1111/j.1532-5415.2004.52171.x

Arroyo MAM, Santos PRDS, Oliveira MFD, Assis Neto ACD (2021) Prolonged use of letrozole causes morphological changes on

gonads in Galea spixii. Animal Rep 18(2):e20200029. https://doi.org/10.1590/1984-3143-AR2020-0029
Asad F, Naz S, Ali T, Gul Y, Jamal R, Shaheen Z, Tasadaq M, Nadeem A, Anwar N, Batool N, Bano S (2023) Effect of natural and synthetic androgen hormone on sex reversal of Nile tilapia (*Oreochromis niloticus*). Brazilian J Biol 84:e272413. https://doi.org/10.1590/1519-6984.272413
Astrazeneca (2000) Arimidex product monograph. Mississauga, Ontario. 7
Baroiller JF, Cotta HD (2018) Sex control in tilapias. In: Wang, H.-P., Piferrer, F., Chen, S.-L. (Eds.), Sex control in aquaculture. John Wiley & Sons Ltd. 191–234. https://doi.org/10.1002/9781119127291.ch9
Bart AN, Athauda ARSB, Fitzpatrick MS, Contreras-Sanchez WM (2003) Ultrasound enhanced immersion protocols for masculinization of Nile tilapia, *Oreochromis niloticus*. J World Aquac Soc 34(2):210-216. https://doi.org/10.1111/j.1749-7345.2003.tb00058.x
Bart AN, Kindschi GA, Ahmed H, Clark J, Young J, Zohar Y (2001) Enhanced transport of calcein into rainbow trout, Oncorhynchus mykiss, larvae using cavitation level ultrasound. Aquaculture 196(1-2):189-97. https://doi.org/10.1016/S0044-8486(00)00579-2
Beardmore JA, Mair GC, Lewis RI (2001) Monosex male production in finfish as exemplified by tilapia: applications, problems and prospects. Aquaculture 197:283–301. https://doi.org/10.1016/B978-0-444-50913-0.50015-1
Betancur López JJ, Quintero Velez JC, Ostos Alfonso H, Barreiro-Sanchez F, Olivera Angel M (2014) Effectiveness of the aromatase (P450 Arom) inhibitors Letrozole and Exemestane for masculinization of red tilapia (*Oreochromis* spp.). Rev Colomb Cienc Pecu 27(1):47-53
Bhandari RK, Komuro H, Nakamura S, Higa M, Nakamura M (2003) Gonadal restructuring and correlative steroid hormone profiles during natural sex change in protogynous honeycomb grouper (*Epinephelus merra*). Zoolog Sci 20(11):1399–1404. https://doi.org/10.2108/zsj.20.1399
Bhatnagar AS (2007) The discovery and mechanism of action of letrozole. Breast Cancer Res Ttreat 105(1):7-17. https://doi.org/10.1007/s10549-007-9696-3
Bound JP, Voulvoulis N (2005) Pharmaceuticals in the aquatic environment—a comparison of risk assessment strategies. Chemosphere 58(7):1037-1045. https://doi.org/10.1016/j.chemosphere.2004.05.010
Brodie A (1991) Aromatase and its inhibitorsan overview. J Steroid Biochem Mol Biol 40:255-261
Brueggemeier RW (2001) Aromatase, aromatase inhibitors and breast cancer. Am J Ther 8(5):333-44
Bye VJ, Lincoln RF (1986) Commercial methods for the control of sexual maturation in rainbow trout (*Salmo gairdneri R.*). Aquaculture 57(1–4):299–309. https://doi.org/10.1016/0044-8486(86)90208-5
Cai P, Yuan H, Gao Z, Daka P, Qiao H, Zhang W, Jiang S, Xiong Y, Gong Y, Wu Y, Jin S (2023) Sex reversal induced by dietary supplementation with 17α-methyltestosterone during the critical period of sex differentiation in oriental river prawn (*Macrobrachium nipponense*). Animal 13(8):1369. https://doi.org/10.3390/ani13081369
Capel B (2017) Vertebrate sex determination: evolutionary plasticity of a fundamental switch. Nat Rev Genet 18:675–689. https://doi.org/10.1038/nrg.2017.60
Carman O, Iskandar A, Chang CF, Wu GC, Muslim M, Ramadhani DE (2023) Accelerated of sex reversal use 17α-methyltestosterone induced female, orange-spotted grouper *Epinephelus coioides*. Sci J Fish Mar 15(2). doi: 10.20473/jipk.v15i2.40624
Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S (2003) Aromatase expression and role of estrogens in male gonad: a review. Reprod Biol Endocrinol 1(1):1-6. https://doi.org/10.1186/1477-7827-1-35
Cek S, Turan F, Atik E (2007a) Masculination of convict cichlid (*Cichlasomanigro fasciatum*) by immersion in *Tribulus terrestris* extract. Aquac Int 15(2):109-119. https://doi.org/10.1007/s10499-006-9071-0
Cek S, Turan F, Atik E (2007b) The effects of gokshura, *Tribulus terrestris* on sex differentiation of guppy, *Poecilia reticulate*. Pak J Biol Sci 10:718-725. https://doi.org/10.3923/pjbs.2007.718.725
Chardard D, Dournon C (1999) Sex reversal by aromatase inhibitor treatment in the newt *Pleurodeles waltl*. J Exp Zool 283:43–50. https://doi.org/10.1002/(SICI)1097-010X(19990101)283:1<43::AID-JEZ6>3.0.CO;2-G
Charnov EL, Bull J (1977) When is sex environmentally determined? Nature 266:828–830. https://doi.org/10.1038/266828a0
Chávez-García R, Contreras-Ramos A, Ortega-Camarillo C, Figureueroa-Lucero G, Prado-Flores G, Mendoza-Martínez G, Vergara-Onofre M (2020) Morphometric comparison of the growth curve in Nile tilapia (*Oreochromis niloticus*) sexually reverted to masculinized and feminized. Latin Am J Aquat Res 48(1):1-6. http://dx.doi.org/10.3856/vol48-issue1-fulltext-2302
Chen L, Wang L, Cheng Q, Tu YX, Yang Z, Li RZ, Luo ZH, Chen ZX (2020) Anti-masculinization induced by aromatase inhibitors in adult female zebrafish. BMC Genomi 21(1):1-0. https://doi.org/10.1186/s12864-019-6437-z
Choueiri TK, Alemany CA, Abou-Jawde RM, Budd GT (2004) Role of aromatase inhibitors in the treatment of breast cancer. Clinical Ther 26(8):1199-214. https://doi.org/10.1016/S0149-2918(04)80017-1
Chumsri S, Howes T, Bao T, Sabnis G, Brodie A (2011) Aromatase, aromatase inhibitors and breast cancer. J Steroid Biochem Mol Biol 125(1-2):13-22. https://doi.org/10.1016/j.jsbmb.2011.02.001
Cohen MH, Johnson JR, Li N, Chen G, Pazdur R (2002) Approval summary: letrozole in the treatment of postmenopausal women with advanced breast cancer. Clin Cancer Res 8(3):665-669
Conover DO (1984) Adaptive significance of temperature-dependent sex determination in a fish. Am Nat 123(3):297-313
Contreras-Sanchez WM, Fitzpatrick MS, Marquescouturier G, Schreck CB (1999) Masculinization of Nile tilapia Oreochromis niloticus by immersion in synthetic androgens: timing and efficacy. Aquaculture 236-239. https://doi.org/10.1016/S0044-8486(99)00136-2
Dank M (2002) The role of aromasin in the hormonal therapy of breast cancer. Pathol Oncol Res 8:87–92. https://doi.org/10.1007/BF03033716
Das R, Rather MA, Basavaraja N, Sharma R, Udit UK (2012) Effect of nonsteroidal aromatase inhibitor on sex reversal of *Oreochromis mossambicus* (Peters 1852). Isr J Aquac 64(703):1-6. http://hdl.handle.net/10524/22926
Das R (2007) Effect of aromatase inhibitor on gonadal development in oreochromis mossambicus (Peters). M.F.Sc. Thesis, KVAFSU. Bidar 57. https://doi.org/10.31018/jans.v7i1.622
De Ronde W, de Jong FH (2011) Aromatase inhibitors in men: effects and therapeutic options. Reprod Biol Endocrinol 9(1):1-7. https://doi.org/10.1186/1477-7827-9-93
Deeks ED, Scott LJ (2009) Exemestane: a review of its use in postmenopausal women with breast cancer. Drug 69:889-918. https://

doi.org/10.2165/00003495-200969070-00007
Dekimpe P. Micha JC (1974) First guidelines of the culture of *Clarias lazera* in Central Africa. Aquaculture 4:227-248. https://doi.org/10.1016/0044-8486(74)90036-2
Delbès G, Blázquez M, Fernandino JI, Grigorova P, Hales BF, Metcalfe C, Navarro-Martin L, Parent L, Robaire B, Rwigemera A, Van Der Kraak G (2022) Effects of endocrine disrupting chemicals on gonad development: Mechanistic insights from fish and mammals. Environ Res 204:112040. https://doi.org/10.1016/j.envres.2021.112040
Demers LM, Melby JC, Wilson TE, Lipton A, Harvey HA, Santen RJ (1990) The effects of CGS 16949A, an aromatase inhibitor on adrenal mineralocorticoid biosynthesis. J Clin Endocrinol Metab 70(4):1162-1166
Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. Aquaculture 208:191-364. https://doi.org/10.1016/S0044-8486(02)00057-1
di Salle E, Briatico G, Giudici D, Ornati G, Zaccheo T, Buzzetti F, Nesi M, Panzeri A (1994) Novel aromatase and 5α-reductase inhibitors. J Steroid Biochem Mol Biol 49(4-6):289-94.https://doi.org/10.1016/0960-0760(94)90270-4
di Salle E, Giudici D, Ornati G, Briatico G, D'Alessio R, Villa V, Lombardi P (1990) 4-aminoandrostenedione derivatives: A Novel class of irreversible aromatase inhibitors comparison with FCE 24304 and 4-hydroxyandrostenedione. J Steriod Biochem Mol Biol 37:369–374. https://doi.org/10.1016/0960-0760(90)90486-5
di Salle E, Ornati G, Giudici D, Lassus M, Evans TR, Coombes RC (1992) Exemestane (FCE 24304), a new steroidal aromatase inhibitor. J Steroid Biochem Mol Biol 43(1-3):137-43. https://doi.org/10.1016/0960-0760(92)90198-R
do Carmo Ota É, Inoue LA, de Castro Silva TS (2024) Concentration and time of feeding with 17-α-methyltestosterone oil diluted and incorporated to the feed for masculinization of Nile tilapia. Acta Scientiarum Anim Sci 46:e62366. https://doi.org/10.4025/actascianimsci.v46i1.62366
Doering JA, Villeneuve DL, Tilton CB, Kittelson AR, Blackwell BR, Kahl MD, Jensen KM, Poole ST, Cavallin JE, Cole AR, Dean KN (2021) Assessing effects of aromatase inhibition on fishes with group-synchronous oocyte development using western mosquitofish (*Gambusia affinis*) as a model. Aquat Toxicol 232:105741
Dutta U, Pant K (2008) Aromatase inhibitors: past, present and future in breast cancer therapy. Med Oncol 25:113-24. https://doi.org/10.1007/s12032-007-9019-x
e Silva RZ, Alvarenga ÉR, Matta SV, de Oliveira Alves GF, Manduca LG, Silva MA, Yoshinaga TT, Fernandes AF, Turra EM (2022) Masculinization protocol for Nile tilapia (*O. niloticus*) in biofloc technology using 17-α-methyltestosterone in the diet. Aquaculture 547:737470. https://doi.org/10.1016/j.aquaculture.2021.737470
Early breast cancer trialists' collaborative group (2015) Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet 386(10001):1341-1352. https://doi.org/10.1016/S0140-6736(15)61074-1
Elbrecht A, Smith RG (1992) Aromatase activity and sex determination in chickens. Science 255:467-470. 10.1126/science.1734525
Enuekwe RC, Okonji VA (2019) Effects of dietary 17-methyl testosterone on sex reversal and growth performance of African mud catfish (*Clarias gariepinus*). Niger J Fish 16:1806-12
Farias RD, Oliveira KR, Souza ME, Ferreira DA, Silva AA, Silva Júnior VA, Dunham R, Coimbra MR (2023) Effect of dosage of orally administered 17α-methyltestosterone on sex reversion of the yellowtail tetra *Astyanax lacustris* (Lütken 1875). Anim Reprod 20:e20220080. https://doi.org/10.1590/1984-3143-AR2022-0080
Feist G, Yeoh CG, Fitzpatrick MS, Schreck CB (1995) The production of functional sex-reversed male rainbow trout with 17α-methyltestosterone and 11 β-hydroxyandrostenedione. Aquaculture 131(1-2):145-52. https://doi.org/10.1016/0044-8486(94)00336-M
Fenske M, Segner H (2004) Aromatase modulation alters gonadal differentation in developing zebrafish (*Danio renio*). Aquat Toxicol 67:105-126. https://doi.org/10.1016/j.aquatox.2003.10.008
Fernando AA, Phang VPE (1994) Freshwater ornamental fish aquaculture in Singapore. Singapore: Singapore Polytechnic Publ 123
Fitzpatrick MS, Contreras-Sanchez WM, Milston RH, Schreck CB (1999) Fate of the masculinization agent methyltestosterone in the pond environment. Central Am Symposium Aquac 249-250. San Pedro Sula
Frisch A (2004) Sex-change and gonadal steroids in sequentially-hermaphroditic teleost fish. Rev Fish Biol Fish 14:481-499. https://doi.org/10.1007/s11160-005-3586-8
Gale WL, Fitzpatrick MS, Lucero M, Contreras-Sánchez WM, Schreck CB (1999) Masculinization of Nile tilapia (*Oreochromis niloticus*) by immersion in androgens. Aquaculture 178(3-4):349-57.https://doi.org/10.1016/S0044-8486(99)00136-2
Gao W, Bohl CE, Dalton JT (2005) Chemistry and structural biology of androgen receptor. Chem Rev 105(9):3352-3370. https://doi.org/10.1021/cr020456u
Gao ZX, Wang HP, Wallat G, Yao H, Rapp D, O'Bryant P, MacDonald R, Wang WM (2010) Effects of a nonsteroidal aromatase inhibitor on gonadal differentiation of bluegill sunfish *Lepomis macrochirus.* Aquac Res 41(9):1282-9. https://doi.org/10.1111/j.1365-2109.2009.02414.x
Garcia CE, Araújo BC, Mello PH, Narcizo AD, Rodrigues-Filho JA, Medrado AT, Zampieri RA, Floeter-Winter LM, Moreira RG (2013) Involvement of pituitary gonadotropins, gonadal steroids and breeding season in sex change of protogynous dusky grouper, *Epinephelus marginatus* (Teleostei: Serranidae), induced by a non-steroidal aromatase inhibitor. Gen Comp Endocrinol 192:170-80. https ://doi.org/10.1016/j.ygcen.2013.06.012
Garcia-Velasco JA, Moreno L, Pacheco A, Guillén A, Duque L, Requena A, Pellicer A (2005) The aromatase inhibitor letrozole increases the concentration of intra ovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. Fertil Steril 84(1):82-7. https://doi.org/10.1016/j.fertnstert.2005.01.117
Geisler J, King N, Dowsett M, Ottestad L, Lundgren S, Walton P, Kormeset PO, Lønning PE (1996) Influence of anastrozole (Arimidex), a selective, non-steroidal aromatase inhibitor, on in vivo aromatisation and plasma oestrogen levels in postmenopausal women with breast cancer. British J Cancer 74(8):1286-91.https://doi.org/10.1038/bjc.1996.531
Geisler J (2011) Differences between the non-steroidal aromatase inhibitors anastrozole and letrozole–of clinical importance? British J Cancer 104(7):1059-66. DOI: 10.1038/bjc.2011.58
Ghosal I, Mukherjee D, Chakraborty SB (2021) The effects of four plant extracts on growth, sex reversal, immunological and haemato-biochemical parameters in Nile tilapia, *Oreochmomis niloticus* (Linnaeus 1758). Aquac Res 52(2):559-76. https://doi.org/10.1111/are.14914
Giudici D, Ornati G, Briatico G, Buzzetti F, Lombardi P, Di Salle E (1988) 6-methylenandrosta-1, 4-diene-3, 17-dione (FCE 24304):

a new irreversible aromatase inhibitor. J Steroid Biochem 30(1-6):391-4.https://doi.org/10.1016/0022-4731(88)90129-X
Glück S (2010) Exemestane as first-line therapy in postmenopausal women with recurrent or metastatic breast cancer. Am J Clin Oncol 33(3):314-9. doi:10.1097/COC.0b013e31819fdf9b
Golan M, Levavi-Sivan B (2014) Artificial masculinization in tilapia involves androgen receptor activation. Gen Comp Endocrinol 207:50-5. https://doi.org/10.1016/j.ygcen.2014.04.026
Gonnelli S, Petrioli R (2008) Aromatase inhibitors, efficacy and metabolic risk in the treatment of postmenopausal women with early breast cancer. Clin Interv Aging 3(4):647-57. https://doi.org/10.2147/cia.s3466
Guiguen Y, Baroiler J, Ricordel M, Iseki K, Mcmeel O, Martin S (1999) Involvement of estrogens in the process of sex differentiation in two fish species: The rainbow trout (*Oncoyhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). Mol Reprod De 54:154-162. https://doi.org/10.1002/(SICI)1098-2795(199910)54:2<154::AID-MRD7>3.0.CO;2-5
Hadji P, Aapro MS, Body JJ, Gnant M, Brandi ML, Reginster JY, Zillikens MC, Glüer CC, de Villiers T, Baber R, Roodman GD (2017) Management of aromatase inhibitor-associated bone loss (AIBL) in postmenopausal women with hormone sensitive breast cancer: Joint position statement of the IOF, CABS, ECTS, IEG, ESCEO, IMS, and SIOG. J Bone Oncol 7:1-2. https://doi.org/10.1016/j.jbo.2017.03.001
Hajibeglou A, Machanlou M (2023) Sex reversal of rainbow trout (*Oncorhynchus mykiss*) using letrozole and combinations of *Asparagus racemosus* and *Tribulus terrestris* extracts. Aquac Int 1-0. https://doi.org/10.1007/s10499-023-01266-6
Han Y, Liu M, Lan Zhang L, Simpson B, Xue Zhang G (2010) Comparison of reproductive development in triploid and diploid female rainbow trout *Oncorhynchus mykiss*. J Fish Biol 76(7):1742-50. https://doi.org/10.1111/j.1095-8649.2010.02613.x
Hayman ES, Fairgrieve WT, Luckenbach JA (2021) Molecular and morphological sex differentiation in sablefish (*Anoplopoma fimbria*), a marine teleost with XX/XY sex determination. Gene 764:145093. https://doi.org/10.1016/j.gene.2020.145093
Haynes BP, Dowsett M, Miller WR, Dixon JM, Bhatnagar AS (2003) The pharmacology of letrozole. J Steroid Biochem Mol Biol 87(1):35-45. https://doi.org/10.1016/S0960-0760(03)00384-4
Heinlein CA, Chang C (2002) Androgen receptor (AR) coregulators: an overview. Endocr Rev 23(2):175-200. https://doi.org/10.1210/edrv.23.2.0460
Hines M (2011) Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior. Front Neuroendocrinol 32(2):170-82. https://doi.org/10.1016/j.yfrne.2011.02.006
Honeycutt JL, Deck CA, Miller SC, Severance ME, Atkins EB, Luckenbach JA, Buckel JA, Daniels HV, Rice JA, Borski RJ, Godwin J (2019) Warmer waters masculinize wild populations of a fish with temperature-dependent sex determination. Sci Rep 9(1):6527. https://doi.org/10.1038/s41598-019-42944-x
Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hoctin-Boes G, Houghton J, Locker GY, Tobias JS (2005) Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 365(9453):60-2.https://doi.org/10.1016/S0140-6736(04)17666-6
Huang J, Liu S, Ouyang H, Han C, Li M, Han L, Li S, Li G, Lin H, Zhang Y (2023) Production of XX neo-male mandarin fish, *Siniperca chuatsi*, after sexual differentiation by oral administration of 17α-methyltestosterone. Water Biol Secur 2(1):100095. https://doi.org/10.1016/j.watbs.2022.100095
Hussain MG (1998) Manipulation of chromosomes in fish: review of various techniques and their implications in aquaculture. Bangladesh J Fish Res 2(1):99-108 http://hdl.handle.net/1834/32223
Irani A, Noori F (2023) Effect of 17α-methyl testosterone, tamoxifen, and letrozole on growth performance and sex reversal of rainbow trout (*Oncorhynchus mykiss*). Iran J Fish Sci 22(3):701-12
Itoh T, Karlsberg K, Kijima I, Yuan YC, Smith D, Ye J, Chen S (2005) Letrozole-, anastrozole- and tamoxifen-responsive genes in MCF-7aro cells: a microarray approach. Mol Cancer Res 3(4):203-18. https://doi.org/10.1158/1541-7786.MCR-04-0122
Iwalewa Megbowon, Mojekwu TO (2014) Tilapia sex reversal using methyl testosterone (MT) and its effect on fish, man and environment. Biotechnology 13:213-216. http://hdl.handle.net/1834/39040
Jeevamani JJ, Kamalakannan B, Nagendran NA, Pandiaraja D, Sundararajan S, Chandrasekaran S (2012) Production of an all-male population of guppy, *Poecilia reticulata* (Schneider). Curr Sci 103(10):1151
Jiang Y, Luo H, Hou M, Chen J, Tao B, Zhu Z, Song Y, Hu W (2022) Aromatase inhibitor induces sex reversal in the protogynous hermaphroditic rice field eel (*Monopterus albus*). Aquaculture 551:737960
Johnston SR, Dowsett M (2003) Aromatase inhibitors for breast cancer: lessons from the laboratory. Nature Rev Cancer 3(11):821-31. doi:10.1038/nrc1211
Joshi HD, Ghode GS, Gore SB (2015) Efficiency of letrozole loaded PLGA nanoparticles on sex reversal of *Poecilia reticulata* (Peters 1859). J Appl Nat Sci 7(1):394-9. https://doi.org/10.31018/jans.v7i1.622
Joshi HD, Tiwari VK, Gupta S, Sharma R, Lakra WS, Sahoo U (2019) Application of nanotechnology for the production of masculinized Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). Aquaculture 511:734206. https://doi.org/10.1016/j.aquaculture.2019.734206
Karaket T, Reungkhajorn A, Ponza P (2023) The optimum dose and period of 17α-methyltestosterone immersion on masculinization of red tilapia (*Oreochromis spp*.). Aquac Fish 8(2):174-9. https://doi.org/10.1016/j.aaf.2021.09.001
Karslı Z (2021) Effects of synthetic androgen (17α-methyltestosterone) and estrogen (17β-estradiol) on growth and skin coloration in emperor red cichlid, *Aulonocara nyassae* (Actinopterygii: Cichliformes: Cichlidae). Acta Ichthyol Piscat 51(4):357-63
Katare MB, Basavaraja N, Joshi HD, Archana C (2015) Effect of letrozole on masculinization of Siamese Figurehting fish (*Betta splendens*). J Appl Nat Sci 7(1):425-33. https://doi.org/10.31018/jans.v7i1.627
Katare MB, Lakra WS, Chadha NK, Kumar M, Basavaraja N, Gupta S, Sawant PB, Pai M, Swain S (2018) Synergistic effect of anastrozole-17α-methyltestosterone on masculinization of dwarf gourami. J Exp Zool India 21(2):633-640
Katare MB, Pai M, Mogalekar HS (2021) Effect of anastrozole on masculinization in ornamental fish, dwarf gourami, *Trichogaster lalius* (Hamilton 1822). Indian J Exp Biol 59(09):581-9. 10.56042/ijeb.v59i09.54935
Khosrow-Khavar F, Filion KB, Bouganim N, Suissa S, Azoulay L (2020) Aromatase inhibitors and the risk of cardiovascular outcomes in women with breast cancer: a population-based cohort study. Circulation 141(7):549-59. https://doi.org/10.1161/CIRCULATIONAHA.119.044750
Kim KH, Moon HN, Yeo IK (2023) Inhibition of sexual maturation inhibition using exemestane and tamoxifen in female olive flounder (*Paralichthys olivaceous*). Res Squa 1-16. https://doi.org/10.21203/rs.3.rs-2856733/v1

Kingsolver JG, Higgins JK, Augustine KE (2015) Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. J Exp Biol 218(14):2218-25. https://doi.org/10.1242/jeb.120733

Kitano T, Takamune K, Nagahama Y, Abe SI (2000) Aromatase inhibitor and 17α-methyltestosterone cause sex-reversal from genetical females to phenotypic males and suppression of P450 aromatase gene expression in Japanese flounder (*Paralichthys olivaceus*). Mol Reprod Dev:Gamete Res 56(1):1-5. https://doi.org/10.1002/(SICI)1098-2795(200005)56:1<1::AID-MRD1>3.0.CO;2-3

Komatsu T, Nakamura S, Nakamura M (2006) Masculinization of female golden rabbitfish *Siganus guttatus* using an aromatase inhibitor treatment during sex differentiation. Comp Biochem Physiol Toxicol Pharmacol 143(4):402-9. https://doi.org/10.1016/j.cbpc.2006.04.015

Krásenská M (2016) Treatment with aromatase inhibitors in postmenopausal women with breast cancer and the possibility of influencing side effects. Klin Onkol: Casopis Ceske Slovenske Onkologicke Opolecnosti 29:S39-49. https://doi.org/10.14735/amko20163s39

Kumkhong S, Chukerd N, Phumrojanar P (2024) The optimum dose and period of synthetic hormones 17-α methyltestosterone during fry stage on masculinization of siamese figurehting fish (*Betta splendens*). Food Agri Sci Technol 10(1):17-28

Kvinnsland S, Anker G, Dirix LY, Bonneterre J, Prove AM, Wilking N, Lobelle JP, Mariani O, Di Salle E, Polli A, Massimini G (2000) High activity and tolerability demonstrated for exemestane in postmenopausal women with metastatic breast cancer who had previously failed on tamoxifen treatment. European J Cancer 36(8):976-82. https://doi.org/10.1016/S0959-8049(00)00041-1

Kwon J, Haghpanah V, Kogson-Hurtado L, McAndrew B, Penman D (2000) Masculinization of genetic female Nile tilapia (*Oreochromis niloticus*) by dietary administration of an aromatase inhibitor during sexual differentiation. J Exp Zool 287(1):46-53. https://doi.org/10.1002/1097-010X(20000615)287:1<46::AID-JEZ6>3.0.CO;2-X

Kwon JY, McAndrew BJ, Penman DJ (2002) Treatment with an aromatase inhibitor suppresses high-temperature feminization of genetic male (YY) Nile tilapia. J Fish Biol 60(3):625-36. https://doi.org/10.1111/j.1095-8649.2002.tb01689.x

Lal J, Biswas P, Singh SK, Debbarma R, Deb S, Yadav NK, Patel AB (2023a) Effects of dietary aromatase inhibitors on masculinization of rosy barb (*Pethia conchonius*): Evidence from growth, coloration and gonado-physiological changes. Plos One 18(11):e0287934. https://doi.org/10.1371/journal.pone.0287934

Lal J, Biswas P, Singh SK, Debbarma R, Mehta NK, Deb S, Sharma S, Waikhom G, Patel AB (2023b) Moving towards gel for fish feeding: Focus on functional properties and its acceptance. Gels 9(4):305. https://doi.org/10.3390/gels9040305

Lance VA, Bogart MH (1992) Disruption of ovarian development in alligator embryos. Gen Comp Endocrinol 86:59–71. https://doi.org/10.1016/0016-6480(92)90126-5

Larsson DJ, Fick J (2009) Transparency throughout the production chain—a way to reduce pollution from the manufacturing of pharmaceuticals? Regul Toxicol Pharmacol 53(3):161-3.https://doi.org/10.1016/j.yrtph.2009.01.008

Leonhardt JH (1997) Efeito da reversão sexual emtilápia do Nilo, *Oreochromis niloticus* (Linnaeus 1757) [Effect of sexual reversal on Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757)] (Doctoral dissertation). Retrieved from https://repositorio.unesp.br/bitstream/handle/11449/144133/000105141.pdf?sequence=1

Li GL, Liu XC, Lin HR (2005) Aromatase inhibitor letrozole induces sex inversion in the protogynous red spotted grouper (*Epinephelus akaara*). Sheng li xue bao: Acta Physiol Sin 57(4):473-9

Liao PH, Chu SH, Tu TY, Wang XH, Lin AY, Chen PJ (2014) Persistent endocrine disruption effects in medaka fish with early life-stage exposure to a triazole-containing aromatase inhibitor (letrozole). J Hazard Mater 277:141-9

Lien EA, Anker G, Lønning PE, Solheim E, Ueland PM (1990) Decreased serum concentrations of tamoxifen and its metabolites induced by aminoglutethimide. Cancer Res 50(18):5851-7

Lincoln RF, Scott AP (1984) Sexual maturation in triploid rainbow trout, Salmo gairdneri Richardson. J Fish Biol 25(4):385-92. https://doi.org/10.1111/j.1095-8649.1984.tb04886.x

Lindqvist AS (2004) Nandrolone decanoate, behaviour and brain: animal experimental studies. rapport nr.: Doctoral Dissertation 141 Avhandling 141. http://hdl.handle.net/2077/68

Lisboa Neto AF, Yoshinaga TT, Sousa Júnior AD, Nichi M, Esteller-Vico A, Assis Neto AC (2023) Letrozole administration effects on the P450aromatase expression and the reproductive parameters in male sheep (*Ovis aries*). Anim Reprod 20:e20230099. https://doi.org/10.1590/1984-3143-AR2023-0099

Liu M, Zhu Q, Li H, Chen R, Hu W, Liu S, Xu D (2023) Characterization of early gonadal differentiation and induction of sex reversal in the rock bream *Oplegnathus fasciatus*. Mar Biotechnol 1-2. https://doi.org/10.1007/s10126-023-10213-8

Liu S, Xu P, Liu X, Guo D, Chen X, Bi S, Lai H, Wang G, Zhao X, Su Y, Yi H (2021) Production of neo-male mandarin fish *Siniperca chuatsi* by masculinization with orally administered 17α-methyltestosterone. Aquaculture 530:735904. https://doi.org/10.1016/j.aquaculture.2020.735904

Lnning PE (1999) Cross-resistance to different aromatase inhibitors in breast cancer treatment. Endocr Relat Cancer 6(2):251-7

Lønning P, Pfister C, Martoni A, Zamagni C (2003) Pharmacokinetics of third-generation aromatase inhibitors. Sem Oncol 30:23-32. https://doi.org/10.1016/S0093-7754(03)00305-1

Lønning PE (2003) Clinical pharmacokinetics of aromatase inhibitors and inactivators. Clin Pharmacokinet 42:619-31. https://doi.org/10.2165/00003088-200342070-00002

Luckenbach JA, Godwin J, Daniels HV, Borski RJ (2003) Gonadal differentiation and effects of temperature on sex determination in southern flounder (*Paralichthys lethostigma*). Aquaculture 216(1-4):315-27. https://doi.org/10.1016/S0044-8486(02)00407-6

M Zaki F, M Said M, Tahoun AA, Amer M (2021) Evaluation of different sex reversal treatments in red tilapia hybrid. Egyptian J Aquat Biol Fish 25(1):279-92. 10.21608/EJABF.2021.140630

Mateen A (2007) Effect of androgen on the sex reversal, growth and meat quality of tilapia, *Oreochromis niloticus*. M. Phil Thesis; Department of zoology and fisheries, University of agriculture, Faisalabad, Pakistan. 166

Matthiessen P, Weltje L (2015) A review of the effects of azole compounds in fish and their possible involvement in masculinization of wild fish populations. Crit Rev Toxicol 45(5):453-67. https://doi.org/10.3109/10408444.2015.1018409

McCullough D, Webb R, Enright KJ, Lane KE, McVeigh J, Stewart CE, Davies IG (2021) How the love of muscle can break a heart: Impact of anabolic androgenic steroids on skeletal muscle hypertrophy, metabolic and cardiovascular health. Rev Endoc. Metab Disord 22(2):389-405. https://doi.org/10.1007/s11154-020-09616-y

McNair A, Lokman PM, Closs GP, Nakagawa S (2015) Ecological and evolutionary applications for environmental sex reversal of

fish. Quart Rev Biol 90(1):23-44
Megbowon I, Mojekwn TO (2014) Tilapia sex reversal using methyl testosterone and its effect fish, man and environment biotechnology. Fison 13(5):213-216. http://hdl.handle.net/1834/39040
Michaud LB, Buzdar AU (1999) Risks and benefits of aromatase inhibitors in postmenopausal breast cancer. Drug Saf 21:297-309. https://doi.org/10.2165/00002018-199921040-00005
Mikolajczyk T, Chyb J, Sokolowska-Mikolajczyk M, Szczerbik P, Socha M, Epler P (2006) The effect of aromatase inhibitor, fadrozole, on sGnRHa-stimulated LH secretion in goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*). Reprod Biol 6(1):195-199
Milani M, Jha G, Potter DA (2009) Anastrozole use in early stage breast cancer of post-menopausal women. Clin Med Therapeut 1:CMT-S9. https://doi.org/10.4137/CMT.S
Miller WR, Bartlett J, Brodie AM, Brueggemeier RW, Di Salle E, Lønning PE, Llombart A, Maass N, Maudelonde T, Sasano H, Goss PE (2008) Aromatase inhibitors: are there differences between steroidal and nonsteroidal aromatase inhibitors and do they matter? Oncol 13(8):829-37. https://doi.org/10.1634/theoncologist.2008-0055
Miller WR (2003) Aromatase inhibitors: mechanism of action and role in the treatment of breast cancer. Sem Oncol 30:3-11. https://doi.org/10.1016/S0093-7754(03)00302-6
Mitragotri S, Edwards DA, Blankschtein D, Langer R (1995) A mechanistic study of ultrasonically-enhanced transdermal drug delivery. J Pharmaceu Sci 84(6):697-706. https://doi.org/10.1002/jps.2600840607
Miura T, Miura CI (2003) Molecular control mechanisms of fish spermatogenesis. Fish Physiol Biochem 28:181-186. https ://doi.org/10.1023/B:FISH.00000 30522.71779.47
Mokbel K (2002) The evolving role of aromatase inhibitors in breast cancer. Int J Clin Oncol 7:279-283. https://doi.org/10.1007/s101470200040
Mor G, Eliza M, Song J, Wiita B, Chen S, Naftolin F (2001) 17α-methyl testosterone is a competitive inhibitor of aromatase activity in Jar choriocarcinoma cells and macrophage-like THP-1 cells in culture. J Steroid Biochem Mol Biol 79(1-5):239-46
Murray MI, Cantrill EL, Farrell GC (1993) Induction of cytochrome P450 2B1 in rat liver by the aromatase inhibitor aminoglutethimide. J Pharmacol Exp Ther 265(1):477-81
Naderi M, Wong MY, Gholami F (2014) Developmental exposure of zebrafish (*Danio rerio*) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. Aquat Toxicol 148:195-203. DOI: 10.1016/j.aquatox.2014.01.009
Noaksson E, Linderoth M, Tjärnlund U, Balk L (2005) Toxicological effects and reproductive impairments in female perch (*Perca fluviatilis*) exposed to leachate from swedish refuse dumps. Aquat Toxicol 75(2):162–177. DOI: 10.1016/j.aquatox.2005.07.011
Núñez NP, Jelovac D, Macedo L, Berrigan D, Perkins SN, Hursting SD, Barrett JC, Brodie A (2004) Effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole on serum hormones and bone characteristics in a preclinical tumor model for breast cancer. Clin Cancer Res 10(16):5375-80. https://doi.org/10.1158/1078-0432.CCR-04-0261
Ospina-Alvarez N, Piferrer F (2008) Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. PloS One 3(7):e2837. https://doi.org/10.1371/journal.pone.0002837
Pandian TJ, Sheela SG (1995) Hormonal induction of sex reversal in fish. Aquaculture 138(1-4):1-22. https://doi.org/10.1016/0044-8486(95)01075-0
Park IS, Oh HS, Koo JG (2003) Effect of oral tamoxifen on growth and survival in the bagrid catfish *Pseudobagrus fulvidraco*. Aquac Res 34(15):1471-4. https://doi.org/10.1111/j.1365-2109.2003.00973.x
Phelps RP, Popma TJ (2000) Sex reversal of tilapia. Pages 34–59 in B.A. Costa-Pierce and J.E. Rakocy, eds. Tilapia Aquaculture in the Americas, Vol. 2. The World Aquaculture Society, Baton Rouge, Louisiana, United States
Piferrer F, Donaldson EM (1989) Gonadal differentiation in coho salmon, *Oncorhynchus kisutch*, after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquaculture 77(2-3):251-62. https://doi.org/10.1016/0044-8486(89)90207-X
Piferrer F, Zanuy S, Carrillo M, Solar II, Devlin RH, Donaldson EM (1994) Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. J Exp Zool 270(3):255-62. https://doi.org/10.1002/jez.1402700304
Raats JI, Falkson G, Falkson HC (1992) A study of fadrozole, a new aromatase inhibitor, in postmenopausal women with advanced metastatic breast cancer. J Clin Oncol 10(1):111-116. 10.1200/JCO.1992.10.1.111
Rahaman MM, Kumagai RI, Tokumoto T (2020) Rapid induction of female-to-male sex change in adult zebrafish by injection of an aromatase inhibitor. Zebrafish 17(4):261-7. https://doi.org/10.1089/zeb.2020.1864
Ranjan R, Xavier B, Dash B, Edward LL, Suresh RD, Kumar PS (2015) Efficacy of 17α-methyl testosterone and letrozole on sex reversal of protogynous grouper, *Epinephelus tauvina* (Forskal 1775) and spawning performance of sex−reversed males. Aquac Res 46(9):2065-2072. https://doi.org/10.1111/are.12360
Rashid H, Kitano H, Hoon Lee K, Nii S, Shigematsu T, Kadomura K, Yamaguchi A, Matsuyama M (2007) Fugu (*Takifugu rubripes*) sexual differentiation: CYP19 regulation and aromatase inhibitor induced testicular development. Sex Dev 1(5):311-22. https://doi.org/10.1159/000108935
Rivero-Wendt CL, Miranda-Vilela AL, Domingues I, Oliveira R, Monteiro MS, Moura-Mello MA, Matias R, Soares AM, Grisolia CK (2020) Steroid androgen 17 alpha methyltestosterone used in fish farming induces biochemical alterations in zebrafish adults. J Environ Sci Health 55(11):1321-32. https://doi.org/10.1080/10934529.2020.1790954
Ruksana S, Pandit NP, Nakamura M (2010) Efficacy of exemestane, a new generation of aromatase inhibitor, on sex differentiation in a gonochoristic fish. Comp Biochem Physiol C Toxicol Pharmacol 152(1):69-74. https://doi.org/10.1016/j.cbpc.2010.02.014
Saartok T, Dahlberg E, Gustafsson JÅ (1984) Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology 114(6):2100-6. https://doi.org/10.1210/endo-114-6-2100
Sarker B, Das B, Chakraborty S, Hossain MA, Alam MM, Mian S, Iqbal MM (2022) Optimization of 17α-methyltestosterone dose to produce quality mono-sex Nile tilapia *Oreochromis niloticus*. Heliyon 8(12). https://doi.org/10.1016/j.heliyon.2022.e12252
Schieweck K, Bhatnagar AS, Matter A (1988) CGS 16949A, a new nonsteroidal aromatase inhibitor: effects on hormone-dependent and-independent tumors in vivo. Cancer Res 48(4):834-8
Schroeder AL, Ankley GT, Habib T, Garcia-Reyero N, Escalon BL, Jensen KM, Kahl MD, Durhan EJ, Makynen EA, Cavallin JE,

Martinovic-Weigelt D (2017) Rapid effects of the aromatase inhibitor fadrozole on steroid production and gene expression in the ovary of female fathead minnows (*Pimephales promelas*). Gen Comp Endocrinol 252:79-87. https://doi.org/10.1016/j.ygcen.2017.07.022

Schumacher CD, Steele RE, Brunner HR (2013) Aldosterone synthase inhibition for the treatment of hypertension and the derived mechanistic requirements for a new therapeutic strategy. J Hypertens 31(10):2085. 10.1097/HJH.0b013e328363570c

Shanthanagouda AH, Patil JG, Nugegoda D (2013) Effects of exposure to oestrogenic compounds on aromatase gene expression are gender dependent in the rainbowfish, *Melanotaenia fluviatilis*. Comp Biochem Physiol C Toxicol Pharmacol 157(2):162-71. https://doi.org/10.1016/j.cbpc.2012.11.004

Shen ZG, Fan QX, Yang W, Zhang YL, Wang HP (2015) Effects of 17α-methyltestosterone and aromatase inhibitor letrozole on sex reversal, gonadal structure and growth in yellow catfish *Pelteobagrus fulvidraco*. Biol Bull 228(2):108-117

Silva RC, Britto DM, Santos CA, Giordani SC, Pedreira MM (2023) Sexual differentiation and sex reversal in tilapia (*Oreochromis niloticus*) by hormone 17 α-methyltestosterone similar to that used in cultivation systems. Aquaculture 574:739624. https://doi.org/10.1016/j.aquaculture.2023.739624

Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR, Bulun SE (1994) Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev 15(3):342-355. https://doi.org/10.1210/edrv-15-3-342

Simpson ER (2003) "Sources of estrogen and their importance". J Steroid Biochem Mol Biol 86(3–5):225–30. https://doi.org/10.1016/S0960-0760(03)00360-1

Singh AK, Singh R (2013) In vivo response of melatonin, gonadal activity and biochemical changes during CYP19 inhibited sex reversal in common carp *Cyprinus carpio* (L). Anim Rep Sci 136(4):317-25. https://doi.org/10.1016/j.anireprosci.2012.11.004

Singh AK (2013) Introduction of modern endocrine techniques for the production of monosex population of fishes. Gen Comp Endocrinol 181:146-55. doi: 10.1016/j.ygcen.2012.08.027S0016-6480(12)00378-4

Singh E, Saini VP, Sharma OP (2018) Orally administered 17 α methyl testosterone at different doses on the sex reversal of the red tilapia (*Oreochromis niloticus*). Int J Fish Aquat Stud 6(3):103-5

Smith IE (1999) Aromatase inhibitors: a dose-response effect? Endocr Relat Cancer 6(2):245-9

Specker JL, Chandlee MK (2003) Methodology for estradiol treatment in marine larval and juvenile fish: uptake and clearance in summer flounder. Aquaculture 217(1-4):663-72. https://doi.org/10.1016/S0044-8486(02)00424-6

Steele RE, Mellor LB, Sawyer WK, Wasvary JM, Browne LJ (1987) In vitro and in vivo studies demonstrating potent and selective estrogen inhibition with the nonsteroidal aromatase inhibitor CGS 16949A. Steroids 50(1-3):147-61. https://doi.org/10.1016/0039-128X(83)90068-5

Sun L, Zha J, Spear PA, Wang Z (2007) Toxicity of the aromatase inhibitor letrozole to Japanese medaka (*Oryzias latipes*) eggs, larvae and breeding adults. Comp Biochem Phys 145(4):533-41.https://doi.org/10.1016/j.cbpc.2007.01.017

Taranger GL, Carrillo M, Schulz RW, Fontaine P, Zanuy S, Felip A, Weltzien FA, Dufour S, Karlsen Ø, Norberg B, Andersson E (2010) Control of puberty in farmed fish. Gen Comp Endocrinol 165(3):483-515. doi: 10.1016/j.ygcen.2009.05.004S0016-6480(09)00211-1

Tenugu S, Senthilkumaran B (2022) Sexual plasticity in bony fishes: Analyzing morphological to molecular changes of sex reversal. Aquac Fish 7(5):525-39. https://doi.org/10.1016/j.aaf.2022.02.007

Trunet PF, Mueller PH, Girard F, Aupetit B, Bhatnagar AS, Zognbi F, Ezzet F, Menard J (1992) The effects of fadrozole hydrochloride on aldosterone secretion in healthy male subjects. J Clin Endocrinol Metab 74(3):571-6. https://doi.org/10.1210/jcem.74.3.1531483

Turan F, Akyurt I (2005) Effect of red clover extract on growth performance and body composition of African catfish *Clarias gariepinus*. Fish Sci 71:618-620. https://doi.org/10.1111/j.1444-2906.2005.01006.x

Tzchori I, Zak T, Sachs O (2004) Masculinization of genetic females of the common carp (*Cyprinus carpio L.*) by dietary administration of an aromatase inhibitor. Isr J Aquac 56: 239-246

Uchida D, Yamashita M, Kitano T, Iguchi T (2004) An aromatase inhibitor or high water temperature induce oocyte apoptosis and depletion of P450 aromatase activity in the gonads of genetic female zebrafish during sex-reversal. Comp Biochem Physiol Mol Integr Physiol 137(1):11-20. https://doi.org/10.1016/S1095-6433(03)00178-8

Venkatasubramanian H, Selvaraj V, Ramasamy M, Santhakumar K (2019) Effect of fadrozole exposure on socioreproductive behaviors and neurochemical parameters in *Betta splendens*. Zebrafish 16(2):139-51. https://doi.org/10.1089/zeb.2018.1595

Verma N, Verma BK, Pushpavanam S (2020) Modeling temperature-dependent sex determination in oviparous species using a dynamical systems approach. Bull Math Biol 82:1-25. https://doi.org/10.1007/s11538-020-00763-6

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJ, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416(6879):389-95. https://doi.org/10.1038/416389a

Wang H, Shi W, Wang L, Zhu C, Pan Z, Yu X, Qiang X (2019) Masculinization under combined administration of an androgen and an aromatase inhibitor in the Ussuri catfish, *Pelteobagrus ussuriensis* (Dybowski 1872). Aquac Res 50(10):3028-36.. https://doi.org/10.1111/are.14261

Weber GM, Leeds TD, Schneider RP (2020) Sex reversal of female rainbow trout by immersion in 17α-methyltestosterone. Aquaculture 528:735535. https://doi.org/10.1016/j.aquaculture.2020.735535

Weber GM, Leeds TD (2022) Effects of duration and timing of immersion in 17α-methyltestosterone on sex reversal of female rainbow trout. Aquac Rep 23:101014. https://doi.org/10.1016/j.aqrep.2022.101014

Wisdom KS, Bhat IA, Kumar P, Pathan MK, Chanu TI, Walke P, Sharma R (2018) Fabrication of chitosan nanoparticles loaded with aromatase inhibitors for the advancement of gonadal development in *Clarias magur* (Hamilton 1822). Aquaculture 497:125-33. https://doi.org/10.1016/j.aquaculture.2018.07.049

Xu G, Huang T, Gu W, Liu E, Wang B (2021) Effects of letrozole and 17α-methyltestosterone on gonadal development in all-female triploid rainbow trout (*Oncorhynchus mykiss*). Aquac Res 52(6):2460-2469. https://doi.org/10.1111/are.15095

Xu J, Dun J, Yang J, Zhang J, Lin Q, Huang M, Ji F, Huang L, You X, Lin Y (2020) Letrozole rat model mimics human polycystic ovarian syndrome and changes in insulin signal pathways. Int Med J Exp Clin Res 26:e923073-1

Yates RA, Dowsett M, Fisher GV, Selen A, Wyld PJ (1996) Arimidex (ZD1033): a selective, potent inhibitor of aromatase in post-

menopausal female volunteers. British J Cancer 73(4):543-8. https://doi.org/10.1038/bjc.1996.94

Ying GG, Kookana RS, Ru YJ (2002) Occurrence and fate of hormone steroids in the environment. Environ Int 28(6):545-51. https://doi.org/10.1016/S0160-4120(02)00075-2

Yostawonkul J, Kitiyodom S, Supchukun K, Thumrongsiri N, Saengkrit N, Pinpimai K, Hajitou A, Thompson KD, Rattanapinyopituk K, Maita M, Kamble MT (2023) Masculinization of red tilapia (*Oreochromis spp*.) Using 17α-methyltestosterone-loaded alkyl polyglucosides integrated into nanostructured lipid carriers. Animal 13(8):1364. https://doi.org/10.3390/ani13081364

Yu NW, Hsu CY, Ku HH, Chang LT, Liu HW (1993) Gonadal differentiation and secretions of estradiol and testosterone of the ovaries of Rana catesbeiana tadpoles treated with 4-hydroxyandrostenedione. J Exp Zool 265(3):252-7. https://doi.org/10.1002/jez.1402650307

Zucchini G, Geuna E, Milani A, Aversa C, Martinello R, Montemurro F (2015) Clinical utility of exemestane in the treatment of breast cancer. Int J Women's Health 551-63.10.2147/IJWH.S69475

Publisher's Note