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Impact of the Anti-Tuberculosis Drug Rifampicin on the Feeding, Growth and Embryonic Developmental Profile of the Mosquitofish *Gambusia affinis*

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Abstract

Although the accumulation of pharmaceutical drugs in aquatic bodies has increased rapidly in recent years, the effect of rifampicin (RIF), a first-line anti-tuberculosis drug, on fish feeding, growth, and embryonic development is unknown. This investigation aimed to determine the impact of RIF on growth and embryonic developmental profile in the mosquitofish *Gambusia affinis*. Experimental groups included controls, which were kept in normal water for 21 days, whereas those in the second, third, and fourth groups were exposed to 50, 200, and 500 mg RIF/L water, respectively. The food intake rate and Specific Growth Rate (SGR) showed a concentration-dependent significant decrease in RIF-treated fish compared with controls, and a strong positive correlation was found between food consumption and SGR. A significant decrease in the number of embryos at an early stage of development and the total number of embryos in RIF-treated fish was associated with several congenital anomalies such as lack of vitellogenin accumulation, yolk sac regression, decreased pigmentation, aggregations of blood vessels, and curvature of the spinal cord compared with controls. Together, these results reveal for the first time that RIF treatment not only impacts feeding and growth, but also exerts potential teratogenic effect on embryonic developmental stages in the mosquitofish *G. affinis*.

Keywords: Anomalies, Embryos, Growth, Mosquitofish, Rifampicin, Viviparity

1. Introduction

Environmental pollution is becoming a serious threat to the lives of organisms throughout the world. Environmental pollutants comprise a wide range of chemicals, such as synthetic organic chemicals, gases from combustion, pesticides, heavy metals, and active pharmaceutical ingredients[1,2]. Among the pollutants, pharmaceutical components in the environment have become a matter of widespread concern because of their continuous and potentially subtle toxicity in different ways[3,4]. These chemicals enter the soil, surface water, and groundwater levels as unmetabolized or active metabolites from humans or animals excretory products[5]. Besides, improper disposal of unused medicines is also one of the key factors contributing to the occurrence of pharmaceuticals in the environment[6]. Active pharmaceutical pollutants are often designed to cross biological membranes, and therefore the rate of uptake and internal concentrations are critically important as they can even interfere with the embryonic development process[7,8]. Rifampicin (RIF) is a bactericidal antibiotic drug of the rifamycin group[9], derived from *Amycolatopsis rifamycinica*[10]. It is used to treat several types of bacterial infections, including tuberculosis (TB), leprosy[11], *Mycobacterium avium* complex[12], and severe community-acquired *Legionella pneumophila* pneumonia[13]. The RIF is the most effective anti-TB drug used to treat TB patients in clinics[14]. A high dose of RIF has common side effects, including nausea, vomiting, diarrhea, loss of appetite, and liver problems or allergic reactions, whereas continuous use of this drug leads to further adverse effects, including abdominal pain, rashes, esophagitis, and other gastrointestinal problems[15,16]. Frequent use of RIF may contribute to an increase in its concentration in water supplies. Using the cloud point extraction method, linear RIF concentrations ranging from 4.12 to 81.41 mg/L have been detected in waste water bodies recently[17].

Our knowledge of the effect of RIF on reproduction and embryonic development is confined to only mammals, and these effects are equivocal in both male and female rats. The combined treatment of RIF with other anti-TB drugs such as ethambutol, isoniazid, and pyrazinamide in male rats causes an increase in oxidative damage with consequent suppression of the fertility index and lowers serum levels of Follicle

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Stimulating Hormone (FSH), Luteinizing Hormone (LH), and testosterone associated with lower sperm counts[18,19]. Similarly, RIF treatment reduces the quality of sperm chromatin structure and motility[20], but increases testosterone levels in male rats by stimulating the production of 17-hydroxylase in the testes[21]. On the other hand, while treatment of RIF in combination with TB drugs affects the weight or number of surviving foetuses concomitant with pre- and post-implantation embryo losses[18,19,22], similar treatment causes an elevation in serum levels of estradiol, progesterone, and prolactin in rats[19]. Likewise, although the relative weights of the uterus and spleen are not significantly affected, the relative weight of the ovary is significantly decreased following oral treatment with RIF in rats[23]. In addition, treatment with RIF causes teratogenic effects and congenital anomalies in rats[24,25], humans[26,27], and birds[28].

Reproduction in fish, like other vertebrates, is predominantly regulated by the Hypothalamic-Pituitary-Gonadal (HPG) axis and a variety of environmental and social cues. Several forms of pharmaceutical drugs can disrupt the normal mechanisms of HPG axis function, leading to the failure of the reproductive success of fish. A few teleosts exhibit viviparity, which is characterized by the ability to give birth to live offspring. The ovaries of viviparous teleosts are associated with both egg production and gestation[29]. *Gambusia affinis* is a viviparous fish belonging to the family Poeciliidae that exhibits intraovarian gestation. It shows high fecundity and short gestation periods, and female *G. affinis* can store sperm for extended periods of time[30]. Upon successful completion of embryonic development, this fish directly gives birth to fries. Although studies in mammals have indicated both stimulatory and inhibitory effects of RIF alone or in combination with other anti-TB drugs on reproductive hormones and embryonic development, the influence of RIF on fish feeding, growth, and embryonic development is unknown. Therefore, the aim of this investigation is to determine the effect of different concentrations of RIF on feeding, growth, and embryonic development in *G. affinis*.

2. Materials and Methods

2.1 Experimental Animals

Adult *G. affinis* was taken from ponds on the campus of Karnataka University, Dharwad. The fishes were transported to the laboratory and acclimated for one month under natural photoperiod conditions. Each aquarium (dimensions: 60×60×16 cm; L×B×H; volume: 2L) included a sex ratio of ten females and four males. The fishes were given commercial pellets twice a day (Taiyo Pet Feed, Chennai, India). Aerators and aquatic plants were provided for the aquariums.

2.2 Experimental Procedure

Pilot studies showed that females with a regressed abdomen lack embryos, whereas females with a bulging abdomen have embryos. Only females with regressed abdomens from the stock were chosen for experimentation. Female fish weighing between 0.20 and 0.30 g were separated into four groups, and their Initial Body Weights (IBWs) were recorded. The fish in the first group (controls: n = 20) were maintained in normal water, whereas those in the second, third, and fourth groups (n = 20 in each group) were exposed to water consisting of 50, 200, and 500 mg RIF (mol. formula, $C_{43}H_{58}N_4O_{12}$; CAS No: 13292-46-1; Salvavidas Pharmaceutical Pvt. Ltd., Gujarat, India)/L in separate aquaria for 21 days. Based on the results of the pilot trials, the concentrations were chosen so that all of the fish survived through the end of the experiment. Each experimental group comprised one replicate (n = 20 per group; n = 10 per replicate per aquarium). Water was replaced daily, and food was provided *ad libitum*.

The fish were kept under natural environmental conditions (photoperiod, 12.32 ± 0.08; water temperature, 22.25 ± 0.05; pH, 7.1 ± 0.05; dissolved oxygen, 6.05 ± 0.44). pH was measured with a professional digital pH meter, while water temperature was measured with a digital thermometer. Dissolved oxygen levels were estimated using Winkler's method. The dry weight of uneaten food was recorded and used to calculate the amount of food consumed by fish in each aquarium. On day 22, the fish were anaesthetized with 2-phenoxyethanol, their Final Body Weights (FBWs) were recorded, and they were euthanized. A small incision was made on the abdomen, and the embryos were taken from the body of the fish and placed in 0.9% saline medium. Specific Growth Rate (SGR) was calculated using the formula: 100 x (FBW – IBW) / number of rearing days.

2.3 Identification and Scoring of Embryos

Embryos in various stages of development were determined using criteria previously available for this fish[31]. Embryonic development in *G. affinis* comprises the blastodisc stage, the shield stage, the optic cup stage, the early eyed stage, the mid eyed stage, the late eyed stage, the very late eyed stage, and the mature embryo, which culminates in the birth of juveniles. On the day of the autopsy, no early-stage embryos were observed, but the subsequent developmental phases are depicted in Figure 3A-H. Embryos of various developmental stages were assessed according to their morphological traits. During the

autopsy, embryos were also examined for abnormalities. The photomicrographs of the embryos were captured using a stereozoom microscope (Olympus, Japan).

2.4 Statistical Analysis

A one-way Analysis of Variance (ANOVA) followed by a *post-hoc* Student-Newman-Keuls test was used to assess the results. The significant difference between IBW and FBW was tested using the student *t*-test. Pearson's correlation method was used to determine the relationship between food consumption and body weight, or SGR, while Pearson's $\chi 2$ - test of homogeneity was applied to determine the variations in embryonic developmental stages across experimental groups. The statistical significance level was set at $p < 0.05$ for all comparisons. All statistical analysis was conducted with Sigma Stat 3.5 (Systat Software Inc., USA).

3. Results

A significant increase ($p = 0.014$) was noticed in the FBW compared with the IBW in the controls; however, no such statistically significant difference ($p > 0.05$) was observed in the 50, 200, or 500 mg RIF-treated fish (Figure 1A). The FBW did not differ significantly ($p = 0.352$) among the different experimental groups (Figure 1B). The food consumption rate and SGR showed a concentration-dependent significant decrease ($p < 0.001$) in 50, 200, or 500 mg RIF-treated fish compared with controls (Figure 1C and D). Analysis of Pearson's correlation revealed no significant interaction between food consumption and body weight ($R = 0.17$; $p = 0.294$), but there was a strong positive correlation between food consumption and SGR ($R = 0.827$; $p < 0.00001$) (Figure 2A and B).

Embryos belonging to different developmental stages, namely, early eye, mid eye, late eye, very late eye, mature embryo, and juvenile stages, were present in controls (Figure 3A–H). Except for very late eye stage embryos and juveniles, the number of embryos in other stages of development, namely, early eye, mid eye, late eye, and mature stages, was significantly decreased ($p < 0.01$) in fish treated with RIF compared to controls (Table 1). Although the numbers of embryos in the early eye stage were higher in fish treated with 50 mg RIF, there was a decrease or absence of embryos in other stages (Table 1). The embryos in the early eye, mid-eye, and late eye stages were completely absent in fish treated with 200 or 500 mg RIF in contrast to their presence in controls (Table 1). Overall, the total numbers of embryos were significantly lower ($p < 0.001$)

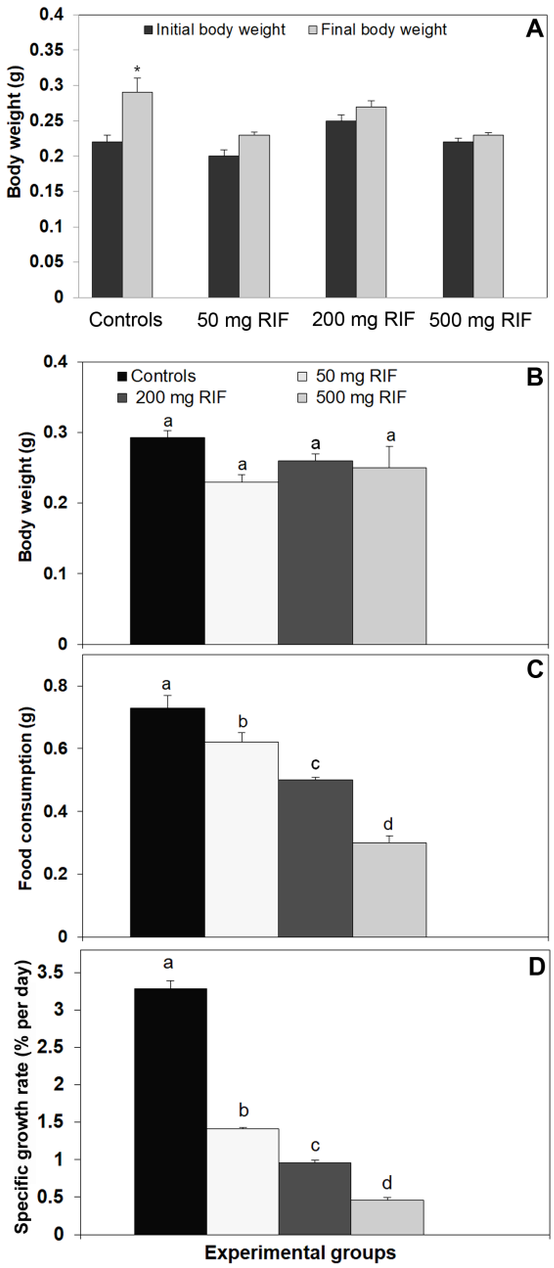

Figure 1. Effect of different concentrations of rifampicin (RIF) treatment on body weight **(A and B)**, food consumption **(C)**, and Specific Growth Rate (SGR) **(D)** in *G. affinis*, (■) – controls, (□) – 50 mg RIF, (■) – 200 mg RIF, (□) – 500 mg RIF-treated. All values are means ± SE. One-way ANOVA followed by a *post-hoc* Student-Newman-Keuls test: groups with the same superscripts are not significantly different, whereas groups with different superscripts significantly ($p < 0.05$) differ from others. The significant difference ($p < 0.05$) between Initial Body Weight (IBW) (■) and Final Body Weight (FBW) (□) is tested using Student *t*-test (A).

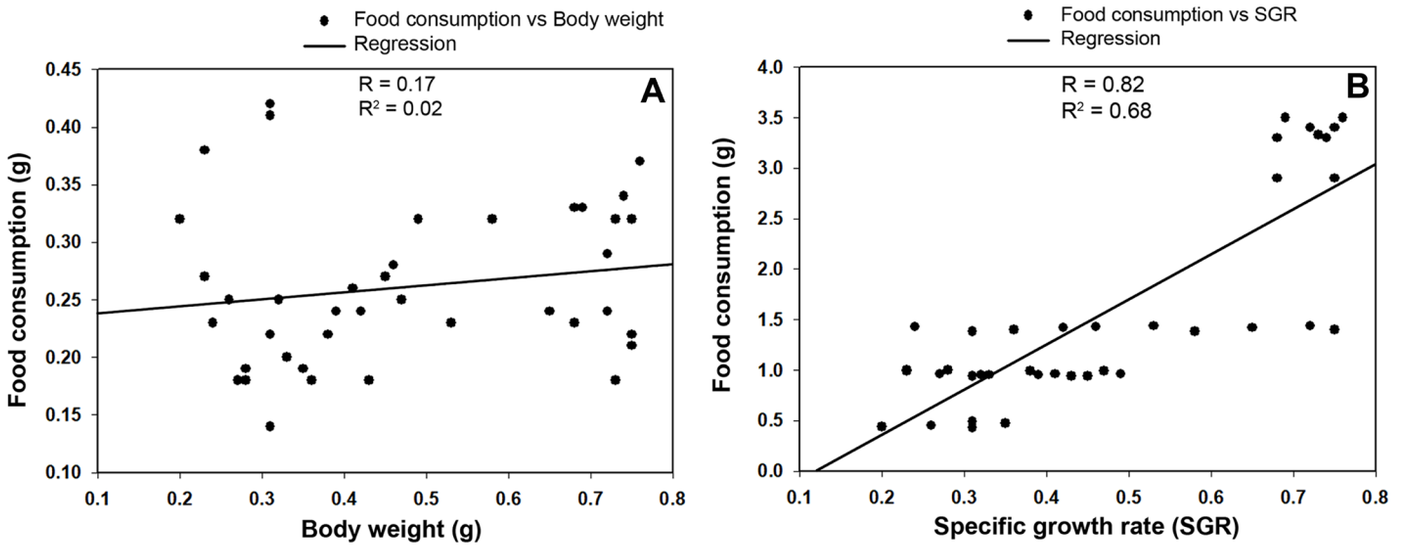

Figure 2. Effect of different concentrations of Rifampicin (RIF) on food consumption vs. body weight **(A)** and food consumption vs. Specific Growth Rate (SGR) **(B)** in *G. affinis*. Pearson's correlation method is used for the analysis of correlation ($p < 0.05$). No significant interaction (R = 0.17; $p = 0.294$) was observed between food consumption and body weight, whereas a strong correlation (R = 0.827; $p < 0.00001$) was found between food consumption and SGR.

Table 1. Effect of Rifampicin on embryonic developmental stages in *G. affinis*

Experimental groups (n=10)	Number of embryos in different developmental stages						
	Early eye	Mid eye	Late eye	Very late eye	Mature	Juvenile	Total
Controls	1	7	14	4	18	10	54
RIF (50mg/L)	6	0	9	0	7	7	29
RIF (200mg/L)	0	0	0	1	4	8	13
RIF (500mg/L)	0	0	0	2	1	7	10
Chi-square test (df = 3)	14.14	15.75	25.17	5	22	0.75	45.9
***p* value**	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p > 0.05$ NS	$p < 0.001$	$p > 0.05$ NS	$p < 0.001$

Note: RIF: Rifampicin; NS: Not Significant

in fish treated with all concentrations of RIF compared to controls (Table 1).

In addition, several abnormalities were also observed in RIF-treated fish. While the deformed yolk sac was commonly observed in the early eye, late eye, and mature embryo stages, the yolk sac was covered with pigmented granules in the juvenile in the 50 mg RIF-treated group (Figure 4A-D) compared to those of controls (Figure 3A-H). In fish treated with 200mg RIF, increased aggregations of blood vessels and incomplete formation of retinal pigments were noticed in the very late embryo stage (Figure 4E and F) compared to controls (Figure 3F). Similar blood vessel aggregation in the yolk sac persisted in mature embryos as well (Figure 4G), whereas juveniles treated with 200 mg RIF showed bending of the vertebral column along with pigmented granules in the yolk sac (Figure 4H) as opposed to controls (Figure 3H). In very late eye stage embryos, aberrant development and diminished pigmented granules were observed (Figure 4I), whereas improper fin formation and bending of the vertebral column at different angles were observed in mature embryos of fish subjected to 500 mg RIF (Figure 4J-L). Similar bending of the vertebral column and protrusion of the eyes were also detected in the juvenile stage in the 500 mg RIF-treated group (Figure 4M and N) compared to their normal development in control fish (Figure 3H).

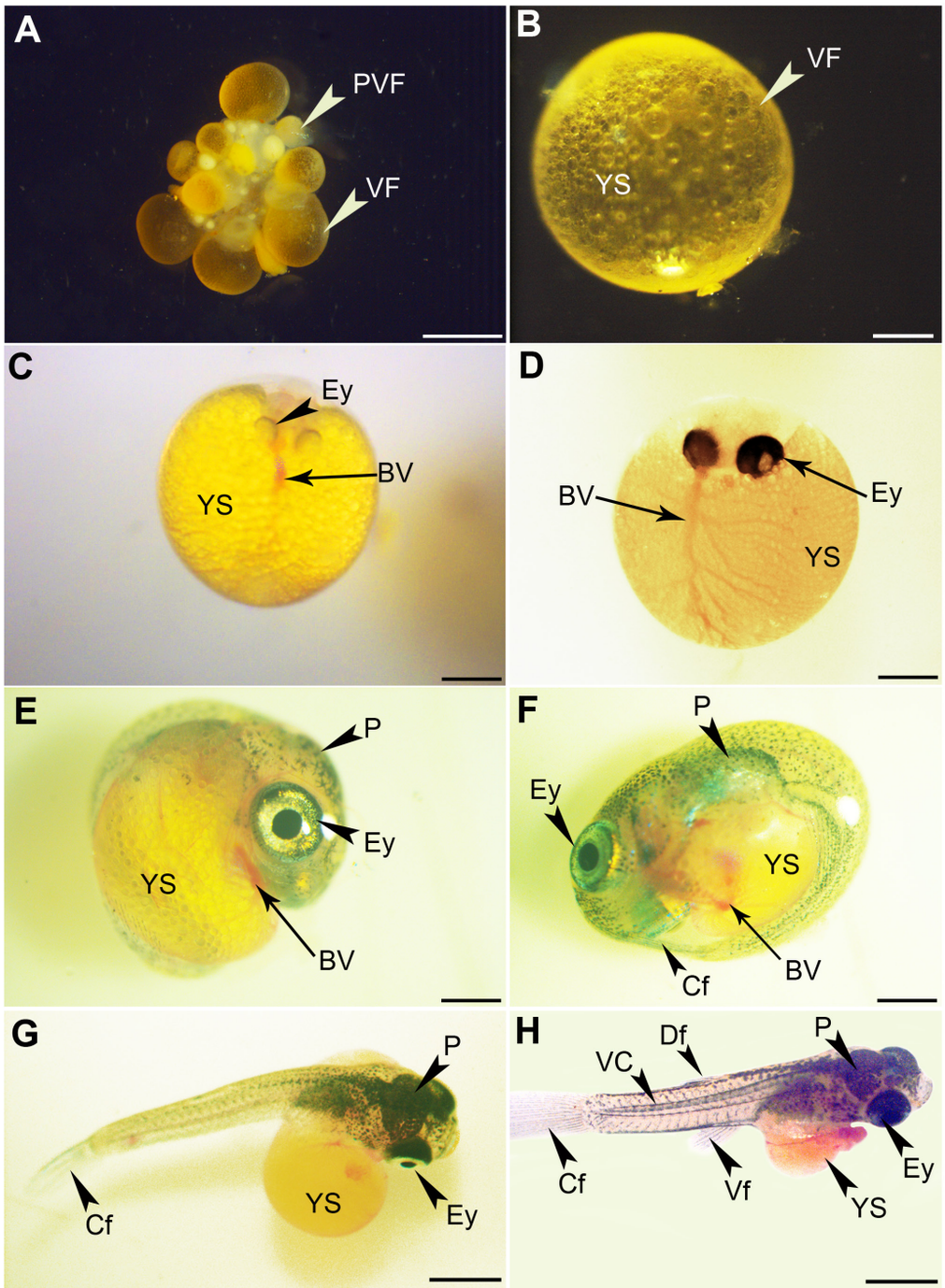

Figure 3. **(A–H)** Photomicrographs showing the ovary and embryos in different stages of development in *G. affinis*. The ovary of controls (A and B) consists of previtellogenic (PVF) and vitellogenic (VF) follicles, and embryos in the early eye **(C)**, mid eye **(D)**, late eye **(E)**, very late eye **(F)**, mature **(G)**, and juvenile **(H)** stages are seen. BV, blood vessel; Cf, caudal fin; Df, dorsal fin; Ey, eye; P, pigmented granules; Vf, ventral fin; VC, vertebral column; YS, yolk sac. Scale bar: A, G, and H, 1 mm; others, 2 mm.

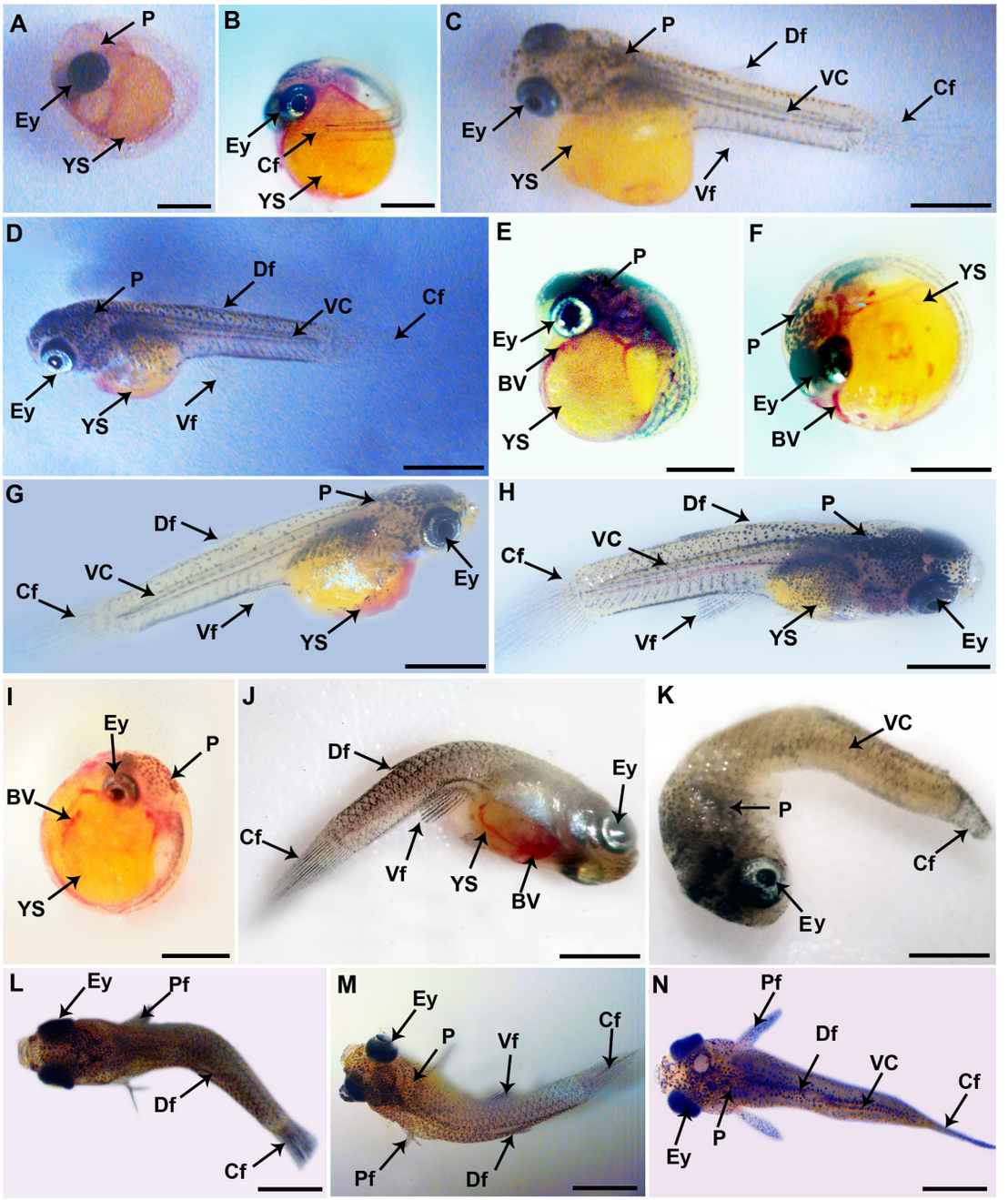

Figure 4. **(A–N)** Photomicrographs showing the effect of 50 mg (A–D), 200 mg (E–H), and 500 mg (I–N) of rifampicin (RIF) on embryonic development in *G. affinis*. Note the degenerating yolk sac (YS) in the early eye **(A)**, late eye **(B)**, and mature embryo **(C)** stages; the yolk sac covered with pigmented granules in the juvenile **(D)** of 50 mg RIF-treated embryos. Increased aggregations of blood vessels **(E)** and incomplete formation of retinal pigments **(F)** in very late embryo stage and aggregation of blood vessels in the yolk sac (YS) in mature embryo **(G)**, and bending of the vertebral column (VC) and yolk sac with pigmented granules (P) in the juvenile **(H)**, are observed in the 200mg RIF-treated group. Abnormal development and decreased pigmented granules (P) in very late eye stage embryo **(I)**, and aggregation of blood vessels (BV) in the yolk sac (YS) or degenerated yolk sac concomitant with abnormal fin formation and bending of the vertebral column (VC) in different angles in mature embryo stage **(J, K, and L)**, bending of the vertebral column (VC), and protrusion of the eyes in the juvenile stage **(M and N)** are observed in the 500 mg RIF-treated group. Cf, caudal fin; Df, dorsal fin; Ey, eye; Vf, ventral fin; Pf, pectoral fin; scale bar, A-N, 1 mm.

4. Discussion

This study reveals the influence of RIF on feeding, growth, and embryonic development in a viviparous fish species. While the increase in fish body weight over time is indicative of their growth[32], many aquaculturists employ SGR (% / day) to report fish growth[33]. In the present study, there was no statistically significant difference in FBW among the experimental groups, although the mean body weight gain was significantly greater in the control group. There was no significant difference between IBW and FBW in the 50, 200, and 500 mg RIF-treated fish groups, but the SGR was significantly lower in RIF-treated fish and decreased concentration-dependently in the 50, 200, and 500 mg RIF-treated fish groups compared to the control group. Therefore, the results of the present study suggest a reduction in growth in the mosquitofish due to RIF treatment. The decrement in growth might also be due to a lack of food availability, as the prolonged fasting decreased the body weights of the immature female Coho salmon *Oncorhynchus kisutch*[34], the juvenile European sea bass *Dicentrarchus labrax*[35], the common dentex *Dentex dentex*[36] and the Nile tilapia *Oreochromis niloticus*[37]. In the present study, although no correlation was observed between food consumption and body weight, a significant positive correlation was noticed between food consumption and SGR. These data imply that the RIF-induced suppression of feeding rate may have a deleterious effect on the growth of mosquitofish.

It is known that ovarian estrogen induces vitellogenin secretion in the liver[38], which is then transferred via blood circulation and integrated into oocytes to form yolk material in fish[39]. The viviparous fish *G. affinis* undergoes intra-ovarian gestation and is presumed to be mostly lecithotrophic, wherein the embryos would depend mainly on the yolk rather than maternal provisioning after fertilization[40]. Therefore, a significant reduction in the overall number of embryos or the absence of some embryos entirely at an early stage of development observed in the current study may be due to impairment in vitellogenin secretion following treatment with RIF in *G. affinis*. This inhibition can be accomplished by the depletion of energy due to RIF-induced suppression of food intake or the direct effect of RIF on vitellogenesis. Fasting is known to modify stored food, especially lipids, which are employed as an endogenous energy source for vital survival activities[41,42]. While the allocation of energy to reproduction depends on the total amount of available energy, an imbalance in stored food such as fat might negatively impact the deposition of vitellogenin in the eggs[43]. In the present study, a significant decrease in food consumption rate, as demonstrated by a concentration-dependent significant decrease in the feeding rate of RIF-treated fish, suggests a decrease in energetic allocation, leading to the depletion of embryos. Alternatively, vitellogenesis is also influenced by growth regulators such as insulin-like growth factor-1 (IGF-1) and growth hormone (GH)[44,45]. While GH acts as the primary regulator of IGF-1, a growth signal essential for the development of embryonic tissues, GH is also implicated in vitellogenesis in some fish species, such as the gilthead sea bream, *Sparus aurata*[46] and the goldfish *Carassius auratus*[47]. Indeed, fasting decreased the levels of circulating IGF-1 and growth hormone GH in the farmed gilthead sea bream[48].

Environmental contaminants can induce anomalies in reproductive structures in fish[49]. Many researchers have observed the adverse effects of anti-TB medications on reproductive parameters and their potential teratogenicity. In the majority of studies, the effect of RIF combined with other anti-TB medications on the reproductive system has been investigated. The co-administration of RIF and other anti-TB drugs such as ethambutol, isoniazid, and pyrazinamide to male rats decreased the levels of sperm count, serum testosterone, LH, and FSH[19], or increased DNA fragmentation, and decreased the fertilizing capacity associated with pre-and post-implantation embryo lethality in females mated with anti-TB drug-treated males[18]. Similar treatment with RIF and isoniazid, either alone or in combination, decreased the foetal weight of female rats[22] and negatively impacted the structure of sperm chromatin and sperm motility in male rats[19]. Furthermore, the treatment of RIF during early pregnancy contributed to the teratogenic effects and congenital anomalies in humans[26,27]. Treatment of RIF in humans during the gestation period resulted in congenital abnormalities, including anencephaly, hydrocephalus, and skeletal abnormalities, such as neural tube defects and limb reduction[25,50–52]. In mammals, although RIF treatment (200 mg/kg) in rabbits did not cause congenital abnormalities, treatment of rats with RIF (150 mg/kg) resulted in spina bifida, anencephaly, and a cleft palate[24,25]. In addition, injections of 2 mg/ml, 4 mg/ml, and 8 mg/ml RIF into the fertilized white leghorn eggs after 21 hrs of incubation followed by three days of incubation resulted in several anomalies, including a constricted heart with U-shaped loop, retardation of the brain vesicle, non-parallel neutral tube lines, and an opened posterior neuropore[28]. In the present investigation, increased aggregations of blood vessels and incomplete formation of retinal pigments in very late embryonic stages, bending of the vertebral column concomitant with pigmented granules in the yolk sac of juveniles, degenerative yolk sacs, or accumulation of the yolk sac with blood vessels following treatment with different concentrations of RIF indicate the teratogenic potential of RIF on embryonic developmental stages in the viviparous fish. However, the mechanism by which RIF induces the teratogenic effect remains unclear in fish. Further studies are required to elucidate this aspect.

5. Conclusions

This study indicates for the first time in viviparous fish that exposure to RIF significantly affects embryonic developmental stages and induces congenital defects comparable to those found in higher vertebrates such as mammals and humans. In addition, this study also reveals that RIF treatment may have a concentration-dependent impact on the feeding rate and growth of fish.

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7. References

1. Beirne P, South N. Introduction: Approaching green criminology. In: Issues in green criminology confronting harms against environments, humanity and other animals. Cullompton, UK: Willan Publishing; 2007. p. 13–22.
2. White R. Environmental crime: A reader. Devon, UK: Willan; 2009.
3. Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environ Health Perspect. 1999; 107:907–38. https://doi.org/10.1289/ehp.99107s6907
4. Chandra R, Saxena G, Kumar V. Phytoremediation of environmental pollutants: an eco-sustainable green technology to environmental management. In: Advances in biodegradation and bioremediation of industrial waste. CRC Press, Taylor and Francis Group, Boca Raton, FL; 2015. p. 1–30. https://doi.org/10.1201/b18218-2
5. Gaso-Sokac D, Habuda-Stanic M, Busic V, Zobundzija D. Occurrence of pharmaceuticals in surface water. Croat J Food Sci Technol. 2017; 9(2):204–10. https://doi.org/10.17508/CJFST.2017.9.2.18
6. Nikolaou A, Meric S, Fatta D. Occurrence patterns of pharmaceuticals in water and wastewater environments. Anal Bioanal Chem. 2007; 387(4):1225–34. https://doi.org/10.1007/s00216-006-1035-8
7. Netzloff ML. The effects of drugs on embryonic development. Ann Clin Lab Sci. 1976; 6(4):332–41. https://doi.org/10.1007/BF02899980
8. Sachdeva P. The effects of drugs on embryonic development. Ann Clin Lab Sci. 1976; 71:1–7.
9. Masters SB, Trevor AJ, Katzung BG. Katzung. Trevor's Pharmacology. 200; New York: Lange Medical Books/McGraw Hill, Medical Pub. Division.
10. Sensi P, Margalith P, Timba, MT. Rifomycin, a new antibiotic preliminary report. Farmaco Ed Sci. 1959; 14:146–7.
11. Tejada FR, Walk AR, Kharel MK. Drugs used in tuberculosis and leprosy. Side Eff Drugs Annu. 2015; 37:349–65. https://doi.org/10.1016/bs.seda.2015.06.009
12. Ruiz P, Gutierrez J, Rodríguez-Cano F, Zerolo FJ, Casal M. Activity of rifampin against Mycobacterium tuberculosis in a reference centre. Microb Drug Resist. 2004; 10(3): 239–42. https://doi.org/10.1089/mdr.2004.10.239
13. Varner TR, Bookstaver PB, Rudisill CN, Albrecht H. Role of rifampin-based combination therapy for severe community-acquired *Legionella pneumophila* pneumonia. Ann Pharmacother. 2011; 45(7–8):967–76. https://doi.org/10.1345/aph.1Q074
14. van Ewijk-Beneken Kolmer EWJ, Teulen MJA, Van den Hombergh ECA, van Erp NE, Te Brake LHM. Determination of protein-unbound, active rifampicin in serum by ultrafiltration and ultra-performance liquid chromatography with UV detection. A method suitable for standard and high doses of rifampicin. J Chromatogr B Analyt Technol Biomed Life Sci. 2017; 1063:42–9. https://doi.org/10.1016/j.jchromb.2017.08.004
15. Pereira MN, Matos BN, Gratieri T, Cunha-Filho M, Gelfuso GM. Development and validation of a simple chromatographic method for simultaneous determination of clindamycin phosphate and rifampicin in skin permeation studies. J Pharm Biomed Anal. 2018; 159:331–40. https://doi.org/10.1016/j.jpba.2018.07.007
16. Sun J, Zhu D, Xu J, Jia R, Chen S, Liu M, *et al*. Rifampin resistance and its fitness cost in *Riemerella anatipestifer*. BMC Microbiol. 2019; 19(1):107. https://doi.org/10.1186/s12866-019-1478-7
17. Khan AU, Shah F, Khan RA, Ismail B, Khan AM, Muhammad H. Preconcentration of rifampicin prior to its efficient spectroscopic determination in the wastewater samples based on a nonionic surfactant. Turk J Chem. 2021; 45(4):1201–9. https://doi.org/10.3906/kim-2102-28
18. Shayakhmetova GM, Bondarenko LB, Kovalenko VM. Damage of testicular cell macromolecules and reproductive capacity of male rats following co-administration of ethambutol, rifampicin, isoniazid and pyrazinamide. Interdiscip Toxicol. 2012; 5(1):9–14. https://doi.org/10.2478/v10102-012-0002-9
19. Awodele O, Momoh AA, Awolola NA, Kale OE, Okunowo WO. The combined fixed-dose antituberculous drugs alter some reproductive functions with oxidative stress involvement in wistar rats. Toxicol Rep. 2016; 3:620–7. https://doi.org/10.1016/j.toxrep.2016.06.007
20. Al-Asady FM, Al-Saray DA. Impacts administration of rifampicin on sperm DNA integrity and male reproductive system parameters in rats. Res J Pharm Technol. 2021; 14 (9):4897–902. https://doi.org/10.52711/0974-360X.2021.00851
21. Nocke-Finck L, Breuer H. Effect of rifampicin on the biosynthesis of testosterone in rat testis. Acta Endocrinol. 1981; 97(4):573–6. https://doi.org/10.1530/acta.0.0970573
22. Ezeuk VC, Ataman JE, Grillo DB. Toxic effects of antituberculosis drugs (Isoniazid and Rifampicin) on feto-placental unit of wistar rats: A morphological, histological and biochemical study. J Clin Exp Tox. 2019; 3(1):1–6.

23. Al-Chalaby AS. Effect of antituberculosis, (Rifampicin and Isoniazide) on female reproductive system performance in adult rats. Kufa J Vet Sci. 2012; 3(2):1–7.
24. Stratford BF. Observations on laboratory rodents treated with "rifamide" during pregnancy. Med J Aust. 1966:10–12. https://doi.org/10.5694/j.1326-5377.1966.tb19426.x
25. Steen JS, Stainton-Ellis DM. Rifampicin in pregnancy. Lancet (London, England), 1977. p. 604-605. https://doi.org/10.1016/S0140-6736(77)91447-7
26. Kalayci T, Erener-Ercan T, Buyukkale G, Cetinkaya M. Limb deformity in a newborn. Is rifampicin just an innocent bystander? Eur Rev Med Pharmacol Sci. 2015; 19(3):517–19.
27. Moro RN, Scott NA, Vernon A, Tepper NK, Goldberg SV, Schwartzman K, *et al*. Exposure to latent tuberculosis treatment during pregnancy. The PREVENT TB and the iAdhere Trials. Trials Ann Am Thorac Soc. 2018; 15(5):570–80. https://doi.org/10.1513/AnnalsATS.201704-326OC
28. Chaisriram N, Plakornkul V, Roongruangchai J. The teratogenic effect of rifampicin on the developing chick embryo. Rangsit Graduate Research Conference, RGRC. 2018; 13:2981–7.
29. Wourms JP, Grove BD, Lombardi J. The maternal-embryonic relationship in viviparous fishes. Fish Physiol. 1988; 11:1-134. https://doi.org/10.1016/S1546-5098(08)60213-7
30. Farr JA. Sexual selection and secondary sexual differentiation in poeciliids: Determinants of male mating success and the evolution of female choice. In: Ecology and Evolution of Live bearing Fishes (Poeciliidae), Meffe GK, Snelson FF Jr., editors. Prentice Hall: USA; 1989. p. 91–123.
31. Bhat SK, Ganesh CB. Dopamine receptor agonist bromocriptine restrains the follicular development, hatchling success and puberty in *Gambusia affinis*. J Appl Ichthyol. 2019; 35(2):501–11. https://doi.org/10.1111/jai.13875
32. De las Heras V, Martos-Sitcha J, Yúfera M, Mancera JM, Martínez-Rodríguez, G. Influence of stocking density on growth, metabolism and stress of thick-lipped grey mullet *Chelon labrosus* Juveniles. Aquaculture. 2015; 448:29–37. https://doi.org/10.1016/j.aquaculture.2015.05.033
33. Hopkins KD. Reporting fish growth: A review of the basics. J World Aquac Soc. 1992; 23:173–9. https://doi.org/10.1111/j.1749-7345.1992.tb00766.x
34. Yamamoto Y, Luckenbach JA, Middleton MA, Swanson P. The spatiotemporal expression of multiple coho salmon ovarian connexin genes and their hormonal regulation in vitro during oogenesis. Reprod Biol Endocrinol. 2011; 9(1):1–16. https://doi.org/10.1186/1477-7827-9-52
35. Adaklı A, Taşbozan O. The effects of different cycles of starvation and refeeding on growth and body composition on European sea bass (*Dicentrarchus labrax*). Turk J Fish Aquat. 2015; 15(3):419–27. http://dx.doi.org/10.13140/RG.2.1.4626.4168
36. Pérez-Jiménez A, Cardenete G, Hidalgo MC, García-Alcázar A, Abellán E, Morales AE. Metabolic adjustments of dentex to prolonged starvation and refeeding. Fish Physiol Biochem. 2015; 38(4):114–57. https://doi.org/10.1007/s10695-011-9600-2
37. Elbialy ZI, Gamal S, Al-Hawary II, Shukry M, Salah AS, Aboshosha AA, Assar DH. Exploring the impacts of different fasting and refeeding regimes on Nile tilapia (*Oreochromis niloticus* L.): Growth performance, histopathological study, and expression levels of some muscle growth-related genes. Fish Physiol Biochem. 2022; 48:973–89. https://doi.org/10.1007/s10695-022-01094-0
38. Kwon HC, Hayashi S, Mugiya Y. Vitellogenin induction by estradiol-17β in primary hepatocyte culture in the rainbow trout, *Oncorhynchus mykiss*. Comp Biochem Physiol B: Comp Biochem. 1993; 104(2):381–6. https://doi.org/10.1016/0305-0491(93)90383-G
39. Tyler CR, Sumpter JP, Witthames PR. The dynamics of oocyte growth during vitellogenesis in the rainbow trout (*Oncorhynchus mykiss*). Biol Reprod. 1990; 43(2):202–9. https://doi.org/10.1095/biolreprod43.2.202
40. Morrison KR, Ngo V, Cardullo RA, Reznick DN. How fish eggs are preadapted for the evolution of matrotrophy. Proceedings of Royal Society B. 2017; 284: 20171342. https://doi.org/10.1098/rspb.2017.1342
41. Navarro I, Gutiérrez J. Fasting and starvation. In: Biochemistry and molecular biology of fishes, ed. P. W. Hochanchka and T. P. Mommsen, Amsterdam: Elsevier Science; 1995. p. 393–434. https://doi.org/10.1016/S1873-0140(06)80020-2
42. Ali M, Nicieza A, Wooton RJ. Compensatory growth in fishes: A response to growth depression, Fish. Fish (Oxf). 2003; 4:147–90. https://doi.org/10.1046/j.1467-2979.2003.00120.x
43. Reading B, Andersen L, Ryu YW, Mushirobira Y, Todo T, Hiramatsu N. Oogenesis and egg quality in finfish: Yolk formation and other factors influencing female fertility. Fishes. 2018; 3(4):45. https://doi.org/10.3390/fishes3040045
44. Amaral IP, Johnston IA. Insulin-like Growth Factor (IGF) signalling and genome-wide transcriptional regulation in fast muscle of zebrafish following a single-satiating meal. J Exp Biol. 2011; 214(13):2125–39. https://doi.org/10.1242/jeb.053298
45. Ndandala CB, Dai M, Mustapha UF, Li X, Liu J, Huang H, Li G, Chen H. Current research and future perspectives of GH and IGFs family genes in somatic growth and reproduction of teleost fish. Aquacult Rep. 2022; 26:1–11. https://doi.org/10.1016/j.aqrep.2022.101289
46. Mosconi G, Carnevali O, Habibi HR, Sanyal R, Polzonetti-Magni AM. Hormonal mechanisms regulating hepatic vitellogenin synthesis in the Gilthead Sea bream, Sparus aurata. Am J Physiol Cell Physiol. 2002; 28(3):C673–8. https://doi.org/10.1152/ajpcell.00411.2001
47. Moussavi M, Nelson ER, Habibi HR. Seasonal regulation of vitellogenin by growth hormone in the goldfish liver. Gen Comp Endocrinol. 2009; 161(1): 79–82. https://doi.org/10.1016/j.ygcen.2008.12.009

48. Perera E, Rosell-Moll E, Martos-Sitcha JA, Naya-Catala F, Simó-Mirabet P, Calduch-Giner J, *et al*. Physiological trade-offs associated with fasting weight loss, resistance to exercise and behavioral traits in farmed gilthead sea bream (*Sparus aurata*) selected by growth. Aquac Rep. 2021; 20. https://doi.org/10.1016/j.aqrep.2021.100645
49. Emel'yanova NG, Pavlov DA, Pavlov ED, Thuan LTB, Ha V T. Anomalies in ovarian condition of manybar goatfish *Parupeneus multifasciatus* (Mullidae) from the coastal zone of south-Central Vietnam. J Ichthyol. 2014; 54:76–84. https://doi.org/10.1134/S0032945214010056
50. Myrianthopoulos NC, Chung CS. Congenital malformations in singletons: Epidemiologic survey. Report from the Collaborative Perinatal project, Birth Defects Orig Artic Ser. 1974; 10(11):1–58.
51. Weinberger SE, Weiss ST, Cohen WR, Weiss JW, Johnson TS. Pregnancy and the lung. Am Rev Respir Dis. 1980; 121(3):559–81. https://doi.org/10.1164/arrd.1980.121.3.559
52. Snider DE, Layde PM, Johnson MW, Lyle MA. Treatment of tuberculosis during pregnancy. Am Rev Respir Dis. 1980; 122(1):65–79.