University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Effects of Metformin in High Concentrations on the Fertility of Female Sailfin Molly *Poecilia latipinna*

[1]Abdul-Ameer M.F. Al-Hachamei ,[2] Hussein A. Saud ,[3] Sajed S. H. Alnoor

[1*3] Department of Fisheries and Marine Resources, College of Agriculture, the University of Basrah, Iraq

[2] Department of Pathological Analyses, College of Sciences, University of Basrah, Iraq

[1] *Email: pgs.abdulameer.mohareb@uobasrah.edu.iq*

[2] *Email: hussein.saud@uobasrah.edu.iq*

[3] *Email: sajd.alnoor@uobasrah.edu.iq*

Abstract

Metformin is a medicine that is used to treat type 2 diabetes and polycystic ovary syndrome (PCOS). This study investigated the effects of exposing the sailfin molly fish to sub-lethal concentrations of 0.008 mol.L^{-1} of metformin. The results showed that metformin caused disturbances in the endocrine glands, especially the pituitary hormones (GTHs). Metformin increased the expression of the *Fshb* and inhibited the *Lhb* genes in the pituitary gland, which led to higher levels of FSH and lower levels of LH hormones in the blood of fish. These hormonal changes led to irregularities in the ovarian development of female fish, such as an increase in the first stage of primary eggs and a delay in the final stage of embryo production.

***Key words: FSH, LH, metformin, Poecilia latipinna*.**

I. INTRODUCTION

Metformin is 1, 1-dimethyl-biguanide hydrochloride with the molecular formula C4H11N5. It is a white, hygroscopic crystalline powder with a bitter taste. Soluble in water and 95% alcohol, but practically insoluble in ether and chloroform. Its molecular weight 129.17 g.mol^{-1} (da Trindade *et al*., 2018). Metformin was derived from a natural compound called guanidine, which is found in the plant *Galega officinalis*. Guanidine was first shown to lower blood glucose in 1918, but it was too toxic to use as a drug. Metformin was discovered as a derivative of guanidine in 1922 AD and its side effects are few compared to guanidine. Metformin was studied for the first time on humans by French physician Jean Stern in the fifties of the last century and was introduced as a drug in France in 1957, then in the United States in 1995 (Bailey *et al*., 2017; Lawler, 2023).

Metformin lowers blood sugar by affecting the way of metabolism in the liver, intestines, and cells handle glucose. It does not change the amount of insulin produced in the body, but it does make cells more sensitive to insulin and lowering the sugar absorption (Rena *et al*., 2017). Metformin also reduces the absorption of sugar by the gastrointestinal tract and reduces the production of glucose by the liver (Aungst, 2022). Some studies suggested that metformin should be included in a group of poly affective, such as penicillin and aspirin, due to its characteristics in treating many diseases (Shmerling, 2021). In addition, it is considered the first choice for patients with type 2 diabetes. It has been used to treat several conditions, including cyst of ovaries (Lashen, 2010.), as well as to reduce body weight and elimination of triglycerides in the blood (Yerevanian, & Soukas, 2019). Improving and treating infections, protecting the heart and blood vessels (Bai & Chen, 2021), delaying the aging (Cheng *et al*., 2022), as well as helping to treat HIV (Planas *et al*., 2021). Promising treatment in types of cancers in humans improved by metformin therapy (Wu *et al*., 2020). Also, it use for treating

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Polycystic Ovary Syndrome (PCOS), a hormonal disorder that affects women of childbearing age and can cause irregular menstruation, infertility, excessive hair growth, acne, improve menstrual cycles, ovulation, and insulin resistance in women with PCOS (Hashim, 2016).

Metformin can also improve male reproductive function, especially in obese men who have low fertility (Tseng, 2022). It can improve sperm quality, motility, and morphology by increasing testosterone production and reducing oxidative stress (Ye *et al*., 2019). According to two studies by Liu *et al*. (2018) and Faure et al. (2018), metformin can act against prostate cancer by reducing its growth and spread

However, metformin did not has serious risks or side effects. It can cause gastrointestinal trouble, such as nausea, diarrhea, and abdominal pain (Drugs.com 2022). Metformin can also interact with other drugs or supplements, such as alcohol, iodinated food, or vitamin B12 (Davies 2022; Gershman, 2022). Moreover, metformin can have long-term effects on offspring exposed to it in the womb, causing increased body weight, altered metabolism, and epigenetic changes (Tseng, 2022).

Other studies cited that metformin has effect on endocrine in aquatic organisms, Niemuth, & Klaper, (2015) studied the effect of metformin at same concentrations recorded in the environment 40µg.L^{-1} on fathead minnows (*Pimephales promelas*) from early life stages to adulthood,. they found that exposure to metformin has transformed male fish to female and reduced the size and fertility of male fish, The study concluded that metformin acts as an endocrine disruptor at environmentally relevant concentrations and they suggested that it may be possible cause hermaphroditismin fish. So that they suggested that it may have negative effects on fish populations and ecosystems.

Mudumbi, *et al*. (2018) indicated that metformin can influence on the growth, reproduction, and survival of aquatic organisms such as fish, algae, bacteria, and zooplankton. Their study concluded that metformin can alter gene expression in aquatic organisms, especially genes involved in the endocrine hormone pathways.

Lee *et al*. (2019) indicated that when applying different concentrations (40, 60, and 360) µg.L^{-1} of metformin on *Oryzias latipes* fish for two consecutive generations, it caused endocrine disruption, hermaphroditism in males, and oxidative stress in fish, subsequently it reduced vitellogenin (VTG2) expression, indicating that metformin has adverse effects on aquatic organisms.Ambrosio-Albuquerque, *et al*. (2021) described the effects of metformin on aquatic organisms showning that metformin can affect on gene expression related to endocrine hormone pathways. Phillips *et al*., (2021) reported that metformin has negatively effects on the development of fish embryos for example, long term exposure to metformin reduced survival rate and caused malformations in zebrafish embryos and larvae. They also found that exposure to metformin for a short period affected on fish movement and genes expression in zebrafish associated with brain and heart development.

It was found that metformin disrupts the gonadal development and reproduction cycle of zebrafish at low concentrations (Barros *et al*., 2022a). Generally fish have been used in medical experiments because they have high matching of genetic similarity with humans, making us using them as useful model for human diseases and understanding the molecular mechanisms of cells (Schaeck, *et al*., 2013). Moreover, fish have several advantages over rodent models, such as large numbers of offspring, visual clarity of embryos, lower maintenance costs, and ease of generation of transgenic animals (Bailone *et al*., 2020 Choi *et al*., 2021; Adhish, & Manjubala 2023). The Sailfin molly, *Poecilia latipinna* is a small viviparous fish that can live in fresh, brackish, and coastal saltwater, they habitats the regions from North Carolina to Mexico (Florida Museum of Natural History, 2023). It is a tolerant species that can survive at low level of oxygen and high salinity (Gonzalez, *et al*., 2005). The Sailfin molly fish have short life period, they become mature and differentiate embryos from four to five weeks for one batch, separating eight to ten weeks between one batch and other, depending on the surrounding conditions (Costa, and Schlupp, 2020). *P. latipinna* used commonly in biological experiments as a model for many disciplines including genetics, ecology, and biochemistry (Yang *et al*., 2009).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

The aim of our study is to determine the effect of metformin on the reproductive path of the sailfin molly fish, *Poecilia latipinna*, through which we can determine the level and type of effects resulting from the release of hospital effluent containing drug residues and sterilization solutions on the environment and its environmental components..

II. Materials and Methods

Fish collection and treatment with metformin

96 fish *Poecilia latipinna* were collected from Basrah body water then they kept in an aquarium and acclimated before experiment start. As a preservative process, all fish were treated with 40 $g.L^{-1}$ of sodium chloride (NaCl) in laboratory aquariums to eliminate any potential parasites (Dewi *et al*., 2018). The captured fish comprised of half males with a total length of 65-81 mm and half females with total length of 59-74 mm. They were acclimated in 100-liter glass tanks for two weeks. For feeding, it was used standard pellets once a day. Fish were divided into 12 groups each contain eight individuals (four males and four females) separated in 30-liter tanks (35 × 30 × 35 cm). Four groups were fixed as control samples (C), four groups of the first treatment were exposed for three hours per day (T1), and other four groups for the second treatment were exposed for five hours per day (T2). It was used one concentration ($1320\mu g.L^{-1}$)(0.008 mol. L^{-1}) of metformin hydrochloride in all treatments.. Water system was changing with new prepared concentrations of metformin daily for period of experiment that extended five weeks. Fish were reared under environmental conditions included photoperiod: 12-hour light/dark, temperature of 26° C (± 2), pH of 8-8.2, and continuous aeration.

Quantitative real-time PCR (qRT-PCR)

Primer Design

We designed primers for *Lhb* and *Fshb* based on the GenBank sequences (Sequence ID: Fshb: XM_015020261.1, Lhb: XM_015023733.1, and eef1a1: housekeeping gene: XM_015020125.1). We used Primer 3 Plus software to design forward and reverse primers, primers synthesed by Macrogen Korea laboratory (Table 1).

Table (1): Primer sequences of *Lhb, Fshb*, and *eef1a1*

Primer sequence	Gene name
F: 5-TGCTTCAGCTGCAACCTGAA-3	***Fshb***
R:5-ATCCTCTGTGTTGCATGTGGT-3	
F:5-TAAGCTCATCTCTGCTGTATAA-3	***Lhb***
R:5- CAACTGGGTAGGTGACGACA-3	
F: 5-GACACCTCATCTACAAGTGTG-3	***eef1a1***
R: 5-GTTTGTCCGTTCTTGGAGATGC-3	

Total RNA extraction

Total RNA was extracted from the brain of four female fish (Four fish from each group. fish were anesthetized with cloves ($400\ mg.L^{-1}$) following the Basrah University regulations for animal research. The upper part of the head (about 100 mg)

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

from each induvial was cut off and stored it in liquid nitrogen until initiation of RNA extraction. AddP rep Total RNA extraction kit (Add Bio Inc.; Addbio, Korea) was applied and DNaseI used to degrade DNA. Quality and quantity of extracted RNA were measured with a nanodrop spectrophotometer (Analytic Jena Germany) at 260/280 nm absorption.

cDNA synthesis

Reverse transcription was performed using AddScript cDNA Synthesis Kit (Add Bio Inc.) to convert the extracted RNA to cDNA for RT-PCR analysis. 5 µL RNA template was mixed with 10 µL of 1X RT master mix, 3 µL of water molecules, 2 µL of random hexamer 10X primer, then the mixture was centrifuged for 5 seconds and transferred it to a PCR thermal cycler. the mixture was incubated at 25°C for 10 minutes, then mixture incubate at 50°C for 60 minutes for reverse transcription, and finally at 80°C for 5 minutes to stop the reaction.

Quantitative real-time polymerase chain reaction analysis

AdScript cDNA Synthesis real-time PCR kit and the Mx3005P real-time PCR system (Agilent; USA) were employed to perform quantitative RT-PCR for the cDNA samples. 20 µL of reaction mixture was mixed with 1X qPCR master mix, 10 pM of each forward and reverse primer, and 320 ng of cDNA product. We used eef1a1 as a reference gene to standardize the relative expression levels of *Lhb* and *fshb*. We ran quantitative real-time PCR of *Lhb*, *fshb*, and eef1a1 with the following thermal cycling scheme: 95°C for 10 minutes for hot-start activation, then 40 cycles of 60°C for 10 sec for annealing and 72°C for 30 sec for extension, and finally 72°C for 2 min for denaturation (Schmittgen & Livak, 2008). Equation was used to analyze the fold change of the target genes.

ELISA Test

LH and FSH hormone concentrations were measured in the blood plasma of the fish treated with metformin. We used 1.0 mL syringe moistened with EDTA to draw blood from the heart. Then the blood was transferred to 1.0 mL micro-centrifuge tubes and left at room temperature for 10-20 minutes. Tubes were centrifuged at 2000-3000 rpm to separate the plasma from the red blood cells and solid particles. ELISA Kits from SunLong Biotech Co., LTD of China (Fish LH ELISA Kit Cata N: SL0024FI and Fish FSH Elisa kit Cata N; SL0019FI) was applied to measure the LH and FSH hormones. samples were read with an absorbance machine (HUMAREADER HS 16670 from Human GmbH, Germany) at 450 nm. Then it was obtained the straight line equation from the x-value curve in terms of y for the calibration concentrations. We multiplied the values by 5 to get the final concentrations of the samples. SPSS statistical analysis was utilized to find out the significant differences among the treatments for FSH and LH hormones concentrations.

Gonado Somatic Index (GSI) and Actual Fertility

For measure GSI, the fish were anesthetized using clove (0.8µg.L^{-1}), then the fish were measured with a sensitive scale, to the nearest 0.001 grams, and their ovaries were removed and weighed with a sensitive balance with an accuracy of 0.0001 grams.

GSI= (GW/BW),

GW = Gondal Weight (g)

BW= Body Weight without entrails (g) (Bahamonde, *et al*., 2013; Rizzo and Bazzoli 2020).

The actual fertility was calculated by keeping the ovariesin a 10 ml test tube, Gilson's solution (100 ml alcohol 60%, 880 ml distilled water, 18 ml glacial acetic acid, 15 ml nitric acid 80%, and 20 g HCl mercury) them. After several days, until the eggs had hardened, they were emptied into an earthenware beaker, and the number of eggs counted under a magnifying glass (Muchlisin, 2014; Chen *et al*., 2022).

Statistical Analyses.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Statistical analysis software (SPSS) version 20 was used to identify statistically significant differences between the values obtained (mean ± SD) by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test. P value < 0.05 was considered significant.

III. Results

Gene Expression of *Fshb*

fshb exhibited relative increase for both treatments (1.20 and 1.23) compared with control, but the increase was not significant (p>0.05) Fig. 1. These results shows that metformin stimulate *fshb* expression

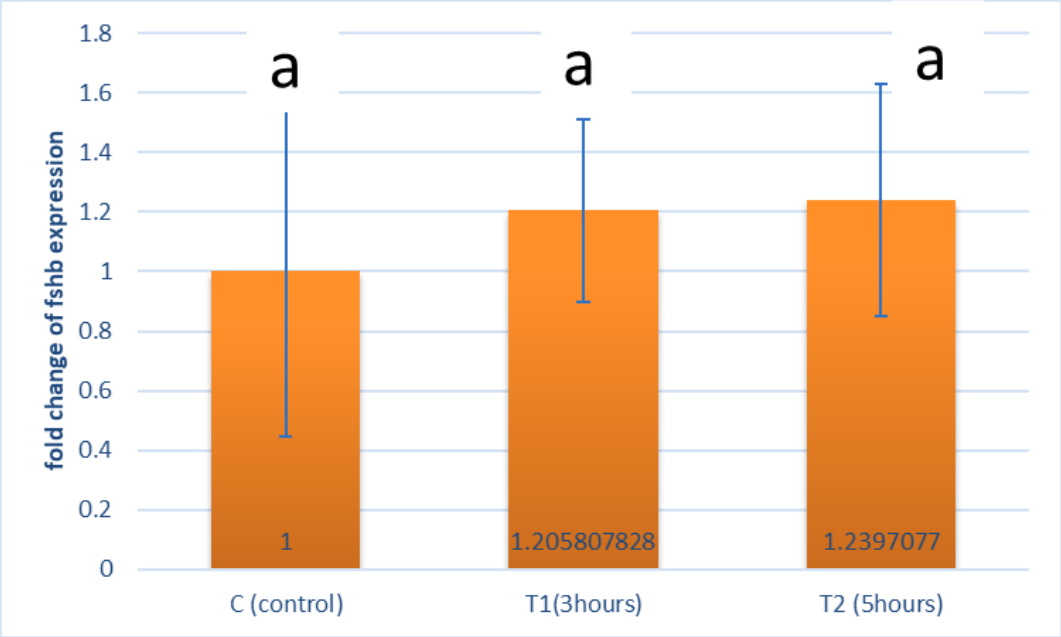


Fig. (1): showed the mean values of fold change and standard deviation of *fshb* expression in the pituitary gland of females sailfin molly (*Poecilia latipinna*), C: control, T1: treatment with 1320 mg.L^{-1} of metformin for three hours per day, T2 treatment with 1320 mg.L^{-1} of metformin for five hours per day . Similar letters mean there were no statistically significant differences between treatments (P≤0.05).

Gene Expression of *lhb*

Lhb expression has been showed dramatically decrease for both treatments (T1: 0.26 and T2: 0.19) of exposure to metformin in comparison with control, and statistically were significant (p≤0.002). Fig. (2) indicating that metformin has inhibition effectiveness on *lhb* expression.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

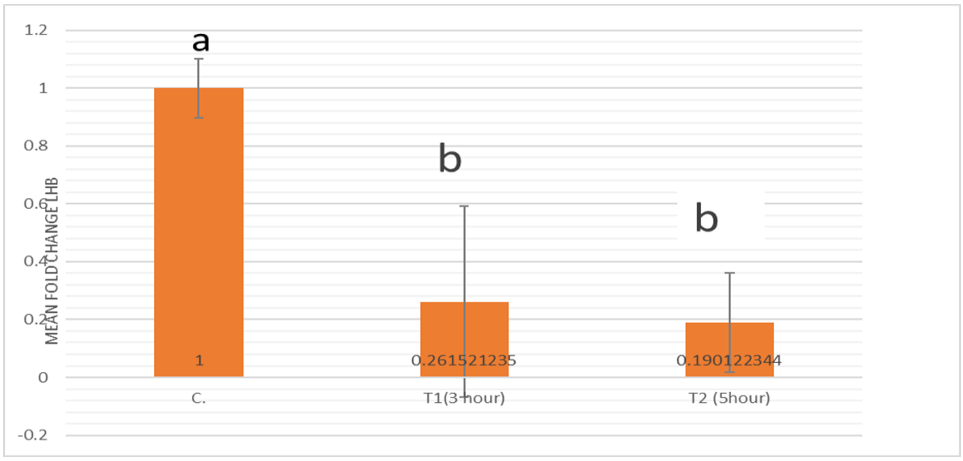


Fig. (2): showed the mean values of fold change and standard deviation of *lhb* expression in the pituitary gland of females sailfin molly (*Poecilia latipinna*), C: control, T1: treatment with 1320 mg.L^{-1} of metformin for three hours per day, T2 treatment with 1320 mg.L^{-1} of metformin for five hours per day. Different letters mean that there are significant differences between the treatments (P≤0.05)

ELISA test of FSH Hormone

ELISA test and statistical analysis (SPSS) using the LSD test at a level of 0.05 confirmed a significant (p≤0.0204) increasing in FSH hormone secretion that tested in the plasma of females fish *Poecilia latipinna*, as it was noticed that there is observed increase in the hormone level with increase the length of the exposure period C (6.379± 0.562 ng.mL^{-1}), T1 (8.707 ±2.56 ng.mL^{-1}) and T2 (18.288 ± 6.079 ng.mL^{-1}) as (Table 2).

Table (2): FSH hormone concentration (ng.mL^{-1}) for control and treatments with metformin in the plasma of females fish *Poecilia latipinna* (mean ± SD).

Group		Concentration (ng.mL^{-1})	Mean	SD
C	1	6.767	6.37875	±0.56
	2	6.897		
	3	6.164		
	4	5.687		
T1	1	7.586	8.707	±2.56
	2	12.543		
	3	7.371		
	4	7.326		

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

T2	1	22.457	18.288	±6.079
	2	15.129		
	3	11.341		
	4	24.224		

ELISA test of LH Hormone

The ELISA test of LH hormone in the blood plasma of *Pocelia latipinna* that treated with metformin showed that there were a strong significant increasing ($P \leq 0.0001$) in comparison with the control table (3) shows the the concentrations of the LH hormone in the blood plasma of *Pocelia latipinna* in units of ng/ml (the mean ± S.D).

Table (3): LH hormone concentration ($ng.mL^{-1}$) for control and treatments in the plasma of females fish *Poecilia latipinna* (mean ± SD), showing the drop of hormone with increase of metformin concentrations.

Group		Concentration ($ng.mL^{-1}$)	Mean	SD
C	1	8.0423	6.374	±1.501
	2	6.670		
	3	6.381		
	4	4.404		
T1	1	4.165	3.521	±0.458
	2	3.328		
	3	3.489		
	4	3.103		
T2	1	1.925	1.978	±0.507
	2	1.318		
	3	2.531		
	4	2.138		

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Gonado Somatic Index (GSI) and Fecundity

However the results of GSI exhibited differences between the control treatment (average ± standard deviation) (0.173 ± 0.05), and the treatments (T1 = 0.149 ± 0.09, =0.121 ± 0.07), Statistical analysis did not show significant differences (P ≥ 0.175, table 4). And between the specialist analysis and the presence of significant differences for the actual fertility between. At the same time the fecundity of treated groups showed significantly high levels (table 4).

Table (4): Shows the groups with the number of samples for each treatment with the average GSI with standard deviation, and the average actual fertility with standard deviation.

Treatments	fish No.	GSI mean(± S.D)	Actual Fecundity, mean (± S.D) embryos number
C	12	0.173(±0.05)	65(±18.56)
T1	9	0.149(± 0.09)	134.67(±79.46)
T2	14	0.121(±0.072)	109.43(±75.98)

Through the morphological examination of eggs for the ovaries of exposed fish to metformin, it observed different stage of embryonic development (Figs. 4 and 5) in comparison with control that showed a certain developmental stage of embryos (Figs. 3). Variety of stages reached %42 in the first treatment (T1), and %44 in the second treatment (T2).

Statistical analysis for the number of embryos between the control and treatments showed high significant (P≤0.018) whereas there was low significant in the number of embryos between treatments (P≤0.047) table (4).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

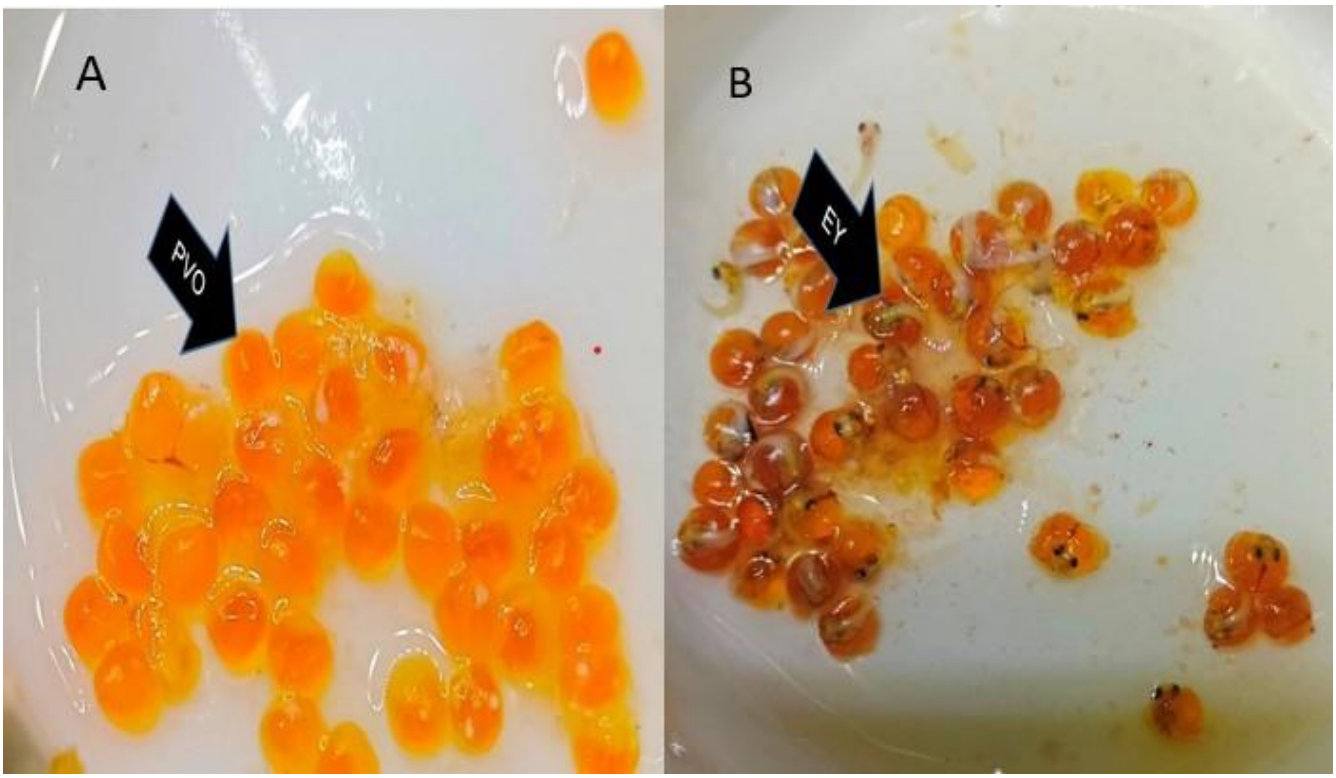


Fig. (3): The ovary of the sailfin molly fish, *Poecilia latipinna*, taken from a control fish, shows one stage of development.A: post-vitellogenic oocyte (PVO) .B: Embryo with yolk (EY).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

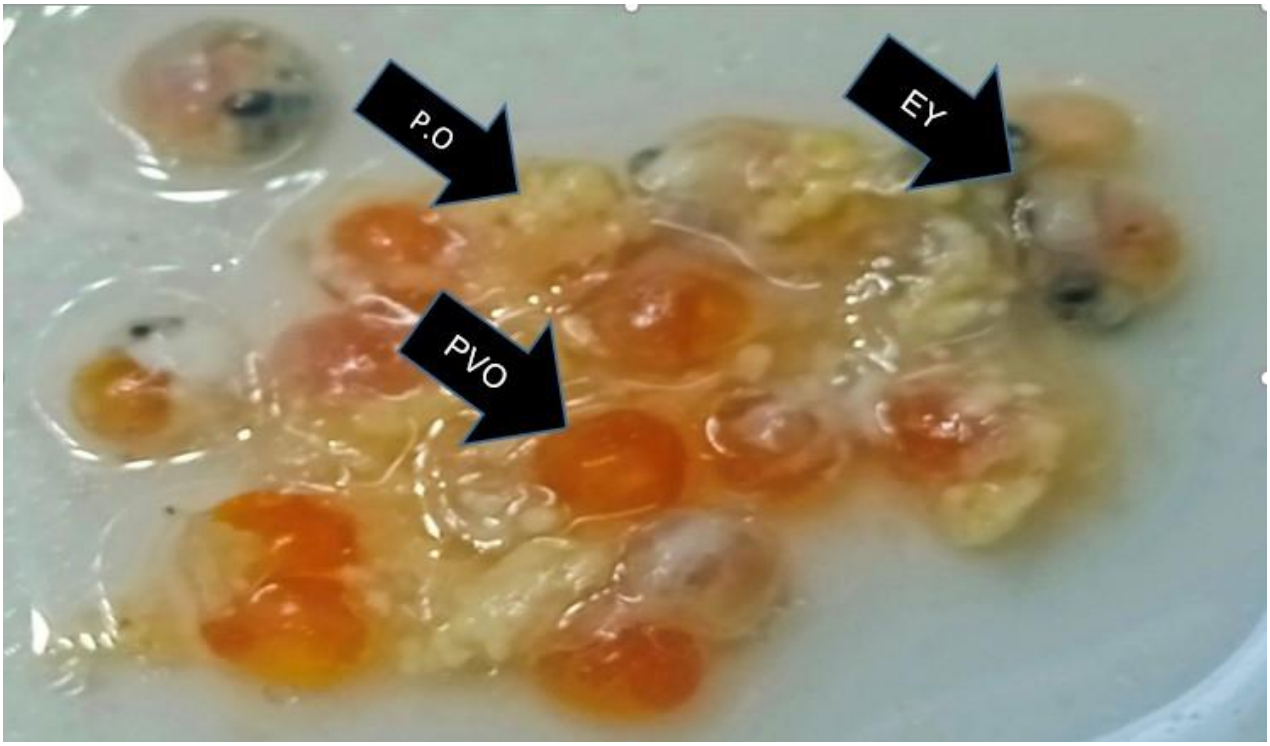


Fig. (4): showed the ovary of the sailfin molly fish *Poecilia latipinna* exposed to metformin, indicating the variety of developmental stages due to effects of metformin on the development process, Embryo with yolk:EY, post-vitellogenic oocyte: PVO , primary oocytes: P.O

IV. Discussion

GHTs (FSH and LH) are two hormones responsible for regulating reproduction in fish, like all vertebrates. FSH stimulates meiosis of germ cells (production of eggs and sperm), while LH triggers maturation (final development and release of eggs and sperm (Hatef, & Unniappan. 2019; Blanco, 2020). FSH and LH regualte the gonads development by activation of signaling pathway and synthesis of sex steroids (Zhang *et al*., 2015; Hollander-Cohen *et al*., 2021). The roles of FSH and LH may vary depending on fish species, sex, and stage of reproduction (Ramos-Júdez *et al*., 2022). It is equally important for males as female (Zhang *et al*., 2015).

Current study showed that exposing molly fish, *Pocelia latipinna*, to high concentrations (0.008 mol.L^{-1}) of metformin, leads to disturbances in the endocrine glands that is responsible for the reproductive , e.g. hypothalamus-pituitary- glands (HPG) which are the main organs for regulating the reproduction process in vertebrates in general, including fish (Niemuth & Klaper 2015; Lee *et al*., 2019; Elizalde-Velázquez *et al* 2020). According to a recent study by Ambrosio-Albuquerque *et al*. (2021), metformin, a drug that affects the pituitary gland, can increase the levels of FSH in fish, FSH is a hormone that helps female fish develop their eggs and produce estrogen, a female sex hormone, FSH also affects the production of vitellogenin, a protein that is important for egg formation (Cabrita *et al*., 2008; Biran & Levavi-Sivan, 2018)

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Metformin increases the incidence of hermaphroditism in males, the presence of eggs in the testicular tissue of male fish *Pimephales promelas*, and the increase in the production of vitellogen from male liver, as a result of increase in the FSH hormone (Niemuth *et al*., 2015). Exposing male fish of *Betta splendens* to metformin reduces their hostility as a result of decrease in the level of LH hormone (MacLaren *et al*., 2018).

Metformin is also a treatment for polycystic ovary syndrome (PCOS), which is a hormonal disorder in FSH/LH hormone levels, a decrease in the level of FSH leads to the cessation or delay of ovulation in the ovary, while an increase in the level of LH causes an raise in androgen and testosterone (Saadia, 2020 ;Mitrašinović-Brulić *et al.*, 2021.). Metformin regulates hormone levels by increasing the secretion of FSH, and reducing of LH (Genazzani *et al*., 2010; Tso, *et al*, 2020; Jiang *et al*., 2022). The current result corresponds the previous authors' results. Recent study proved that via gene expression and ELISA analyses. The morphological test of the ovary also reflected the gene expression and ELISA result, showing presence different stage of embryonic development at variance untreated fish (control) that showed up ovary contain a similar stage of embryonic development. This explains the relationship between GHTs and metformin, where FSH stimulates germ cells division and LH has an important role in reproduction by regulating the final maturation of gametes (eggs and sperm). LH is secreted by the pituitary gland targeting the gonads to stimulate other production hormones, such as MIH or MIS that control oocyte maturation. It plays an important role in the final stages of ovulation (Hollander-Cohen *et al*., 2021; Kumar *et al*., 2021; Zohar *et al*., 2021)

Endocrine-disrupting chemicals can interfere with the normal function of the endocrine system by mimicking the action of hormones, preventing their stimulation through binding to hormone receptors, or altering the normal action of hormones in stimulating hormones (Yilmaz *et al*., 2020). Endocrine-disrupting chemicals (EDCs) can affect on some organs or endocrine glands, such as the thyroid, ovaries, testes, adrenal glands, and pituitary gland. Endocrine-disrupting chemicals can also disrupt energy metabolism and cause obesity and metabolic syndrome (Lauretta *et al.*, 2019; Lacouture *et al*., 2022).

The most common mechanism of action for metformin is inhibition of mitochondrial respiratory complex 1, this leads to reduced oxidative phosphorylation, reduced ATP production, and an increase in the AMP to ATP ratio that activates AMP-activated kinase (AMPK), which inhibits mechanistic target of rapamycin (mTOR) and activates the catabolism of ATP for energy (Wang *et al*., 2019 ; Agius *et al*., 2020; Ma *et al*., 2022). Metformin-induced activation of AMPK, inhibition and decrease in expression of mTOR, a protein kinase belonging to the family of phosphatidylinositol-3 kinases (PIKKs). Which regulates various cellular processes such as metabolism, growth, and reproduction (Murugan, 2019), that is, it stops the building processes of proteins and fats and accelerates their metabolism (Lamming *et al*., 2013; Weichhart, 2018). This suggest the reduction of GSI in treated fish. Despite the increase in the hormone FSH, the volume of the egg yolk derived from vitellogen did not increase as a result of the effect of mechanistic target of rapamycin (mTOR). A study on fish about the effect of metformin on the reproduction activity found that it interferes with the energy pathway, which is an important in the regulation and success of the reproduction process (Volkoff & London, 2018; Barros *et al.*, 2022b).

Another hypothesized pathway by which metformin may influence on endocrine secretions is DNA methylation, This process means adding a methyl group (carbon with three hydrogen atoms) to a DNA base, usually cytosine To activate or suppress specific genes (Moore *et al*., 2013). Hassler *et al*. (2016) and Lin *et al*. (2020) cited that metformin accumulated in zebrafish tissues, acting DNA methylation by its interaction with DNA methy ltransferase 1 (DNMT1) that is an enzyme regulates DNA methylation and alters the state of gene expression.

We conclude that metformin leads to disrupt the endocrine glands, especially the pituitary gland hormones (GTH), as it increased the level of the hormone FSH, subsequently lead to change cycle and inhibit LH hormone. The latter in turn act to delay the ovulation. The metformin reproductive effectiveness also can be influenced by the species and the physiological state of fish, as it acts to interference with the energy metabolism pathway via other genetic pathways.

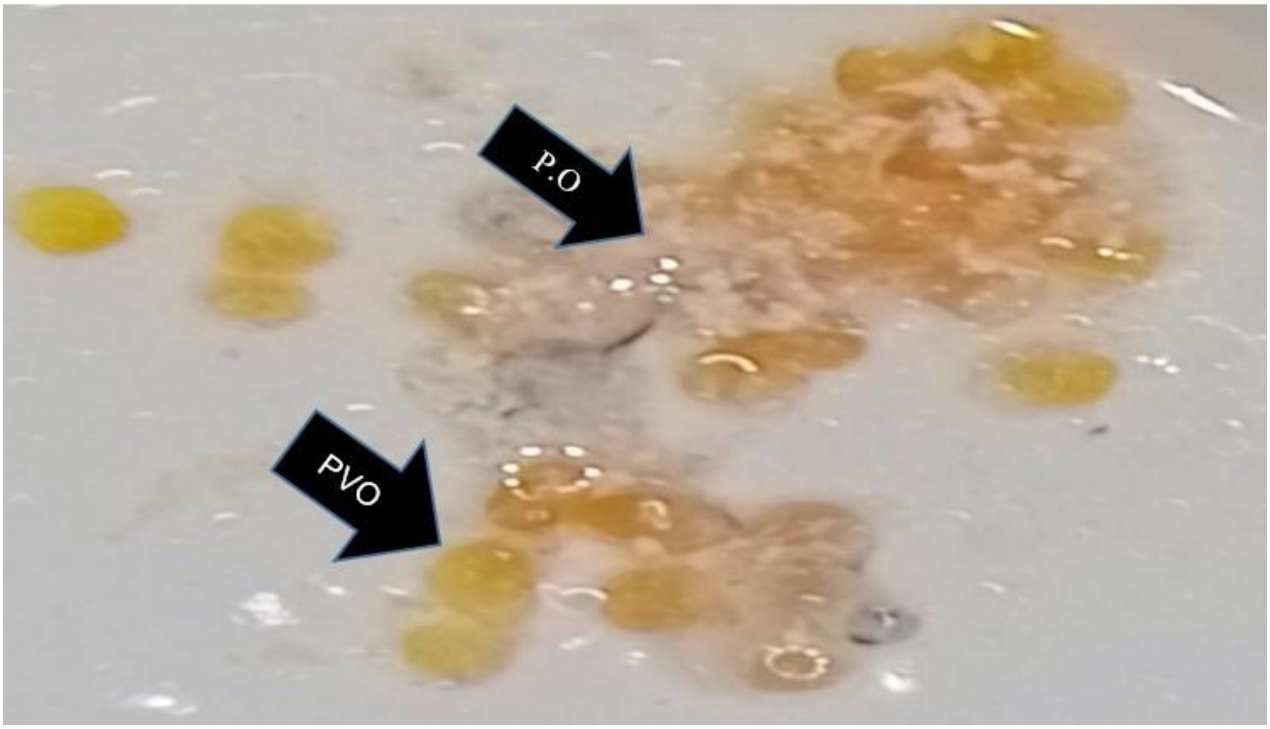
University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Fig. (6): showed the ovary of the sailfin molly fish *Poecilia latipinna* that exposed to metformin, pointing out early stages and late stage of embryonic development, post-vitellogenic oocyte: PVO , primary oocytes: P.O



University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

V. Conclusion

Our study shows that metformin affects the reproductive cycle of female molly fish by enhancing the expression of *Fshb* gene and suppressing that of *Lhb* gene. These two effects lead to ovarian changes in the process of egg formation and maturation.

Acknowledgments

We would like to express our sincere gratitude to our colleagues from the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, for their support and assistance.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical approval

This study was conducted in accordance with all relevant institutional, national and international ethical guidelines for the care and use of animals.

VI. REFERENCES

Adhish, M., & Manjubala, I. (2023). Effectiveness of zebrafish models in understanding human diseases—A review of models. *Heliyon*. https://doi.org/10.1016/j.heliyon.2023.e14557

Agius, L., Ford, B. E., & Chachra, S. S. (2020). The metformin mechanism on gluconeogenesis and AMPK activation: the metabolite perspective. *International journal of molecular sciences*, *21*(9), 3240. https://doi.org/10.3390/ijms21093240

Ambrosio-Albuquerque, E. P., Cusioli, L. F., Bergamasco, R., Gigliolli, A. A. S., Lupepsa, L., Paupitz, B. R., Barbieri, P.A., Borin-Carvalho, L.A., & de Brito Portela-Castro, A. L. (2021). Metformin environmental exposure: A systematic review. *Environmental Toxicology and Pharmacology*, *83*, 103588. https://doi.org/10.1016/j.etap.2021.103588

Aungst .Christina, (2022). What Is Metformin Used for, and How Does It Work Exactly? https://www.goodrx.com/metformin/metformin-how-does-it-work-what-does-it-do

Bahamonde, P. A., Munkittrick, K. R., & Martyniuk, C. J. (2013). Intersex in teleost fish: are we distinguishing endocrine disruption from natural phenomena?. *General and comparative endocrinology*, *192*(1), 25-35. http://dx.doi.org/10.1016/j.ygcen.2013.04.005

Bai, B., & Chen, H. (2021). Metformin: A novel weapon against inflammation. *Frontiers in pharmacology*, *12*, 622262. https://doi.org/10.3389/fphar.2021.622262

Bailey, C. J. (2017). Metformin: historical overview. *Diabetologia*, *60*(9), 1566-1576. https://doi.org/10.1007/s00125-017-4318-z

Bailone, R. L., Fukushima, H. C. S., Ventura Fernandes, B. H., De Aguiar, L. K., Corrêa, T., Janke, H., Setti,P.G., Roça, R. De O. & Borra, R. C. (2020). Zebrafish as an alternative animal model in human and animal vaccination research. Laboratory animal research, 36, 1-10. https://doi.org/10.1186/s42826-020-00042-4

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Barros, S., Alves, N., Pinheiro, M., Ribeiro, M., Morais, H., Montes, R., Rodil, R., Quintana, J.B., Coimbra, A. M., Santos, M. M., & Neuparth, T. (2022a). Are fish populations at risk? Metformin disrupts zebrafish development and reproductive processes at chronic environmentally relevant concentrations. *Environmental Science & Technology*, *57*(2), 1049-1059 https://doi.org/10.1021/acs.est.2c05719

Barros, S., Ribeiro, M., Coimbra, A. M., Pinheiro, M., Morais, H., Alves, N., Montes, R ., Rodil, R., Quintana, J. B., Santos ,M. M., & Neuparth, T. (2022b). Metformin disrupts *Danio rerio* metabolism at environmentally relevant concentrations: A full life-cycle study. *Science of The Total Environment*, *846*, 157361. https://doi.org/10.1016/j.scitotenv.2022.157361

Biran, J., & Levavi-Sivan, B. (2018). *Endocrine Control of Reproduction Fish*. Pp. 362–368. In Skinner, M. K. (Ed.). *Encyclopedia of Reproduction.* Vol. 6, 2nd ed. Academic Press: Elsevier. http://dx.doi.org/10.1016/B978-0-12-809633-8.20579-7

Blanco, A. M. (2020). Hypothalamic-and pituitary-derived growth and reproductive hormones and the control of energy balance in fish. *General and Comparative Endocrinology*, *287*, 113322. https://doi.org/10.1016/j.ygcen.2019.113322

Cabrita, E., Robles, V., & Herráez, P. (Eds.). (2008). Methods in reproductive aquaculture: marine and freshwater species. CRC press.p.p(34-37) https://books.google.iq/books?hl=ar&lr=&id=e5NcUsHvTOwC&oi=fnd&pg=PP1&dq=.+Methods+in+reproductive+aquaculture:+marine+and+freshwater+species&ots=AWQpexKIuK&sig=WMpzFJt5diOfDdTqQN8NWLVJgVk&redir_esc=y#v=onepage&q=.%20Methods%20in%20reproductive%20aquaculture%3A%20marine%20and%20freshwater%20species&f=false

Campbell, J. M., Bellman, S. M., Stephenson, M. D., & Lisy, K. (2017). Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. *Ageing Research Reviews*, *40*, 31-44. https://doi.org/10.1016/j.arr.2017.08.003

Chen, X., Liu, B., & Lin, D. (2022). Sexual Maturation, Reproductive Habits, and Fecundity of Fish. Biology of Fishery Resources, 113-142. https://doi.org/10.1007/978-981-16-6948-4_5

Cheng, F. F., Liu, Y. L., Du, J., & Lin, J. T. (2022). Metformin's Mechanisms in Attenuating Hallmarks of Aging and Age-Related Disease. *Aging and Disease*, *13*(4), 970-986. https://doi.org/10.14336/ad.2021.1213

Choi, T. Y., Choi, T. I., Lee, Y. R., Choe, S. K., & Kim, C. H. (2021). Zebrafish as an animal model for biomedical research. *Experimental & Molecular Medicine*, *53*(3), 310-317. https://doi.org/10.1038/s12276-021-00571-5

Costa, G. C., & Schlupp, I. (2020). Placing the hybrid origin of the asexual Amazon molly (*Poecilia formosa*) based on historical climate data. *Biological Journal of the Linnean Society*, *129*(4), 835-843. https://doi.org/10.1093/biolinnean/blaa010

da Trindade, M. T., Kogawa, A. C., & Salgado, H. R. N. (2018). Metformin: a review of characteristics, properties, analytical methods and impact in the green chemistry. *Critical reviews in analytical chemistry*, *48*(1), 66-72. https://doi.org/10.1080/10408347.2017.1374165

Davies, S. (2022). Metformin use and vitamin B12 deficiency: New MHRA guidance. *Journal of Diabetes Nursing*, *26*(5). 259. https://diabetesonthenet.com/journal-diabetes-nursing/metformin-vitamin-b12-deficiency-mhra/

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Dewi, R. R., Siallagan, W., & Suryanto, D. (2018). The efficacy of sodium chloride application in the control of fish lice (*Argulus* sp.) infection on tilapia (*Oreochromis niloticus*). *Acta Aquatica: Aquatic Sciences Journal*, *5*(1), 4-7. https://doi.org/10.1016/B978-0-12-809633-8.20624-9

Drugs.com (2022), Metformin Side Effects, https://www.drugs.com/sfx/metformin-side-effects.html

Elizalde-Velázquez, G. A., & Gómez-Oliván, L. M. (2020). Occurrence, toxic effects and removal of metformin in the aquatic environments in the world: Recent trends and perspectives. *Science of The Total Environment*, *702*, 134924. https://doi.org/10.1016/j.scitotenv.2019.134924

Faure, M., Bertoldo, M. J., Khoueiry, R., Bongrani, A., Brion, F., Giulivi, C., Dupont, J., & Froment, P. (2018). Metformin in Reproductive Biology. *Frontiers in Endocrinology*, *9*, 393399. https://doi.org/10.3389/fendo.2018.00675

Florida Museum of Natural History, (2023). *Poecilia latipinna*, DISCOVER FISHES, Available https://www.floridamuseum.ufl.edu/discover-fish/species-profiles/poecilia-latipinna/

Fontaine, E. (2018). Metformin-Induced Mitochondrial Complex I Inhibition: Facts, Uncertainties, and Consequences. *Frontiers in Endocrinology*, *9*, 390473. https://doi.org/10.3389/fendo.2018.00753

Foretz, M., Guigas, B., & Viollet, B. (2019). Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nature Reviews Endocrinology*, *15*(10), 569-589. https://doi.org/10.1038/s41574-019-0242-2

Genazzani, A. D., Ricchieri, F., & Lanzoni, C. (2010). Use of Metformin in the Treatment of Polycystic Ovary Syndrome. *Women's Health.* 6(4), 577–593. https://doi.org/10.2217/WHE.10.43

Gershman, J. (2022). What to Know About Taking Vitamin B12 and Metformin, https://www.goodrx.com/metformin/metformin-and-vitamin-b12

Gonzalez, R., Cooper, J., & Head, D. (2005). Physiological responses to hyper-saline waters in sailfin mollies (*Poecilia latipinna*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *142*(4), 397-403. https://doi.org/10.1016/j.cbpa.2005.08.008

Guzmán, J. M., Luckenbach, J. A., Yamamoto, Y., & Swanson, P. (2014). Expression profiles of Fsh-regulated ovarian genes during oogenesis in coho salmon. *PloS one*, *9*(12), e114176. https://doi.org/10.1371/journal.pone.0114176

Hashim, H. A. (2016). Twenty years of ovulation induction with metformin for PCOS; what is the best available evidence?. *Reproductive biomedicine online*, *32*(1), 44-53. https://doi.org/10.1016/j.rbmo.2015.09.015

Hassler, M. R., Redl, E., Hudson, Q. J., Miller, W. J., & Egger, G. (2016). Basic Epigenetic Mechanisms and Phenomena. *Drug Discovery in Cancer Epigenetics*, 3-40. https://doi.org/10.1016/B978-0-12-802208-5.00001-1

Hatef, A., & Unniappan, S. (2019). Metabolic hormones and the regulation of spermatogenesis in fishes. *Theriogenology*, *134*, 121-128. https://doi.org/10.1016/j.theriogenology.2019.05.021

Herzig, S., & Shaw, R. J. (2018). AMPK: Guardian of metabolism and mitochondrial homeostasis. *Nature Reviews Molecular Cell Biology*, *19*(2), 121-135. https://doi.org/10.1038/nrm.2017.95

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Hollander-Cohen, L., Golan, M., & Levavi-Sivan, B. (2021). Differential regulation of gonadotropins as revealed by transcriptomes of distinct LH and FSH cells of fish pituitary. *International Journal of Molecular Sciences*, *22*(12), 6478. https://doi.org/10.3390/ijms22126478

Huhtala, M. S., Tertti, K., & Rönnemaa, T. (2020). Serum lipids and their association with birth weight in metformin and insulin treated patients with gestational diabetes. *diabetes research and clinical practice*, *170*, 108456 https://doi.org/10.1016/j.diabres.2020.108456

Jiang, S., Tang, T., Sheng, Y., Li, R., & Xu, H. (2022). The Effects of Letrozole and Metformin Combined with Targeted Nursing Care on Ovarian Function, LH, and FSH in Infertile Patients with Polycystic Ovary Syndrome. *Journal of Healthcare Engineering*, *2022*. https://doi.org/10.1155/2022/3712166

Kumar, P., Behera, P., Christina, L., & Kailasam, M. (2021). Sex Hormones and Their Role in Gonad Development and Reproductive Cycle of Fishes. *Recent updates in molecular Endocrinology and Reproductive Physiology of Fish: An Imperative step in Aquaculture*, 1-22. https://doi.org/10.1007/978-981-15-8369-8_1

Lacouture, A., Lafront, C., Peillex, C., Pelletier, M., & Audet-Walsh, É. (2022). Impacts of endocrine-disrupting chemicals on prostate function and cancer. *Environmental Research*, *204*, 112085. https://doi.org/10.1016/j.envres.2021.112085

Lamming, D. W., Ye, L., Sabatini, D. M., & Baur, J. A. (2013). Rapalogs and mTOR inhibitors as anti-aging therapeutics. *The Journal of clinical investigation*, *123*(3), 980-989. https://doi.org/10.1172/jci64099

Lashen, H. (2010). Role of metformin in the management of polycystic ovary syndrome. Therapeutic advances in endocrinology and metabolism, 1(3), 117-128. https://doi.org/10.1177/2042018810380215

Lauretta, R., Sansone, A., Sansone, M., Romanelli, F., & Appetecchia, M. (2019). Endocrine disrupting chemicals: effects on endocrine glands. *Frontiers in endocrinology*, *10*, 178. | https://doi.org/10.3389/fendo.2019.00178

Lawler, M., (2023). The Possible Benefits of Metformin for Type 2 Diabetes and Other Health Conditions, https://www.everydayhealth.com/type-2-diabetes/metformin-health-benefits-why-they-likely-go-beyond-type-2-diabetes

Lee, J. W., Shin, Y. J., Kim, H., Kim, H., Kim, J., Min, S. A., ... & Park, K. (2019). Metformin-induced endocrine disruption and oxidative stress of Oryzias latipes on two-generational condition. *Journal of hazardous materials*, *367*, 171-181. https://doi.org/10.1016/j.jhazmat.2018.12.084

Lin, W., Yan, Y., Ping, S., Li, P., Li, D., Hu, J., Liu, W., Wen, X., & Ren, Y. (2020). Metformin-induced epigenetic toxicity in zebrafish: experimental and molecular dynamics simulation studies. *Environmental science & technology*, *55*(3), 1672-1681. https://doi.org/10.1021/acs.est.0c06052

Liu, Q., Tong, D., Liu, G., Gao, J., Wang, L. A., Xu, J., Yang, X., Xie, Q., Huang, Y., Pang, J., Wang, L., He, Yong., Zhang, D., Ma, Q., Lan, W., & Jiang, J. (2018). Metformin Inhibits Prostate Cancer Progression by Targeting Tumor-Associated Inflammatory Infiltration Metformin Inhibits Inflammatory Infiltration in Prostate Cancer. *Clinical Cancer Research*, *24*(22), 5622-5634. https://doi.org/10.1158/1078-0432.CCR-18-0420

Lubzens, E., Young, G., Bobe, J., & Cerdà, J. (2010). Oogenesis in teleosts: how fish eggs are formed. *General and comparative endocrinology*, *165*(3), 367-389. https://doi.org/10.1016/j.ygcen.2009.05.022

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Ma, T., Tian, X., Zhang, B., Li, M., Wang, Y., Yang, C., , Wu, J., Wei, X., Qu, Q., Yu, Y., Long, S., Feng, J. W., Li, C., Zhang, C., Xie, C., Wu, Y. Xu, Z., Chen, J., He, Y., Yu, Y., Huang, X., Yao, L., Zhang, L., Zhu, M., & Lin, S. C. (2022). Low-dose metformin targets the lysosomal AMPK pathway through PEN2. *Nature*, *603*(7899), 159-165. https://doi.org/10.1038/s41586-022-04431-8

MacLaren, R. D., Wisniewski, K., & MacLaren, C. (2018). Environmental concentrations of metformin exposure affect aggressive behavior in the Siamese fighting fish, *Betta splendens*. *PLoS One*, *13*(5), e019725 https://doi.org/10.1371/journal.pone.0197259

Madiraju, A. K., Erion, D. M., Rahimi, Y., Zhang, X. M., Braddock, D. T., Albright, R. A., Prigaro,B.J., . Wood, J. L, Bhanot, S. , . MacDonald, M. J., Jurczak, Mi. J., Camporez, J.P., Lee, H.Yo., Cline, G. W., Samuel, V. T., Kibbey, R.G., Shulman, G. I., Madiraju, A. K., Erion, D. M., Rahimi, Y., Zhang, X.M., . Albright, R. A.Braddock, D. T., Prigaro, B. J., Wood, J. L., Bhanot, S., MacDonald, M. J., Jurczak, M. J., Camporez, J.P., Lee, H. Y., . Cline, G. W., Samuel, V. T., Kibbey, R. G.& Shulman, G. I. (2014). Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature*, *510*(7506), 542-546, https://doi.org/10.1038/nature13270

Mitrašinović-Brulić, M., Buljan, M., & Suljević, D. (2021). Association of LH/FSH ratio with menstrual cycle regularity and clinical features of patients with polycystic ovary syndrome. *Middle East Fertility Society Journal*, *26*(1), 40. https://doi.org/10.1186/s43043-021-00085-0

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology*, *38*(1), 23-38. https://doi.org/10.1038/npp.2012.112

Muchlisin, Z. A. (2014). A general overview on some aspects of fish reproduction. *Aceh International Journal of Science and Technology*, *3*(1), 43-52.. https://doi.org/10.13170/aijst.3.1.1355

Mudumbi, J. B. N., Ntwampe, S. K. O., Mekuto, L., Matsha, T., & Itoba-Tombo, E. F. (2018). The role of pollutants in type 2 diabetes mellitus (T2DM) and their prospective impact on phytomedicinal treatment strategies. *Environmental monitoring and assessment*, *190*, 1-23. https://doi.org/10.1007/s10661-018-6634-2

Murugan, A. K. (2019). mTOR: Role in cancer, metastasis and drug resistance. In *Seminars in cancer biology* Vol. 59, pp. 92-111). Academic Press. https://doi.org/10.1016/j.semcancer.2019.07.003

Niemuth, N. J., & Klaper, R. D. (2015). Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. *Chemosphere*, *135*, 38-45. https://doi.org/10.1016/j.chemosphere.2015.03.060

Niemuth, N. J., Jordan, R., Crago, J., Blanksma, C., Johnson, R., & Klaper, R. D. (2015). Metformin exposure at environmentally relevant concentrations causes potential endocrine disruption in adult male fish. *Environmental toxicology and chemistry*, *34*(2), 291-296. https://doi.org/10.1002/etc.2793

Phillips, J., Akemann, C., Shields, J. N., Wu, C. C., Meyer, D. N., Baker, B. B., Pitts, D., & Baker, T. R. (2021). Developmental phenotypic and transcriptomic effects of exposure to nanomolar levels of metformin in zebrafish. *Environmental toxicology and pharmacology*, *87*, 103716. https://doi.org/10.1016/j.etap.2021.103716

Planas, D., Pagliuzza, A., Ponte, R., Fert, A., Marchand, L. R., Massanella, M., Gosselin, A., Mehraj,V., Dupuy, F. P., Isnard, S., Goulet, J., Lesage, S., Cohen, E. E. A., Ghali, M. P., Angel, J. B., Chomont, N., Routy, J. P., & Ancuta, P.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

(2021). LILAC pilot study: effects of metformin on mTOR activation and HIV reservoir persistence during antiretroviral therapy. *EBioMedicine*, *65*, 103270. https://doi.org/10.1016/j.ebiom.2021.103270

Ramos-Júdez, S., Giménez, I., Gumbau-Pous, J., Arnold-Cruañes, L. S., Estévez, A., & Duncan, N. (2022). Recombinant Fish and Lh therapy for spawning induction of previtellogenic and early spermatogenic arrested teleost, the flathead grey mullet (*Mugil cephalus*). *Scientific Reports*, *12*(1), 6563. https://doi.org/10.1038/s41598-022-10371-0

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*(9), 1577-1585. https://doi.org/10.1007/s00125-017-4342-z

Rizzo, E., & Bazzoli, N. (2020). Reproduction and embryogenesis. In *Biology and physiology of freshwater neotropical fish* (pp. 287-313). Academic Press. https://doi.org/10.1016/B978-0-12-815872-2.00013-0

Saadia, Z. (2020). Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS)-obese vs. Non-obese women. *Medical Archives*, *74*(4), 289. https://doi.org/10.5455/medarh.2020.74.289-293

Schaeck, M., Van den Broeck, W., Hermans, K., & Decostere, A. (2013). Fish as research tools: alternatives to *in vivo* experiments. *Alternatives to Laboratory Animals*, *41*(3), 219-229. https://doi.org/10.1177/026119291304100305

Schaffer, R. (2017). *Beyond diabetes, metformin may prove to be a 'wonder drug, endocrinology*. https://www.healio.com/news/ /20170207/beyond-diabetes-metformin-may-prove-to-be-a-wonder-drug,

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature protocols*, *3*(6), 1101-1108. https://doi.org/10.1038/nprot.2008.73

Shakoori, A. R. (2017). Fluorescence in situ hybridization (FISH) and its applications. *Chromosome Structure and Aberrations*, 343-367. https://doi.org/10.1007/978-81-322-3673-3_16

Shmerling, R. H., (2021). Is metformin a wonder drug? - Harvard Health. https://www.health.harvard.edu/blog/is-metformin-a-wonder-drug-202109222605

Shpakov, A. O. (2021). Improvement Effect of Metformin on Female and Male Reproduction in Endocrine Pathologies and Its Mechanisms. *Pharmaceuticals*, *14*(1), 42. https://doi.org/10.3390/ph14010042

Tseng, C. H. (2022). The effect of metformin on male reproductive function and prostate: An updated review. *The World Journal of Men's Health*, *40*(1), 11. https://doi.org/10.5534/wjmh.210001

Tso, L. O., Costello, M. F., Albuquerque, L. E. T., Andriolo, R. B., & Macedo, C. R. (2020). Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. *Cochrane Database of Systematic Reviews*, (12). https://doi.org/10.1002/14651858.CD006105.pub4

Vial, G., Detaille, D., & Guigas, B. (2019). Role of mitochondria in the mechanism (s) of action of metformin. *Frontiers in endocrinology*, *10*, 294. https://doi.org/10.3389/fendo.2019.00294

Volkoff, H., & London, S. (2018). *Nutrition and reproduction in fish*. Pp, 743-748. In *Encyclopedia of reproduction*. Volume 6, 2nd ed. https://doi.org/10.1016/B978-0-12-809633-8.20624-9

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 272-290

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/hfpbwr68*

Wang, Y., An, H., Liu, T., Qin, C., Sesaki, H., Guo, S., Radovick, S., Hussain, M., Maheshwari, A., Wondisford, F.E., Rourke, B. O., & He, L. (2019). Metformin improves mitochondrial respiratory activity through activation of AMPK. *Cell reports*, *29*(6), 1511-1523. https://doi.org/10.1016/j.celrep.2019.09.070

Bridges, H. R., Jones, A. J., Pollak, M. N., & Hirst, J. (2014). Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *Biochemical Journal*, *462*(3), 475-487.

Weichhart, T. (2018). mTOR as regulator of lifespan, aging, and cellular senescence: a mini-review. *Gerontology*, *64*(2), 127-134. https://doi.org/10.1159/000484629

Wu, H., Huang, D., Zhou, H., Sima, X., Wu, Z., Sun, Y., Wu, Z., Wu, Z., Sun, Y., Wang, L., Ruan, Y., Wu, Q., Wu, F., She, T., Chu, Y., Huang, Q., Ning, Z., & Zhang, H. (2022). Metformin: A promising drug for human cancers. *Oncology Letters*, *24*(1), 1-9 https://doi.org/10.3892/ol.2022.13325

Yang, W. K., Hseu, J. R., Tang, C. H., Chung, M. J., Wu, S. M., & Lee, T. H. (2009). Na+/K+-ATPase expression in gills of the euryhaline sailfin molly, *Poecilia latipinna*, is altered in response to salinity challenge. *Journal of Experimental Marine Biology and Ecology*, *375*(1-2), 41-50. https://doi.org/10.1016/j.jembe.2009.05.004

Ye, J., Luo, D., Xu, X., Sun, M., Su, X., Tian, Z., Zhang, M., & Guan, Q. (2019). Metformin improves fertility in obese males by alleviating oxidative stress-induced blood-testis barrier damage. *Oxidative medicine and cellular longevity*, *2019*. Article ID 9151067. https://doi.org/10.1155/2019/9151067

Yerevanian, A., & Soukas, A. A. (2019). Metformin: mechanisms in human obesity and weight loss. *Current obesity reports*, *8*, 156-164. https://doi.org/10.1007/s13679-019-00335-3

Yilmaz, B., Terekeci, H., Sandal, S., & Kelestimur, F. (2020). Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Reviews in endocrine and metabolic disorders*, *21*, 127-147. https://doi.org/10.1007/s11154-019-09521-z

Zhang, Z., Zhu, B., & Ge, W. (2015). Genetic analysis of zebrafish gonadotropin (FSH and LH) functions by TALEN-mediated gene disruption. *Molecular Endocrinology*, *29*(1), 76-98. https://doi.org/10.1210/me.2014-1256

Zohar, Y. (2021). Fish reproductive biology–Reflecting on five decades of fundamental and translational research. *General and Comparative Endocrinology*, *300*, 113544. https://doi.org/10.1016/j.ygcen.2020.113544