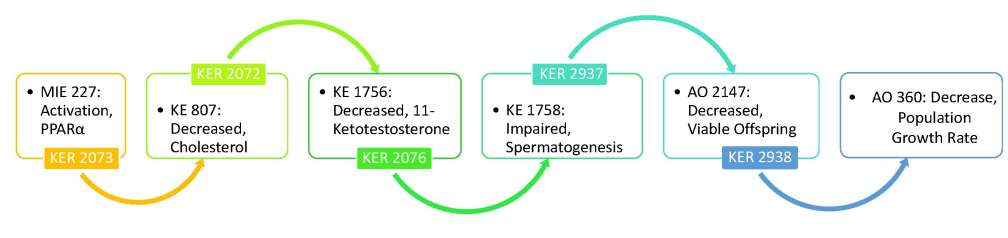
AOP ID and Title:

AOP 323: PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone
Short Title: PPARa Agonism Impairs Fish Reproduction

Graphical Representation



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Abstract

This adverse outcome pathway details the linkage from peroxisome proliferator-activated receptor alpha (PPARα) activation to the adverse effects of decreased viable offspring and decrease in population growth rate in fish. PPARα is a ligand-activated nuclear receptor that, after forming a heterodimer with retinoid X receptor (RXR), promotes transcription of many genes including those involved in fatty acid β-oxidation and cholesterol metabolism. Synthetic ligands have been designed as pharmaceuticals to target PPARα for treatment of human metabolic diseases. Exposure to these pharmaceuticals or other contaminants in environment can disrupt metabolic processes in fish, including the activation of PPARα. In fish, this can lead to decreased cholesterol which in turn causes a decrease in reproductive hormones, notably 11-ketotestosterone (11-KT). A decrease in reproductive hormones impairs the fish's ability to reproduce. Described here is the pathway in which decreased 11-KT impairs inducement of spermatogenesis and sperm production which results in a reduced number of viable offspring. This can lead to impacts on population growth rate due to the decreased number of viable offspring resulting in a decline in recruitment and contribution of offspring to the next generation.

Background

This AOP was developed to address one potential effect of per- and polyfluoroalkyl substances (PFAS) on fish. Through review of the human health and *in vitro* toxicity data on conserved pathways and molecular targets for PFAS disruption, activation of PPARα was identified as a potential target of several PFAS which could result in altered lipid metabolism. This AOP focused primarily on teleost fish using experimental data from prototypical stressors, along with knock-out and genetic mutation experiments, for evidence of causality and essentiality for existing and newly developed KEs and KERs.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name

Sequence	Type	Event ID	Title	Short name
	MIE	227	Activation, PPARα	Activation, PPARα
	KE	807	Decreased, cholesterol	Decreased, cholesterol
	KE	1756	Decreased, plasma 11-ketotestosterone level	Decreased, 11KT
	KE	1758	Impaired, Spermatogenesis	Impaired, Spermatogenesis
	AO	2147	Decreased, Viable Offspring	Decreased, Viable Offspring
	AO	360	Decrease, Population growth rate	Decrease, Population growth rate

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Activation, PPARα	adjacent	Decreased, cholesterol	High	Low
Decreased, cholesterol	adjacent	Decreased, plasma 11-ketotestosterone level	High	Low
Decreased, plasma 11-ketotestosterone level	adjacent	Impaired, Spermatogenesis	High	Low
Impaired, Spermatogenesis	adjacent	Decreased, Viable Offspring	Moderate	Low
Decreased, Viable Offspring	adjacent	Decrease, Population growth rate	Moderate	Low

Stressors

Name	Evidence
Clofibrate	
Gemfibrozil	
Fenofibrate	

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI

Sex Applicability

Sex	Evidence
Male	High

The empirical evidence suggests that this AOP is applicable to adult, reproductively mature, male teleost fish.

Life Stage

The life stage applicable to this AOP is adult, reproductively mature organisms.

Sex

The process of spermatogenesis occurs in reproductively mature males. Therefore, this AOP is only applicable to males.

Taxonomic

This AOP is considered most relevant for teleost fish. Most of the experimental evidence compiled for this AOP is from teleost fish, for which 11-KT is the dominant androgen. However, PPARs including PPARα are highly conserved across humans, rodents, and fish. An evaluation of protein sequence conservation via SeqAPASS (https://seqapass.epa.gov/seqapass/) predicted similarity in cross-species susceptibility to PPARα agonists among humans, zebrafish, medaka, and other fish species. Thus, PPARα agonism and downstream effects on cholesterol, hormone production (not limited to 11-KT), spermatogenesis (a highly conserved biological process), and production of offspring could have more broad taxonomic relevance.

Essentiality of the Key Events

Essentiality of most of key events in this AOP is supported with experimental exposures with prototypical stressors that demonstrate modification of a more upstream KE associated with a corresponding change in downstream KE(s). Several of the key events have further support for essentiality with knock-out and genetic mutations experiments as well as rescue studies. Key studies are listed below.

Although it is challenging to directly measure PPARα activation in fish *in vivo* studies, there are multiple studies that have shown that fish exposed to fibrates (and thus assumed activation of PPARα) have decreased cholesterol. This relationship has been demonstrated in a variety of fish species [fathead minnow (Runnalls et al., 2007), grass carp (Du et al., 2008; Guo et al., 2015), Nile tilapia (Ning et al., 2017), rainbow trout (Prindiville et al., 2011), medaka (Lee et al., 2019), zebrafish (AL-Habsi et al., 2016; Velasco-Santamaria et al., 2011; Fraz et al., 2018), turbot (Urbatzka et al., 2015)], with temporal and dose concordance in one study (Velasco-Santamaria et al., 2011).

The process of steroid hormone biosynthesis is well understood, and cholesterol is the precursor for all steroid hormones, including 11-KT. The relationship between decreased cholesterol and decreased 11-KT is well-established. There are several experimental exposure studies that showed decreased 11-KT associated with decreased cholesterol with dose and temporal concordance (Lee et al., 2019; Velasco-Santamaria et al., 2011). The essentiality of cholesterol for production of 11-KT is further supported by an *ex vivo* study which showed that exposure to gemfibrozil (a known PPARα agonist) resulted in decreased 11-KT production unless supplemented with 25OH-cholesterol (Fraz et al., 2018), demonstrating that decreased cholesterol availability was the cause of the decreased steroid synthesis.

11-KT is well documented as a critical androgen for proper male reproduction in teleost fish and has well-documented involvement in spermatogenesis and spermiation. The essentiality of 11-KT for spermatogenesis has been documented in zebrafish knock-out studies with rescue (Zhang et al., 2020) which showed that zebrafish with *cyp11c1* knockout have reduced 11-KT levels, smaller genitalia, inability naturally mate, defective Leydig and Sertoli cells, and insufficient spermatogenesis. The treatment of100 nM 11-KA (which is converted to 11-KT *in vivo*) for 4 hours per day for 10 days corrected these effects, demonstrating that insufficient 11-KT levels was the cause of arrested spermatogenesis.

Successful oocyte fertilization and production of viable offspring is dependent on spermatogenesis and the production of sufficient quality and quantity of sperm. Essentiality is strongly supported by gene modification studies, such knock-out studies targeting genes associated with spermatogenesis and meiotic division as well as exposure studies with known endocrine disruptors (e.g., DEHP, EE2). Multiple studies with zebrafish have shown that knockouts targeting genes associated with spermatogenesis (e.g., Tdrd12, AR) and meiotic division (e.g., E2f5, Mettl3, mlh1) resulted in interference with spermatogenesis (i.e., delayed or arrested progression, apoptosis, and decrease in sperm density, quality and/or motility) and male zebrafish that were either infertile or exhibited decreased fertilization rates when mated with WT females (Dai et al., 2017; Leal et al, 2008; Tang et al., 2018; Xia et al., 2018; Xie et al., 2020).

By definition, there must be viable offspring to maintain a population. However, there are other vital rates that are essential here as well, such as survival to reproductive age.

Weight of Evidence Summary

The weight of evidence for each of the KERs within this AOP are ranked moderate to high. Each of the KERs is biologically plausible, with the highest ratings for the intermediate KERs (Decreased, cholesterol leading to Decreased, 11-KT and Decreased, 11-KT leading to Impaired, Spermatogenesis). The relationship between the MIE and the first key event is considered moderate for biological plausibility due to challenges in directly measuring the PPARα activation in *in vivo* studies. Whereas the links to the individual adverse outcome (Decreased, viable offspring) and the population level adverse outcome are considered moderate for biological plausibility due to the other factors that can influence each of these outcomes. There is substantial experimental evidence in fish to support this AOP, however, few studies measured multiple sequential key events and the final link to decreased population growth rate is based on biological plausibility and population modeling. Overall weight of evidence is moderate.

Biological Plausibility

This AOP is considered highly plausible, based on the evaluation of available evidence for the mechanistic (structural or functional) relationships between upstream and downstream KEs that are consistent with established biological knowledge. There is a broad understanding of lipid metabolism pathways and supporting *in vivo* and *in vitro* experimental data on the role of PPARα in lipid metabolism. PPARα is conserved across vertebrates and has been documented in multiple fish species, therefore biological plausibility is considered moderate for activation of PPARα leading to decreased cholesterol. The next two KERs are considered highly plausible. The process of steroid hormone biosynthesis is well understood, and cholesterol is the precursor for all steroid hormones including testosterone and 11-ketotestosterone (Norris and Carr, 2020). Similarly, the 11-ketotestosterone is well documented as necessary for spermatogenesis and sperm production (Amer et al., 2001; Borg, 1994; Geraudie et al., 2010).

Because there are multiple factors required to produce viable offspring, biological plausibility is considered moderate for the process of impaired spermatogenesis leading to decreased viable offspring. The link from the individual level adverse outcome (decreased viable offspring) to the population level adverse outcome (decrease in population growth rate) is also influenced by multiple factors.

Empirical Support

There is substantial experimental evidence to support this AOP. Experimental results from a variety of fish studies with prototypical stressors demonstrate concordance and consistency throughout the AOP. However, there were few studies that measured multiple sequential KEs and limited concentration or dose-response data and temporal measurements across diversity of taxa. Due to these limitations, response-response relationships for a quantitative understanding of this AOP could not be evaluated. Concordance of empirical support across the AOP is summarized in Attachment A.

There are multiple studies in fish that demonstrate exposure to known PPARα agonists (considered prototypical stressors or model chemicals) resulted in decreased total cholesterol. These studies include experimental exposure of seven different fish species [fathead minnow (Runnalls et al., 2007), grass carp (Du et al., 2008; Guo et al., 2015), Nile tilapia (Ning et al., 2017), rainbow trout (Prindiville et al., 2011), medaka (Lee et al., 2019), zebrafish (AL-Habsi et al., 2016; Velasco-Santamaria et al., 2011; Fraz et al., 2018), turbot (Urbatzka et al., 2015)] to several different fibrates (clofibrate, clofibric acid, gemfibrozil, fenofibrate, WY-14643). Temporal and dose concordance was demonstrated in one study (Velasco-Santamaría et al., 2011); however, there is insufficient empirical evidence for development of a quantitative relationship between the KEs.

While the following KER (decreased cholesterol leading to decreased 11-KT) has strong biological plausibility, there are relatively few fish experimental exposure studies that measured both cholesterol and 11-KT. Two exposure studies that measured both KEs showed dose and temporal concordance (Lee et al., 2019; Velasco-Santamaria et al., 2011), and the third study provided strong evidence essentiality of cholesterol for the production of 11-KT (Fraz et al., 2018).

There is substantial empirical evidence showing spermatogenesis in numerous fish species is dependent on 11-KT, with several studies demonstrating temporal and dose concordance for this relationship. These studies include testing of both higher 11-KT (treatments with 11-KT or increased production) and decreased 11-KT. For example, increased 11-KT has been related to measures of successful spermatogenesis such as greater number of spermatids (Agulleiro et al., 2007; Selvaraj et al., 2013), more advanced testicular stages (Cavaco et al., 1998, 2001), and more differentiated and later type spermatogonia (Melo et al., 2015; Miura et al., 1991). Whereas, decreased 11-KT in fish has been associated with negative impacts or delays in spermatogenesis including decreased number of spermatocytes, spermatids, and/or spermatozoa (Agbohessi et al., 2015; Chen et al., 2017; de Waal et al., 2009; Liu et al., 2018; Pereira et al., 2015; Sales et al., 2020; Xia et al,. 2018). Melo et al. (2015) is one example of studies that demonstrated temporal concordance; in this study exposure to adrenosterone (ketoandrostenedione; which is converted to 11-KT *in vivo*) caused an increase in 11-KT levels at 7 and 14 d, with Type A differentiated spermatogonial numbers also increased 14 d after treatment.

There is substantial empirical evidence demonstrating that impaired spermatogenesis results in decreased oocyte fertilization and a reduction in viable offspring. Much of the cited literature is from fish exposed to prototypical stressors (endocrine disruptors), with several studies demonstrating dose and temporal concordance. In addition to the gene modification studies previously described for essentiality, exposure studies with endocrine disruptors [e.g., di(2-ethylhexyl) phthalate (DEHP), 17α-ethinylestradiol (EE2), nonylphenol] provide evidence of concordance and consistency of this KER. These include studies with zebrafish, Nile tilapia, Japanese medaka, and marine medaka (Corradetti et al., 2013; Hill & Janz, 2003; Kang et al., 2002, Nash et al., 2004; Seki et al., 2002). Several studies provide evidence of dose-response concordance such as a concentration dependent effect on both spermatogenesis and fertilization rate of when male fish exposed to DEHP are mated with wild-type females (Ma et al., 2018; Uren-Webster et al., 2010; Ye et al., 2014).

Direct empirical evidence on population size decreases associated with decreased viable offspring is very limited. There are no empirical data suitable for evaluating the dose-response, temporal, or incidence concordance between these two adverse outcomes. This relationship is based on biological plausibility and population modeling (e.g., Miller & Ankley, 2004; Miller et al., 2020).

Uncertainties, inconsistencies, and data gaps

- There were no notable inconsistencies in the literature that was reviewed for development of this AOP. However, there are several areas of uncertainty. These include:
- It is challenging to directly measure PPARα agonism in fish *in vivo* studies. Therefore, we relied on fish exposure studies with pharmaceuticals designed to activate PPARα in humans. However, there is uncertainty of whether all fibrates shown effective in humans are PPARα agonists in fish. This AOP was developed on the assumption that these pharmaceutical also activate PPARα in fish, which is supported by a cross-species comparison *in vitro* and susceptibility evaluation based on gene sequences support similarity in responses across vertebrates.
- 11-KT levels can be highly variable between fish species and have seasonal fluctuations within a species (with highest levels at spawning).
- For the relationship between 11-KT and spermatogenesis, a few studies documented a significant change in one without a significant change in the other, highlighting the complexity of this relationship.
- Both of the adverse outcomes (Decreased, viable offspring and Decrease, population growth rate) are influenced by multiple factors. The key events in this AOP are just one potential path to these outcomes. In addition, PPARα agonism could result in other toxicity pathways, such as decreased juvenile growth, which were not included in the development of this AOP.
- Finally, few studies measured multiple sequential key events; thus evidence had to be compiled KER by KER to support this

AOP.

Quantitative Consideration

At this time available data are insufficient to develop a quantitative AOP linking PPARα agonism with decreased viable offspring or decreased population growth rate.

Considerations for Potential Applications of the AOP (optional)

- The present AOP can inform a tiered testing approach for PPARα agonists (including some PFAS) based on in vitro screening results (e.g., Houck et al., 2021) and targeted in vivo testing (illustrated by Villeneuve et al., 2023).
- The present AOP can inform the development of microphysiological or computational systems models to evaluated probable effects on reproduction.
- The present AOP can aid in prediction of potential effects when PPAR agonists are measured in environmental samples and interpretation (along with selection of additional endpoints to measure) when PPAR activity is detected with effects-based environmental monitoring (e.g., Blackwell et al., 2019).

References

Agbohessi, P.T., Imorou Toko I., Ouédraogo, A., Jauniaux, T., Mandiki, S.N., & Kestemont, P. (2015). Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in Benin (West Africa). *Science of the Total Environment*, *506-507*, 567-584. https://doi.org/10.1016/j.scitotenv.2014.11.047

Agulleiro, M.J., Scott, A.P., Duncan, N., Mylonas, C.C., & Cerdà, J. (2007). Treatment of GnRHa-implanted Senegalese sole (*Solea senegalensis*) with 11-ketoandrostenedione stimulates spermatogenesis and increases sperm motility. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *147*(4), 885-92. https://doi.org/10.1016/j.cbpa.2007.02.008

Al-Habsi, A.A., A. Massarsky, T.W. Moon (2016) “Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (*Danio rerio*)”, *Comparative Biochemistry and Physiology, Part B,* Vol. 199, Elsevier, pp. 87-96. http://dx.doi.org/10.1016/j.cbpb.2015.11.009

Amer, M.A., Miura, T., Miura, C., & Yamauchi, K. (2001). Involvement of Sex Steroid Hormones in the Early Stages of Spermatogenesis in Japanese Huchen (*Hucho perryi*). *Biology of Reproduction, 65*(4), 1057–1066. https://doi.org/10.1095/biolreprod65.4.1057

Blackwell, B. R., Ankley, G. T., Bradley, P. M., Houck, K. A., Makarov, S. S., Medvedev, A. V., Swintek, J., & Villeneuve, D. L. (2019). Potential Toxicity of Complex Mixtures in Surface Waters from a Nationwide Survey of United States Streams: Identifying in Vitro Bioactivities and Causative Chemicals. *Environmental science & technology*, *53*(2), 973–983. https://doi.org/10.1021/acs.est.8b05304

Borg, B. (1994). Androgens in teleost fishes. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology, and Endocrinology*, *109*(3), 219-245. https://doi.org/10.1016/0742-8413(94)00063-G

Cavaco, J.E.B., Bogerd, J., Goos, H., & Schulz, R.W. (2001). Testosterone inhibits 11-ketotestosterone-induced spermatogenesis in African catfish (*Clarias gariepinus*). *Biology of Reproduction*, *65*(6), 1807-1812. https://doi.org/10.1095/biolreprod65.6.1807

Cavaco, J.E.B., Vilrokx, C., Trudeau, V.L., Schulz, R.W., & Goos, H.J.T. (1998). Sex steroids and the initiation of puberty in male African catfish, Clarias gariepinus. *American Journal of Physiology*, *275*(6), 1793-1802. https://doi.org/10.1152/ajpregu.1998.275.6.R1793

Chen, J., Jiang, D., Tan, D., Fan, Z., Wei, Y., Li, M., & Wang, D. (2017). Heterozygous mutation of eEF1A1b resulted in spermatogenesis arrest and infertility in male tilapia, Oreochromis niloticus. *Scientific Reports*, *7,* 43733. https://doi.org/10.1038/srep43733

Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., & Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (*Danio rerio*). *Reproductive biology*, *13*(3), 195–202. https://doi.org/10.1016/j.repbio.2013.07.003

Dai, X., Shu, Y., Lou, Q., Tian, Q., Zhai, G., Song, J., Lu, S., Yu, H., He, J., & Yin, Z. (2017). Tdrd12 Is Essential for Germ Cell Development and Maintenance in Zebrafish. *International journal of molecular sciences*, *18*(6), 1127. https://doi.org/10.3390/ijms18061127 de Waal, P.P., Leal, M.C., García-López, A., Liarte, S., de Jonge, H., Hinfray, N., Brion, F., Schulz, R.W., & Bogerd, J. (2009). Oestrogen-induced androgen insufficiency results in a reduction of proliferation and differentiation of spermatogonia in the zebrafish testis. *Journal of Endocrinology*, *202*(2), 287-97. https://doi.org/10.1677/JOE-09-0050

Du, Z. et al. (2008) “Hypolipidaemic effects of fenofibrate and fasting in the herbivorous grass carp (*Ctenopharyngodon Idella*) fed a high-fat diet”, *British Journal of Nutrition*, Vol. 100, Cambridge University Press, pp. 1200-1212. doi:10.1017/S0007114508986840

Fraz, S., A.H. Lee, J.Y. Wilson (2018) “Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (*Danio*

rerio)", *Aquatic Toxicology*, Vol. 198, Elsevier, pp. 1-9. https://doi.org/10.1016/j.aquatox.2018.02.006

Geraudie, P., Gerbron, M., & Minier, C. (2010). Seasonal variations and alterations of sex steroid levels during the reproductive cycle of male roach (*Rutilus rutilus*). *Marine Environmental Research*, *69*(S1), S53-S55. https://doi.org/10.1016/j.marenvres.2009.11.008

Guo, X. et al. (2015) "Effects of lipid-lowering pharmaceutical clofibrate on lipid and lipoprotein metabolism of grass carp (*Ctenopharyngodon idella* Val.) fed with the high non-protein energy diets", *Fish Physiology and Biochemistry,* Vol. 41, Springer, pp. 331-343. doi: 10.1007/s10695-014-9986-8

Hill, R. L., Jr, & Janz, D. M. (2003). Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. *Aquatic toxicology (Amsterdam, Netherlands)*, *63*(4), 417–429. https://doi.org/10.1016/s0166-445x(02)00207-2

Houck, K. A., Patlewicz, G., Richard, A. M., Williams, A. J., Shobair, M. A., Smeltz, M., Clifton, M. S., Wetmore, B., Medvedev, A., & Makarov, S. (2021). Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure. *Toxicology*, *457*, 152789. https://doi.org/10.1016/j.tox.2021.152789

Kang, I. J., Yokota, H., Oshima, Y., Tsuruda, Y., Yamaguchi, T., Maeda, M., Imada, N., Tadokoro, H., & Honjo, T. (2002). Effect of 17beta-estradiol on the reproduction of Japanese medaka (Oryzias latipes). *Chemosphere*, *47*(1), 71–80. https://doi.org/10.1016/s0045-6535(01)00205-3

Leal, M. C., Feitsma, H., Cuppen, E., França, L. R., & Schulz, R. W. (2008). Completion of meiosis in male zebrafish (*Danio rerio*) despite lack of DNA mismatch repair gene mlh1. *Cell and tissue research*, *332*(1), 133–139. https://doi.org/10.1007/s00441-007-0550-z

Lee, G. et al. (2019) Effects of gemfibrozil on sex hormones and reproduction related performances of *Oryzias latipes* following long-term (155 d) and short-term (21 d) exposure, *Ecotoxicology and Environmental Safety*, Vol. 173, Elsevier, pp. 174-181. https://doi.org/10.1016/j.ecoenv.2019.02.015

Liu, Z.H., Chen, Q.L., Chen, Q., Li, F., & Li, Y.W. (2018). Diethylstilbestrol arrested spermatogenesis and somatic growth in the juveniles of yellow catfish (*Pelteobagrus fulvidraco*), a fish with sexual dimorphic growth. *Fish Physiology and Biochemistry*, *44*(3), 789-803. https://doi.org/10.1007/s10695-018-0469-1

Ma, Yan-Bo, Jia, Pan-Pan, Junaid, Muhammad, Yang, Li, Lu, Chun-Jiao, & Pei, De-Sheng. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution (1987), 237, 1050-1061.

Melo, M.C., van Dijk, P., Andersson, E., Nilsen, T.O., Fjelldal, P.G., Male, R., Nijenhuis, W., Bogerd, J., de França, L.R., Taranger, G.L., & Schulz R.W. (2015). Androgens directly stimulate spermatogonial differentiation in juvenile Atlantic salmon (*Salmo sala*). *General and Comparative Endocrinology*, 211, 52-61. https://doi.org/10.1016/j.ygcen.2014.11.015.

Miller, D. H., & Ankley, G. T. (2004). Modeling impacts on populations: fathead minnow (*Pimephales promelas*) exposure to the endocrine disruptor 17beta-trenbolone as a case study. *Ecotoxicology and environmental safety*, *59*(1), 1–9. https://doi.org/10.1016/j.ecoenv.2004.05.005

Miller, D. H., Clark, B. W., & Nacci, D. E. (2020). A multidimensional density dependent matrix population model for assessing risk of stressors to fish populations. *Ecotoxicology and environmental safety*, *201*, 110786. https://doi.org/10.1016/j.ecoenv.2020.110786

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. (1991). Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). *Proceedings of the National Academy of Sciences of the United States of America*, *88*(13), 5774–5778. https://doi.org/10.1073/pnas.88.13.5774

Ning, L. et al. (2017) "Nutritional background changes the hypolipidemic effects of fenofibrate in Nile tilapia (*Oreochromis niloticus*)", *Scientific Reports,* Vol. 7(41706), Nature. https://doi.org/10.1038/srep41706

Norris, D. & Carr, J. A. 2020. Vertebrate Endocrinology, 6th Edition. Academic Press. Cambridge, MA, USA, 656 pp. ISBN: 9780128200933

Pereira, T.S., Boscolo, C.N., Silva, D.G., Batlouni, S.R., Schlenk, D., & Almeida, E.A. (2015). Anti-androgenic activities of diuron and its metabolites in male Nile tilapia (*Oreochromis niloticus*). *Aquatic Toxicology*, *164*, 10-15. https://doi.org/10.1016/j.aquatox.2015.04.013

Prindiville, J.S. et al. (2011). The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout. *Toxicology and Applied Pharmacology,* Vol. 251, Elsevier, pp. 201-238. doi:10.1016/j.taap.2010.12.013

Runnalls, T.J., Hala, D.N., & Sumpter, J.P. (2007). Preliminary studies into the effects of the human pharmaceutical Clofibric acid on sperm parameters in adult Fathead minnow. *Aquatic Toxicology*, *84*(1), 111-118. https://doi.org/10.1016/j.aquatox.2007.06.005

Sales, C.F., Barbosa Pinheiro, A.P., Ribeiro, Y.M., Weber, A.A., Paes-Leme, F.O., Luz, R.K., Bazzoli, N., Rizzo, E., & Melo, R.M.C. (2020). Effects of starvation and refeeding cycles on spermatogenesis and sex steroids in the Nile tilapia *Oreochromis niloticus*. *Molecular and Cellular Endocrinology*, *500*, 110643. https://doi.org/10.1016/j.mce.2019.110643

Seki, M., Yokota, H., Matsubara, H., Tsuruda, Y., Maeda, M., Tadokoro, H., & Kobayashi, K. (2002). Effect of ethinylestradiol on the reproduction and induction of vitellogenin and testis-ova in medaka (*Oryzias latipes*). *Environmental toxicology and chemistry*, *21*(8), 1692–1698.

Selvaraj, S., Ohga, H., Nyuji, M., Kitano, H., Nagano, N., Yamaguchi, A., & Matsuyama, M. (2013). Subcutaneous administration of Kiss1 pentadecapeptide accelerates spermatogenesis in prepubertal male chub mackerel (*Scomber japonicus*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *166*(2), 228-36. https://doi.org/10.1016/j.cbpa.2013.06.007

Tang, H., Chen, Y., Wang, L., Yin, Y., Li, G., Guo, Y., Liu, Y., Lin, H., Cheng, C.H.K., & Liu, X. (2018). Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor. *Biology of Reproduction*, *98*(2), 227-238. https://doi.org/10.1093/biolre/iox165

Urbatzka, R., Galante-Oliveira, S., Rocha, E., Lobo-da-Cunha, A., Castro, L. F., & Cunha, I. (2015). Effects of the PPARα agonist WY-14,643 on plasma lipids, enzymatic activities and mRNA expression of lipid metabolism genes in a marine flatfish, *Scophthalmus maximus*. *Aquatic toxicology (Amsterdam, Netherlands)*, *164*, 155–162. https://doi.org/10.1016/j.aquatox.2015.05.004

Uren-Webster, Tamsyn M, Lewis, Ceri, Filby, Amy L, Paull, Gregory C, & Santos, Eduarda M. (2010). Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. Aquatic Toxicology, 99(3), 360-369.

Vandermeer, J.H. & Goldberg, D.E. (2013). Population ecology: first principles. Princeton University Press, Princeton, NJ USA.

Velasco-Santamaría, Y.M., Korsgaard, B., Madsen, S.S., & Bjerregaard, P. (2011). Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (Danio rerio). *Aquatic Toxicology*, *105*(1-2), 107-118. https://doi.org/10.1016/j.aquatox.2011.05.018

Villeneuve, D. L., Blackwell, B. R., Cavallin, J. E., Collins, J., Hoang, J. X., Hofer, R. N., Houck, K. A., Jensen, K. M., Kahl, M. D., Kutsi, R. N., Opseth, A. S., Santana Rodriguez, K. J., Schaupp, C., Stacy, E. H., & Ankley, G. T. (2023). Verification of In Vivo Estrogenic Activity for Four Per- and Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists via New Approach Methodologies. *Environmental science & technology*, *57*(9), 3794–3803. https://doi.org/10.1021/acs.est.2c09315

Xia, H., Zhong, C., Wu, X., Chen, J., Tao, B., Xia, X., Shi, M., Zhu, Z., Trudeau, V. L., & Hu, W. (2018). *Mettl3* mutation disrupts gamete maturation and reduced fertility in zebrafish. *Genetics, 208*(2), 729-743. doi: 10.1534/genetics.117.300574

Xie, H., Kang, Y., Wang, S., Zheng, P., Chen, Z., Roy, S., & Zhao, C. (2020). E2f5 is a versatile transcriptional activator required for spermatogenesis and multiciliated cell differentiation in zebrafish. PLoS genetics, 16(3), e1008655. https://doi.org/10.1371/journal.pgen.1008655

Ye, Ting, Kang, Mei, Huang, Qiansheng, Fang, Chao, Chen, Yajie, Shen, Heqing, & Dong, Sijun. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (*Oryzias melastigma*). Aquatic Toxicology, 146, 115-126.

Zhang, Q., Ye, D., Wang, H., Wang, Y., Hu, W., Sun, Y. (2020). Zebrafish cyp11c1 Knockout Reveals the Roles of 11-ketotestosterone and Cortisol in Sexual Development and Reproduction. *Endocrinology*, *161*(6). https://doi.org/10.1210/endocr/bqaa048

Appendix 1

List of MIEs in this AOP

Event: 227: Activation, PPARα

Short Name: Activation, PPARα

Key Event Component

Process	Object	Action
peroxisome proliferator activated receptor signaling pathway	peroxisome proliferator-activated receptor alpha	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:18 - PPARα activation in utero leading to impaired fertility in males	MolecularInitiatingEvent
Aop:51 - PPARα activation leading to impaired fertility in adult male rodents	MolecularInitiatingEvent

AOP ID and Name	Event Type
Aop:61 - NFE2L2/FXR activation leading to hepatic steatosis	KeyEvent
Aop:37 - PPARα activation leading to hepatocellular adenomas and carcinomas in rodents	MolecularInitiatingEvent
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	MolecularInitiatingEvent
Aop:401 - G protein-coupled estrogen receptor 1 (GPER) signal pathway in the lipid metabolism disrupting effects	KeyEvent

Stressors

Name
Di(2-ethylhexyl) phthalate
Mono(2-ethylhexyl) phthalate
Stressor:205 pirinixic acid (WY-14,643)
Clofibrate
Nafenopin
ciprofibrate
Gemfibrozil
PERFLUOROOCTANOIC ACID
Bezafibrate
Fenofibrate
Simvastatin

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Organ term

Organ term
liver

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Fibrates are ligands of PPARα (Staels et al. 1998).

Phthalates

MHEP (CAS 4376-20-9) directly binds *in vitro* to PPARα (Lapinskas et al. 2005) and activates this receptor in transactivation assays PPARα (Lapinskas et al. 2005), (Maloney and Waxman 1999), (Hurst and Waxman 2003), (Bility et al. 2004), (Lampen, Zimnik, and Nau 2003), (Venkata et al. 2006)]. DEHP (CAS 117-81-7) has not been found to bind and activate PPARα (Lapinskas et al. 2005), (Maloney and Waxman 1999). However, the recent studies shown activation of PPARα (ToxCastTM Data).

Notably, PPARα are responsive to DEHP *in vitro* as they are translocated to the nucleus (in primary Sertoli cells) (Dufour et al. 2003), (Bhattacharya et al. 2005). Expression of PPARα [mRNA and protein] has been reported to be also modulated by

phthtalates: (to be up-regulated *in vivo* upon DEHP treatment (Xu et al. 2010) and down-regulated by Diisobutyl phthalate (DiBP) (Boberg et al. 2008)).

Perfluorooctanoic Acid (PFOA) is known to activate PPARα (Vanden Heuvel et al. 2006).

Organotin

Tributyltin (TBT) activates all three heterodimers of PPAR with RXR, primarily through its interaction with RXR (le Maire et al. 2009)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

PPARα has been identified in frog (Xenopus laevis), mouse, human, rat, fish, hamster and chicken (reviewed in (Wahli and Desvergne 1999)).

Key Event Description

Gene expression occurs in a coordinated fashion (Judson et al., 2012). The many observations of altered gene expression following binding of ligand to PPARα led to systematic investigations of the genomic signature that corresponds to PPARα activation (Tamura et al., 2006; Kupershmidt et al., 2010; Rosen et al., 2017; Rooney et al., 2018; Corton et al., 2020; Hill et al., 2020; Lewis et al., 2020). Specific gene with increased expression following PPARα activation include Cyp4a1, Cpt1B, and Lpl. More generally, the pathways activated include:

- Genes involved in Metabolism of lipids and lipoproteins
- Fatty acid metabolism
- Genes involved in Fatty acid, triacylglycerol, and ketone body metabolism
- PPAR signaling pathway
- Peroxisome
- Genes involved in Cell Cycle

Biological state

The Peroxisome Proliferator Activated receptor α (PPARα) belongs to the Peroxisome Proliferator Activated receptors (PPARs; NR1C) steroid/thyroid/retinoid receptor superfamily of transcription factors.

Biological compartments

PPARα is expressed in high levels in tissues that perform significant catabolism of fatty acids (FAs), such as brown adipose tissue, liver, heart, kidney, and intestine (Michalik et al. 2006). The receptor is present also in skeletal muscle, intestine, pancreas, lung, placenta and testes (Mukherjee et al. 1997), (Schultz et al. 1999).

General role in biology

PPARs are activated by fatty acids and their derivatives; they are sensors of dietary lipids and are involved in lipid and carbohydrate metabolism, immune response and peroxisome proliferation (Wahli and Desvergne 1999), (Evans, Barish, & Wang, 2004). PAPRα is a also a target of hypothalamic hormone signalling and was found to play a role in embryonic development (Yessoufou and Wahli 2010).

Fibrates, activators of PPARα, are commonly used to treat hypertriglyceridemia and other dyslipidemic states as they have been shown to decrease circulating lipid levels (Lefebvre et al. 2006).

How it is Measured or Detected

Binding of ligands to PPARα is measured using binding assays in vitro and in silico, whereas the information about functional activation is derived from transactivation assays (e.g. transactivation assay with reporter gene) that demonstrate functional activation of a nuclear receptor by a specific compound. Binding of agonists within the ligand-binding site of PPARs causes a conformational change of nuclear receptor that promotes binding to transcriptional co-activators. Conversely, binding of antagonists results in a conformation that favours the binding of co-repressors (Yu and Reddy 2007), (Viswakarma et al. 2010). Transactivation assays are performed using transient or stably transfected cells with the PPARα expression plasmid and a reporter plasmid, respectively. There are also other methods that have been used to measure PPARα activity, such as the Electrophoretic Mobility Shift Assay (EMSA) or commercially available PPARα transcription factor assay kits, see Table 1. The transactivation (stable

transfection) assay provides the most applicable OECD Level 2 assay (i.e. In vitro assays providing mechanistic data) aimed at identifying the initiating event leading to an adverse outcome (LeBlanc, Norris, and Kloas 2011). A recent study characterized the PPARα ligand binding domain for the purpose of next-generation metabolic disease drugs (Kamata et al. 2020).

The most direct measure of this MIE is microarray profiling from large gene expression databases TG-GATEs and DrugMatrix coupled with t statistical analysis of whole genome expression profiles (Svoboda et al., 2019; Igarashi et al., 2015) From these data, A gene expression signature of 131 PPARα-dependent genes was built using microarray profiles from the livers of wild-type and PPARα-null mice. A quantitative measure of this expression signature is a measure of similarity/correlation between the PPARα signature and positive and negative test sets is provided by the Running Fisher test (Corton et al., 2020; Hill et al., 2020; Kupershmidt et al., 2010; Lewis et al., 2020; Rooney et al., 2018).

A gene expression signature of 131 PPARα-dependent genes was built using microarray profiles from the livers of wild-type and PPARα-null mice. A quantitative measure of this expression signature would be a measure of similarity/correlation between the PPARα signature and positive and negative test sets is provided by the Running Fisher test (Kupershmidt et al., 2010; Rooney et al., 2018; Corton et al., 2020).

For all substances, MIE activation does not rise monotonically over dose or time. These fluctuations are likely due to variations in cofactor availability or access to the site of transcription (Gaillard et al., 2006; Koppen et al., 2009; Kupershmidt et al., 2010; Ong et al., 2010; Chow et al., 2011; De Vos et al., 2011; Simon et al., 2015).

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Measurements of PPARα Activation

Method/Test	Test Principle	Test Environment	Test Outcome	Assay Type/Domain
molecular modelling; docking simulation	Computational simulation of ligand binding	In silico	Prediction off binding interaction	Quantitative virtual screeings
Scintillation proximity binding assay	Direct binding of ligand	In vitro	Identifies compouds that bind to PPARα	Qualitative in vitro screening
PPARα reporter gene assay	Quantify changes in in PPARα activation via a sensitive surrogate	In vitro, Ex vivo	Measures changes in activity of genes linked to a PPARα receptor element	Quantitative in vitro screening
Electrophoretic Band Shift	determines if a protein or protein mixture will bind to a specific DNA or RNA sequence	In vitro	Measures cofactor binding by changes in gel mobility	Quantitative in vitro screening
Microarray profiling	Develop MIE-specific sets of gene expression biomarkers	In vivo	Classification of PPARα biomarker genes with statistical methods	Quantitative in vivo screening

References

Bhattacharya, Nandini, Jannette M Dufour, My-Nuong Vo, Janice Okita, Richard Okita, and Kwan Hee Kim. 2005. “Differential Effects of Phthalates on the Testis and the Liver.” Biology of Reproduction 72 (3) (March): 745–54. doi:10.1095/biolreprod.104.031583.

Bility, Moses T, Jerry T Thompson, Richard H McKee, Raymond M David, John H Butala, John P Vanden Heuvel, and Jeffrey M Peters. 2004. “Activation of Mouse and Human Peroxisome Proliferator-Activated Receptors (PPARs) by Phthalate Monoesters.” Toxicological Sciences : An Official Journal of the Society of Toxicology 82 (1) (November): 170–82. doi:10.1093/toxsci/kfh253.

Chow, C. C., Ong, K. M., Dougherty, E. J., & Simons, S. S. (2011). Inferring mechanisms from dose-response curves. Methods Enzymol, 487, 465-483. https://doi.org/10.1016/B978-0-12-381270-4.00016-0

Corton, J. C., Hill, T., Sutherland, J. J., Stevens, J. L., & Rooney, J. (2020). A Set of Six Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. Toxicol Sci. https://doi.org/10.1093/toxsci/kfaa101

De Vos, D., Bruggeman, F. J., Westerhoff, H. V., & Bakker, B. M. (2011). How molecular competition influences fluxes in gene expression networks. PLoS One, 6(12), e28494. https://doi.org/10.1371/journal.pone.0028494

Dufour, Jannette M, My-Nuong Vo, Nandini Bhattacharya, Janice Okita, Richard Okita, and Kwan Hee Kim. 2003. “Peroxisome Proliferators Disrupt Retinoic Acid Receptor Alpha Signaling in the Testis.” Biology of Reproduction 68 (4) (April): 1215–24. doi:10.1095/biolreprod.102.010488.

Feige, Jérôme N, Laurent Gelman, Daniel Rossi, Vincent Zoete, Raphaël Métivier, Cicerone Tudor, Silvia I Anghel, et al. 2007. “The Endocrine Disruptor Monoethyl-Hexyl-Phthalate Is a Selective Peroxisome Proliferator-Activated Receptor Gamma Modulator That Promotes Adipogenesis.” The Journal of Biological Chemistry 282 (26) (June 29): 19152–66. doi:10.1074/jbc.M702724200.

Gaillard, S., Grasfeder, L. L., Haeffele, C. L., Lobenhofer, E. K., Chu, T.-M., Wolfinger, R., Kazmin, D., Koves, T. R., Muoio, D. M., Chang, C.-y., & McDonnell, D. P. (2006). Receptor-selective coactivators as tools to define the biology of specific receptor-coactivator pairs. Mol Cell, 24(5), 797-803. https://doi.org/10.1016/j.molcel.2006.10.012

Hill, T., Rooney, J., Abedini, J., El-Masri, H., Wood, C. E., & Corton, J. C. (2020). Gene Expression Thresholds Derived From Short-Term Exposures Identify Rat Liver Tumorigens. Toxicol Sci. https://doi.org/10.1093/toxsci/kfaa102

Hurst, Christopher H, and David J Waxman. 2003. “Activation of PPARalpha and PPARgamma by Environmental Phthalate Monoesters.” Toxicological Sciences : An Official Journal of the Society of Toxicology 74 (2) (August): 297–308. doi:10.1093/toxsci/kfg145.

Igarashi, Y., Nakatsu, N., Yamashita, T., Ono, A., Ohno, Y., Urushidani, T., & Yamada, H. (2015). Open TG-GATEs: a large-scale toxicogenomics database. Nucleic Acids Res, 43(Database issue), D921-7. https://doi.org/10.1093/nar/gku955

Kamata S, Oyama T, Saito K, Honda A, Yamamoto Y, Suda K, Ishikawa R, Itoh T, Watanabe Y, Shibata T, Uchida K, Suematsu M, Ishii I. PPARα Ligand-Binding Domain Structures with Endogenous Fatty Acids and Fibrates. iScience. 2020;23(11):101727. 10.1016/j.isci.2020.101727

Kaya, Taner, Scott C Mohr, David J Waxman, and Sandor Vajda. 2006. “Computational Screening of Phthalate Monoesters for Binding to PPARgamma.” Chemical Research in Toxicology 19 (8) (August): 999–1009. doi:10.1021/tx050301s.

Koppen, A., Houtman, R., Pijnenburg, D., Jeninga, E. H., Ruijtenbeek, R., & Kalkhoven, E. (2009). Nuclear receptor-coregulator interaction profiling identifies TRIP3 as a novel peroxisome proliferator-activated receptor gamma cofactor. Mol Cell Proteomics, 8(10), 2212-2226. https://doi.org/10.1074/mcp.M900209-MCP200

Kupershmidt, I., Su, Q. J., Grewal, A., Sundaresh, S., Halperin, I., Flynn, J., Shekar, M., Wang, H., Park, J., Cui, W., Wall, G. D., Wisotzkey, R., Alag, S., Akhtari, S., & Ronaghi, M. (2010). Ontology-based meta-analysis of global collections of high-throughput public data. PLoS One, 5(9). https://doi.org/10.1371/journal.pone.0013066

Lampen, Alfonso, Susan Zimnik, and Heinz Nau. 2003. “Teratogenic Phthalate Esters and Metabolites Activate the Nuclear Receptors PPARs and Induce Differentiation of F9 Cells.” Toxicology and Applied Pharmacology 188 (1) (April): 14–23. doi:10.1016/S0041-008X(03)00014-0.

Lapinskas, Paula J., Sherri Brown, Lisa M. Leesnitzer, Steven Blanchard, Cyndi Swanson, Russell C. Cattley, and J. Christopher Corton. 2005. “Role of PPARα in Mediating the Effects of Phthalates and Metabolites in the Liver.” Toxicology 207 (1): 149–163.

Le Maire, Albane, Marina Grimaldi, Dominique Roecklin, Sonia Dagnino, Valérie Vivat-Hannah, Patrick Balaguer, and William Bourguet. 2009. “Activation of RXR-PPAR Heterodimers by Organotin Environmental Endocrine Disruptors.” EMBO Reports 10 (4) (April): 367–73. doi:10.1038/embor.2009.8.

LeBlanc, GA, DO Norris, and W Kloas. 2011. “Detailed Review Paper State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors” (178).

Lefebvre, Philippe, Giulia Chinetti, Jean-Charles Fruchart, and Bart Staels. 2006. “Sorting out the Roles of PPAR Alpha in Energy Metabolism and Vascular Homeostasis.” The Journal of Clinical Investigation 116 (3) (March): 571–80. doi:10.1172/JCI27989.

Lewis, R. W., Hill, T., & Corton, J. C. (2020). A set of six Gene expression biomarkers and their thresholds identify rat liver tumorigens in short-term assays. Toxicology, 443, 152547. https://doi.org/10.1016/j.tox.2020.152547

Maloney, Erin K., and David J. Waxman. 1999. “Trans-Activation of PPARα and PPARγ by Structurally Diverse Environmental Chemicals.” Toxicology and Applied Pharmacology 161 (2): 209–218.

Michalik, Liliane, Johan Auwerx, Joel P Berger, V Krishna Chatterjee, Christopher K Glass, Frank J Gonzalez, Paul A Grimaldi, et al. 2006. “International Union of Pharmacology. LXI. Peroxisome Proliferator-Activated Receptors.” Pharmacological Reviews 58 (4) (December): 726–41. doi:10.1124/pr.58.4.5.

Mukherjee, R, L Jow, G E Croston, and J R Paterniti. 1997. “Identification, Characterization, and Tissue Distribution of Human Peroxisome Proliferator-Activated Receptor (PPAR) Isoforms PPARgamma2 versus PPARgamma1 and Activation with Retinoid X Receptor Agonists and Antagonists.” The Journal of Biological Chemistry 272 (12) (March 21): 8071–6.

Ong, K. M., Blackford, J. A., Kagan, B. L., Simons, S. S., & Chow, C. C. (2010). A theoretical framework for gene induction and experimental comparisons. Proc Natl Acad Sci U S A, 107(15), 7107-7112. https://doi.org/10.1073/pnas.0911095107

Rooney, J., Hill, T., Qin, C., Sistare, F. D., & Corton, J. C. (2018). Adverse outcome pathway-driven identification of rat liver tumorigens in short-term assays. Toxicol Appl Pharmacol, 356, 99-113. https://doi.org/10.1016/j.taap.2018.07.023

Schultz, R, W Yan, J Toppari, A Völkl, J A Gustafsson, and M Pelto-Huikko. 1999. “Expression of Peroxisome Proliferator-Activated Receptor Alpha Messenger Ribonucleic Acid and Protein in Human and Rat Testis.” Endocrinology 140 (7) (July): 2968–75. doi:10.1210/endo.140.7.6858.

Simon, T. W., Budinsky, R. A., & Rowlands, J. C. (2015). A model for aryl hydrocarbon receptor-activated gene expression shows potency and efficacy changes and predicts squelching due to competition for transcription co-activators. PLoS One, 10(6), e0127952. https://doi.org/10.1371/journal.pone.0127952.

Staels, B., J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, and J.-C. Fruchart. 1998. “Mechanism of Action of Fibrates on Lipid and Lipoprotein Metabolism.” Circulation 98 (19) (November 10): 2088–2093. doi:10.1161/01.CIR.98.19.2088.

Svoboda, D. L., Saddler, T., & Auerbach, S. S. (2019). An Overview of National Toxicology Program’s Toxicogenomic Applications: DrugMatrix and ToxFX. In Advances in Computational Toxicology (pp. 141-157). Springer. https://link.springer.com/chapter/10.1007/978-3-030-16443-0_8

ToxCastTM Data. “ToxCastTM Data.” US Environmental Protection Agency. http://www.epa.gov/ncct/toxcast/data.html

Vanden Heuvel, John P, Jerry T Thompson, Steven R Frame, and Peter J Gillies. 2006. “Differential Activation of Nuclear Receptors by Perfluorinated Fatty Acid Analogs and Natural Fatty Acids: A Comparison of Human, Mouse, and Rat Peroxisome Proliferator-Activated Receptor-Alpha, -Beta, and -Gamma, Liver X Receptor-Beta, and Retinoid X Rec.” Toxicological Sciences : An Official Journal of the Society of Toxicology 92 (2) (August): 476–89. doi:10.1093/toxsci/kfl014.

Venkata, Nagaraj Gopisetty, Jodie a Robinson, Peter J Cabot, Barbara Davis, Greg R Monteith, and Sarah J Roberts-Thomson. 2006. “Mono(2-Ethylhexyl)phthalate and Mono-N-Butyl Phthalate Activation of Peroxisome Proliferator Activated-Receptors Alpha and Gamma in Breast.” Toxicology Letters 163 (3) (June 1): 224–34. doi:10.1016/j.toxlet.2005.11.001.

Viswakarma, Navin, Yuzhi Jia, Liang Bai, Aurore Vluggens, Jayme Borensztajn, Jianming Xu, and Janardan K Reddy. 2010. “Coactivators in PPAR-Regulated Gene Expression.” PPAR Research 2010 (January). doi:10.1155/2010/250126.

Wahli, Walter, and B Desvergne. 1999. “Peroxisome Proliferator-Activated Receptors: Nuclear Control of Metabolism.” Endocrine Reviews 20 (5) (October): 649–88. Wu, Bin, Jie Gao, and Ming-wei Wang. 2005. “Development of a Complex Scintillation Proximity Assay for High-Throughput Screening of PPARgamma Modulators.” Acta Pharmacologica Sinica 26 (3) (March): 339–44. doi:10.1111/j.1745-7254.2005.00040.x.

Xu, Chuan, Ji-An Chen, Zhiqun Qiu, Qing Zhao, Jiaohua Luo, Lan Yang, Hui Zeng, et al. 2010. “Ovotoxicity and PPAR-Mediated Aromatase Downregulation in Female Sprague-Dawley Rats Following Combined Oral Exposure to Benzo[a]pyrene and Di-(2-Ethylhexyl) Phthalate.” Toxicology Letters 199 (3) (December 15): 323–32. doi:10.1016/j.toxlet.2010.09.015.

Yessoufou, a, and W Wahli. 2010. “Multifaceted Roles of Peroxisome Proliferator-Activated Receptors (PPARs) at the Cellular and Whole Organism Levels.” Swiss Medical Weekly 140 (September) (January): w13071. doi:10.4414/smw.2010.13071.

Yu, Songtao, and Janardan K Reddy. 2007. “Transcription Coactivators for Peroxisome Proliferator-Activated Receptors.” Biochimica et Biophysica Acta 1771 (8) (August): 936–51. doi:10.1016/j.bbalip.2007.01.008.

List of Key Events in the AOP

Event: 807: Decreased, cholesterol

Short Name: Decreased, cholesterol

Key Event Component

Process	Object	Action
cholesterol biosynthetic process	cholesterol	decreased
cholesterol transport	cholesterol	decreased
cholesterol transport	cholesteryl ester	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:124 - HMG-CoA reductase inhibition leading to decreased fertility	KeyEvent
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	KeyEvent

Stressors

Name
Gemfibrozil
Bezafibrate
Clofibrate
Fenofibrate
Atorvastatin
Simvastatin

Biological Context

Level of Biological Organization

Tissue

Organ term

Organ term

blood plasma

Evidence for Perturbation by Stressor

Gemfibrozil

Juvenile female rainbow trout have decreased cholesterol (including total, HDL, LDL, & VLDL) after exposure to gemfibrozil (Prindiville et al. 2011)

Male and female zebrafish fed gemfibrozil alone or in combination with atorvastatin have decreased cholesterol (Al-Habsi et al. 2016)

Bezafibrate

Adult male zebrafish fed bezafibrate have decreased cholesterol (Velasco-Santamaría et al. 2011)

Clofibrate

Feeding grass carp either a high-fat or high-carbohydrate diet causes increases in total cholesterol, HDL, and LDL. Clofibrate reduces the high cholesterol levels caused by these diets to levels similar to controls (Guo et al. 2015)

Fenofibrate

Feeding fenofibrate to grass carp on a high fat diet causes a decrease in cholesterol, LDL, body weight, and whole-body lipid content (Du et al. 2008)

Atorvastatin

Male and female zebrafish fed atorvastatin alone or in combination with gemfibrozil have decreased whole-body cholesterol (Al-Habsi et al., 2016)

Atorvastatin is a statin drug that lowers cholesterol by inhibiting HMG-CoA reductase. Other chemical that work by the same mechanism can be found at: https://comptox.epa.gov/dashboard/chemical_lists/STATINS

Simvastatin

Larval Zebrafish fed a high fat and high cholesterol diet show reduced liver cholesterol when given simvastatin (Dai et al., 2015)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High
All life stages	Moderate

Sex Applicability

Sex	Evidence

Sex	Evidence
Male	High
Female	High

Taxonomic Applicability: Cholesterol is synthesized in plants but acts as a precursor for different products than in animals (Sonawane et al. 2016). Within the animal kingdom most deuterostomes (including vertebrata, cyclostomata, cephalochordate, and echinodermata, but not chordata) possess the genes necessary for cholesterol biosynthesis. However, most protostomes (including arthropoda and nematomorpha) have lost these genes (Zhang et al., 2019). Thus far vertebrates are the primary consideration for this KE.

Lifestage Applicability: Cholesterol can be measured in organisms at all life stages. However, the size of young organisms may limit the ability to collect plasma for cholesterol analysis. Whole-body measurements or pooled samples may be more feasible.

Sex Applicability: Cholesterol measurements are applicable for all sexes

Key Event Description

Most cholesterol synthesis in vertebrates occurs within the endoplasmic reticulum of hepatic cells. First, acetyl-CoA is converted to HMG-CoA via HMG-CoA synthase. Next, HMG-CoA is converted to mevalonate via HMG-CoA reductase. Several other steps follow, but conversion of HMG-CoA to mevalonate is the rate-limiting step of cholesterol synthesis (Cerqueira et al. 2016; Risley 2002). Consequently, Statin drugs inhibit HMG-CoA reductase to reduce cholesterol (Pahan 2006).

Cholesterol synthesis may also occur to a limited extent in steroidogenic cells where it's used to produce steroid hormones (Azhar et al., 2007)

Once cholesterol is produced in the liver, it's transported in the plasma. Hydrophobic lipids like cholesterol, cholesteryl ester (a cholesterol molecule bound to a fatty acid), and triglycerides are transported via lipoprotein complexes. There are different groups of lipoproteins which use different proteins and ratios of lipids including high-density lipoprotein (HDL), low-density (LDL), and very low-density (VLDL).

Cholesterol metabolism KEGG Pathway ko04979

How it is Measured or Detected

Commerical assay kits are available for measuring cholesterol using either colorimetric or fluorometric detection. Total cholesterol assay kits often include cholesteryl esters in the measurement (Cell Bio Labs, ThermoFisher). Additional kits are availalbe for measuring the cholesterol in the different lipoprotein complexes (Cell Bio Labs).

Oil Red O staining can be used for organisms such as zebrafish larvae that are clear, however it stains triglycerides and lipids not just cholesterol (Zhou et al., 2015).

Plasma cholesterol is a common clinical measurement in humans and the Abell-Kendall technique is the standard chemical determination method (Cox et al. 1990), although there are a wide variety of viable methods.

References

Al-Habsi, A.A., A. Massarsky, T.W. Moon (2016) "Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (*Danio rerio*)", *Comparative Biochemistry and Physiology, Part B,* Vol. 199, Elsevier, pp. 87-96. http://dx.doi.org/10.1016/j.cbpb.2015.11.009

Azhar, S., E. Reaven (2007) "Regulation of Leydig cell cholesterol metabolism", in A.H. Payne, M.P. Hardy (eds.) *The Leydig Cell in Health and Disease,* Humana Press. https://doi.org/10.1007/978-1-59745-453-7

Cox RA, García-Palmieri MR. Cholesterol, Triglycerides, and Associated Lipoproteins. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 31. Available from: https://www.ncbi.nlm.nih.gov/books/NBK351/

Dai, W. et al. (2015) "High fat plus high cholesterol diet lead to hepatic steatosis in zebrafish larvae: a novel model for screening anti-hepatic steatosis drugs", *Nutrition and Metabolism*, Vol. 12(42), Springer Nature. DOI 10.1186/s12986-015-0036-z

Du, Z.Y. et al. (2008) "Hypolipidaemic effect of fenofibrate and fasting in the herbivorous grass carp (*Ctenopharyngodon idella)* fed a high-fat diet", *British Journal of Nutrition,* Vol. 100, Cambridge University Press, pp. 1200-1212. doi:10.1017/S0007114508986840

Guo, X. et al. (2015) "Effects of lipid-lowering pharmaceutical clofibrate on lipid and lipoprotein metabolism of grass carp (*Ctenopharyngodon idellal* Val.) fed with the high non-protein energy diets", *Fish Physiology and Biochemistry,* Vol. 41, Springer, pp. 331-343. doi: 10.1007/s10695-014-9986-8

Cerqueira, N. M., Oliveira, E. F., Gesto, D. S., Santos-Martins, D., Moreira, C., Moorthy, H. N., ... & Fernandes, P. A. (2016).

Cholesterol biosynthesis: a mechanistic overview. *Biochemistry*, *55*(39), 5483-5506.

Prindiville, J.S. et al. (2011) “The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout”, *Toxicology and Applied Pharmacology,* Vol. 251, Elsevier, pp. 201-238. doi:10.1016/j.taap.2010.12.013

Pahan, K. (2006). Lipid-lowering drugs. *Cellular and molecular life sciences CMLS*, *63*(10), 1165-1178.

Risley, J. M. (2002). Cholesterol biosynthesis: Lanosterol to cholesterol. *Journal of chemical education*, *79*(3), 377.

Sonawane, P.D. et al. (2016) “Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism”, *Nature Plants,* Vol. 3, Nature Publishing Group, https://doi.org/10.1038/nplants.2016.205

Velasco-Santamaría, Y.M. et al. (2011) “Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (*Danio rerio*)”, *Aquatic Toxicology,* Vol. 105, Elsevier, pp. 107-118. doi:10.1016/j.aquatox.2011.05.018

Zhang, T. et al. (2019) “Evolution of the cholesterol biosynthesis pathway in animals”, *Molecular Biology and Evolution,* Vol. 36(11), Oxford University Press, pp. 2548-2556. doi:10.1093/molbev/msz167

Zhou, J. et al. (2015) "Rapid analysis of hypolipidemic drugs in a live zebrafish assay", *Journal of Pharmacological and Toxicological Methods,* Vol. 72, Elsevier, pp. 47-52. http://dx.doi.org/10.1016/j.vascn.2014.12.002

Event: 1756: Decreased, plasma 11-ketotestosterone level

Short Name: Decreased, 11KT

Key Event Component

Process	Object	Action
androgen biosynthetic process	11-Keto-testosterone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	KeyEvent
Aop:349 - Inhibition of 11β-hydroxylase leading to decresed population trajectory	KeyEvent
Aop:348 - Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	KeyEvent

Stressors

Name
beta-Sitosterol
Bezafibrate
Gemfibrozil
Bis(2-ethylhexyl) phthalate
Cypermethrin
Carbamazepine

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term

blood plasma
~~Organ term~~

Evidence for Perturbation by Stressor

beta-Sitosterol

Beta-sitosterol causes a dose-depended reduction in 11KT in male goldfish (MacLatchy & Van Der Kraak 1995)

Bezafibrate

Bezafibrate reduces 11-KT in the plasma of adult male zebrafish (Velasco-Santamaría et al. 2011)

Gemfibrozil

Gemfibrozil reduced 11KT in the plasma of adult male medaka (Lee et al. 2019)

Gemfibrozil expsoure caused reduced 11KT in the testes, plasma, and whole-body samples of adult male zebrafish (Fraz et al., 2018)

Bis(2-ethylhexyl) phthalate

A review of androgen signaling in fish cites several studies showing DEHP decreased 11KT (Golshan et al., 2019)

Cypermethrin

Cypermethrin causes decreased 11KT in catfish (Singh & Singh, 2008)

Carbamazepine

Carbamazepine decreased 11KT in the testes, plasma, and whole-body samples of adult male zebrafish (Fraz et al., 2018)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI
Order carcharhiniformes	carcharhiniformes	Moderate	NCBI
mammals	mammals	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
Juvenile	Moderate
Adult, reproductively mature	High
Larvae	Moderate

Sex Applicability

Sex	Evidence
Male	High
Female	High

Taxanomic Applicability: Most understand of 11KT comes from studies involving teleost fish as it is their dominant androgen. Some studies have measured 11KT in sharks of the order carcharhiniformes, but there is less research in this area (Manire et al., 1999; Garnier et al. 1999; Mills et al. 2010). Many mammals possess the genes necessary to produce 11KT (NCBI), but 11KT may not be as relevant when it's not the dominant androgen.

Sex Applicability: Males and females use the same biological processes to produce steroids. However, sexual dimorphism in 11KT production varies between species. In humans, plasma levels of 11KT do not differ between sexes (Imamichi et al., 2016). In Zebrafish, gonad levels of 11KT are approximately two magnitudes higher in males than females (Wang & Orban, 2007). Of the 30 other fish species sampled by Lokman et al. (2002), 11KT levels are typically dramatically lower in females than in males, but a few

species of the order Perciformes show no sexual dimorphism.

Life Stage Applicability: 11KT can be measured in fish larvae however individuals must be pooled for sufficient sample size (Hattori et al., 2009). Lokman et al. (2002) measured plasma levels of 11-KT in several species of juvenile and adult fish. 11KT levels tend to be higher in males although some fish species don't show sexual dimorphism. Levels of 11KT in juveniles are similar to levels in females regardless of if the species shows sexual dimorphism in 11KT levels. In males, 11KT increases for spawning and decreases afterwards (Kindler et al., 1989; Páll et al., 2002). Because of it's involvement in reproduction, 11KT levels may not be meaningful in juveniles.

Key Event Description

11-ketotestosterone (11KT; CAS 564-35-2 | DTXSID8036499) is an oxygenated steroidal androgen with a keto group at the C11 position (Pretorius et al. 2017).

11-ketotestosterone is a dominant androgen in teleost fish (Borg 1994). It is synthesized from testosterone using the enzymes CYP11b1 and HSD11b (Yazawa et al., 2008; Swart et al., 2013). Zebrafish studies also show that cyp17a1 and cyp11c1 knockouts have dramatically reduced levels of 11KT (Shu et al., 2020; Zhang et al., 2020)

11KT is also produced by other vertebrates, although the site of its biosynthesis and physiological signficance in different taxa can vary widely. In humans, 11KT is primarily synthesized in the adrenal glands (Pretorius et al. 2017; Turcu et al. 2018).

Although mutations in the *mettl3* gene usually cause embryonic lethality, one particular mutation in non-lethal and causes significantly reduced 11KT levels in zebrafish (Xia et al., 2018)

How it is Measured or Detected

11KT production can be measured in an ex vivo steroidogenesis assay using the organism's gonad after it has been exposed to a compound.

The concentration of 11KT can be measured in a radioimmunoassay or enzyme-linked immunosorbent assay (ELISA).

Several papers show that in fish, 11KT is correlated with testosterone levels (Spanò et al., 2004; Maclatchy & Vanderkraak, 1995; Lorenzi et al., 2008).

References

Borg, B. (1994). Androgens in teleost fishes. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *109*(3), 219-245.

Fraz, S. et al. (2018) "Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (*Danio rerio*)", *Aquatic Toxicology*, Vol. 198, Elsevier, pp. 1-9. https://doi.org/10.1016/j.aquatox.2018.02.006

Golshan, M. & S.M.H. Alvai (2019) "Androgen signaling in male fishes: Examples of anti-androgenic chemicals that cause reproductive disorders", *Theriogenology*, Vol. 139, Elsevier, pp. 58-71. https://doi.org/10.1016/j.theriogenology.2019.07.020

Hattori, R.S. et al. (2009) "Cortisol-induced masculinization: Does thermal stress affect gonadal fate in pejerrey, a teleost fish with temperature-dependent sex determination?", *PLoS ONE*, Vol. 4(8), pp. 1-7. doi:10.1371/journal.pone.0006548

Imamichi, Y. et al. (2016) "11-Ketotestosterone is a major androgen produced in human gonads", *The Journal of Clinical Endocrinology & Metabolism,* Vol. 101(10), Oxford Academic, pp. 3582-3591. https://doi.org/10.1210/jc.2016-2311

Kindler, P. M. et al. (1989) "Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus:* Centrarchidae)", *General and Comparative Endocrinology,* Vol. 75(3), Elsevier, pp. 446-453. https://doi.org/10.1016/0016-6480(89)90180-9

Lee, G. et al. (2019) "Effects of gemfibrozil on sex hormones and reproduction related performances of *Oryzias latipes* following long-term (155 d) and short-term (21 d) exposure", *Ecotoxicology and Environmental Safety,* Vol. 173, Elsevier, pp. 174-181. https://doi.org/10.1016/j.ecoenv.2019.02.015

Lokman, P.M. et al. (2002) "11-Oxygenated androgens in female teleosts: prevalence, abundance, and life history implications", *General and Comparative Endocrinology,* Vol. 129, Academic Press, pp. 1-12. doi: 10.1016/s0016-6480(02)00562-2

Lorenzi, V. et al. (2008) "Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone, and 17β-estradiol in the bluebanded goby (*Lythrypnus dalli)*", *General and Comparative Endocrinology,* Vol. 155(2)., Elsevier, pp. 438-446. https://doi.org/10.1016/j.ygcen.2007.07.010

MacLatchy, D.L. and G.J. Vanderkraak (1995) "The phytoestrogen β-sitosterol alters the reproductive endocrine status of goldfish", *Toxicology and Applied Pharmacology,* Vol. 134(2), Elsevier, pp. 305-312. https://doi.org/10.1006/taap.1995.1196

Manire, C.A., L.E. Rasmussen & T.S. Gross (1999) “Serum steroid hormones including 11-ketotestosterone, 11-ketoandrostenedione, and dihydroprogesterone in juvenile and adult bonnethead sharks, *Sphyrna tiburo*”, *Journal of Experimental Zoology*, Vol. 284(5), Wiley-Blackwell, pp. 595-603. DOI: 10.1002/(sici)1097-010x(19991001)284:5<595::aid-jez15>3.0.co

Páll, M. K., I. Mayer and B. Borg (2002) “Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus* l. – Changes in 11-ketotestosterone levels during nesting cycle”, *Hormones and Behavior,* Vol. 41(4), Elsevier, pp. 377-383. https://doi.org/10.1006/hbeh.2002.1777

Pretorius, E, Arlt, W & Storbeck, K-H 2016, 'A new dawn for androgens: novel lessons from 11-oxygenated C19 steroids', Molecular and Cellular Endocrinology. https://doi.org/10.1016/j.mce.2016.08.014

Shu, T. et al. (2020) “Zebrafish cyp17a1 knockout reveals that androgen-mediated signaling is important for male brain sex differentiation”, *General and Comparative Endocrinology*, Vol. 295. doi:10.1016/j.ygcen.2020.113490

Singh, P.B. & V. Singh (2008) “Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17beta and 11-ketotestosterone, and sperm motility in *Heteropneustes fossilis* (Bloch)”, *Chemosphere*, Vol. 72(3), Elsevier, pp. 422-431. DOI: 10.1016/j.chemosphere.2008.02.026

Spanó, L. et al. (2004) “Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*)”, *Aquatic Toxicology,* Vol. 66(4), Elsevier, pp. 369-379. https://doi.org/10.1016/j.aquatox.2003.10.009

Swart, A.C. et al. (2013) “11β-hydroxyandrostenedione, the product of androstenedione metabolism in the adrenal, is metabolized in LNCaP cells by 5α-reductase yielding 11β-hydroxy-5α-androstanedione”, *The Journal of Steroid Biochemistry and Molecular Biology,* Vol 138, Elsevier, pp. 132-142. https://doi.org/10.1016/j.jsbmb.2013.04.010

Turcu AF, Nanba AT, Auchus RJ. The Rise, Fall, and Resurrection of 11-Oxygenated Androgens in Human Physiology and Disease. Horm Res Paediatr. 2018;89(5):284-291. doi: 10.1159/000486036. Epub 2018 May 9. PMID: 29742491; PMCID: PMC6031471.

Velasco-Santamaría, Y.M. et al. (2011) “Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (*Danio rerio*)”, *Aquatic Toxicology,* Vol. 105, Elsevier, pp. 107-118. doi:10.1016/j.aquatox.2011.05.018

Wang, X.G. and L. Orban (2007) “Anti-Müllerian hormone and 11β-hydroxylase show reciprocal expression to that of aromatase in the transforming gonad of zebrafish males”, *Developmental Dynamics,* Vol 236(5), Wiley-Liss, pp. 1329-1338. https://doi.org/10.1002/dvdy.21129

Xia, H. et al. (2018) “*Mettl3* mutation disrupts gamete maturation and reduced fertility in zebrafish”, *Genetics*, Vol. 208(2), Genetics Society of America, pp. 729-743. DOI: 10.1534/genetics.117.300574

Yazawa, T. (2008) “Cyp11b1 is induced in the murine gonad by luteinizing hormone/human chorionic gonadotropin and involved in the production of 11-ketotestosterone, a major fish androgen: Conservation and evolution of the androgen metabolic pathway”, *Endocrinology,* Vol. 149(4), Oxford Academy, pp. 1786-1792. https://doi.org/10.1210/en.2007-1015

Zheng, Q. et al. (2020) “Loss of cyp11c1 causes delayed spermatogenesis due to the absence of 11-ketotestosterone", *Journal of Endocrinology,* Vol. 244(3), Bioscientifica, pp. 487-499. https://doi.org/10.1530/JOE-19-0438

Event: 1758: Impaired, Spermatogenesis

Short Name: Impaired, Spermatogenesis

Key Event Component

Process	Object	Action
Abnormal spermatogenesis	Mature sperm cell	abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	KeyEvent
Aop:348 - Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	KeyEvent

Stressors

Name
Flutamide
Vinclozolin
Bis(2-ethylhexyl) phthalate

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
testis

Evidence for Perturbation by Stressor

Flutamide

Flutamide impairs spermatogenesis in adult male zebrafish (Yin et al., 2017)

Male fathead minnows exposed to flutamide show spermatocyte degredation and necrosis in their testis (Jensen et al., 2004)

Vinclozolin

A review of androgen signaling in male fish cites several studies showing vinclozolin decreases sperm quality (Golshan et al., 2019)

Bis(2-ethylhexyl) phthalate

A review of androgen signaling in male fish cites several studies showing DEHP decreases sperm quality (Golshan et al., 2019)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Male	High

Taxonomic Applicability: The relevance for invertebrates has not been evaluated.

Life Stage Applicability: Only applicable for sexually mature adults

Sex Applicability: Only applicable to males

Key Event Description

Spermatogenesis is a multiphase process of cellular transformation that produces mature male gametes known as sperm for sexual reproduction (Xu et al., 2015). The process of spermatogenesis can be broken down into 3 phases: the mitotic proliferation of spermatogonia, meiosis, and post-meiotic differentiation(spermiogenesis) (Boulanger et al., 2015). Spermatogenesis can be impaired within these phases or due to external factors such as chemical exposures or the

gonadal tissue environment. For example, zebrafish and fathead minnow exposed to flutamide, an antiandrogen, have shown signs of impaired spermatogenesis such as spermatocyte degradation(Jensen et al., 2004, Yin et al., 2017).

How it is Measured or Detected

Impairment of spermatogenesis can be measured and detected in a multitude of ways. One example of this is qualitative histological assessments (Jensen et al., 2004). Through histology, sperm morphology can be examined and quantified through the number and stage of the sperm. Sperm morphology, overall quantity, and quantity within each stage can be ways to detect impaired spermatogenesis(Uhrin et al., 2000, Xie et al., 2020). Additionally, sperm quality can also be another assessment of impaired spermatogenesis such as sperm motility, velocity, ATP content, and lipid peroxidation(Gage et al., 2004, Xia et al., 2018, Chen et al., 2015). Impaired spermatogenesis can also be seen by measuring sperm density(Chen et al., 2015).

References

Boulanger, G., Cibois, M., Viet, J., Fostier, A., Deschamps, S., Pastezeur, S., Massart, C., Gschloessl, B., Gautier-Courteille, C., & Paillard, L. (2015). Hypogonadism Associated with Cyp19a1 (Aromatase) Posttranscriptional Upregulation in Celf1 Knockout Mice. Molecular and cellular biology, 35(18), 3244–3253. https://doi.org/10.1128/MCB.00074-15

Chen, J., Xiao, Y., Gai, Z., Li, R., Zhu, Z., Bai, C., Tanguay, R. L., Xu, X., Huang, C., & Dong, Q. (2015). Reproductive toxicity of low level bisphenol A exposures in a two-generation zebrafish assay: Evidence of male-specific effects. Aquatic toxicology (Amsterdam, Netherlands), 169, 204–214. https://doi.org/10.1016/j.aquatox.2015.10.020

Golshan, M. & S.M.H. Alvai (2019) “Androgen signaling in male fishes: Examples of anti-androgenic chemicals that cause reproductive disorders”, Theriogenology, Vol. 139, Elsevier, pp. 58-71. https://doi.org/10.1016/j.theriogenology.2019.07.020

Jensen, K.M. et al. (2004) “Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow”, Aquatic Toxicology, Vol. 70(2), Elsevier, pp. 99-110. https://doi.org/10.1016/j.aquatox.2004.06.012

Uhrin, P., Dewerchin, M., Hilpert, M., Chrenek, P., Schöfer, C., Zechmeister-Machhart, M., Krönke, G., Vales, A., Carmeliet, P., Binder, B. R., & Geiger, M. (2000). Disruption of the protein C inhibitor gene results in impaired spermatogenesis and male infertility. The Journal of clinical investigation, 106(12), 1531–1539. https://doi.org/10.1172/JCI10768

Xia, H., Zhong, C., Wu, X., Chen, J., Tao, B., Xia, X., Shi, M., Zhu, Z., Trudeau, V. L., & Hu, W. (2018). Mettl3 Mutation Disrupts Gamete Maturation and Reduces Fertility in Zebrafish. Genetics, 208(2), 729–743. https://doi.org/10.1534/genetics.117.300574

Xie, H., Kang, Y., Wang, S., Zheng, P., Chen, Z., Roy, S., & Zhao, C. (2020). E2f5 is a versatile transcriptional activator required for spermatogenesis and multiciliated cell differentiation in zebrafish. PLoS genetics, 16(3), e1008655. https://doi.org/10.1371/journal.pgen.1008655

Xu, K., Wen, M., Duan, W., Ren, L., Hu, F., Xiao, J., Wang, J., Tao, M., Zhang, C., Wang, J., Zhou, Y., Zhang, Y., Liu, Y., & Liu, S. (2015). Comparative analysis of testis transcriptomes from triploid and fertile diploid cyprinid fish. Biology of reproduction, 92(4), 95. https://doi.org/10.1095/biolreprod.114.125609

Yin, P. et al. (2017) “Diethylstilbestrol, flutamide and their combination impaired the spermatogenesis of male adult zebrafish through disrupting HPG axis, meiosis and apoptosis”, Aquatic Toxicology, Vol. 185, Elsevier, pp. 129-137. https://doi.org/10.1016/j.aquatox.2017.02.013

List of Adverse Outcomes in this AOP

Event: 2147: Decreased, Viable Offspring

Short Name: Decreased, Viable Offspring

Key Event Component

Process	Object	Action
sexual reproduction		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	AdverseOutcome

Biological Context

Level of Biological Organization

Individual

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Unspecific	

***Taxonomic applicability*:** Decrease in viable offspring may have relevance for species with sexual reproduction, including fish, mammals, amphibians, reptiles, birds, and invertebrates.

Life stage applicability: Decrease in viable offspring is relevant for reproductively mature individuals.

Sex applicability: Decrease in viable offspring can be measured for both males and females.

Key Event Description

The production of viable offspring in sexual reproduction is through fertilization of oocytes that then develop into offspring. Producing viable offspring is dependent on multiple factors, including but not limited to, oocyte maturation and ovulation, spermatogenesis and sperm production, successful fertilization of oocytes, development including successful organogenesis, and adequate nutrition.

How it is Measured or Detected

Effects on the production of viable offspring is measured or detected through the ability (or inability) of reproductively mature organisms to produce offspring, number of offspring produced (per pair, individual, or population), and/or percent of fertilized, viable embryos.

Event: 360: Decrease, Population growth rate

Short Name: Decrease, Population growth rate

Key Event Component

Process	Object	Action
population growth rate	population of organisms	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish)	AdverseOutcome
Aop:25 - Aromatase inhibition leading to reproductive dysfunction	AdverseOutcome
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction	AdverseOutcome
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction	AdverseOutcome
Aop:100 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of female spawning behavior	AdverseOutcome

AOP ID and Name	Event Type
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation	AdverseOutcome
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription	AdverseOutcome
Aop:155 - Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:101 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of pheromone release	AdverseOutcome
Aop:102 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with meiotic prophase I /metaphase I transition	AdverseOutcome
Aop:63 - Cyclooxygenase inhibition leading to reproductive dysfunction	AdverseOutcome
Aop:103 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with spindle assembly checkpoint	AdverseOutcome
Aop:292 - Inhibition of tyrosinase leads to decreased population in fish	AdverseOutcome
Aop:310 - Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR	AdverseOutcome
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality	AdverseOutcome
Aop:312 - Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement	AdverseOutcome
Aop:334 - Glucocorticoid Receptor Agonism Leading to Impaired Fin Regeneration	AdverseOutcome
Aop:336 - DNA methyltransferase inhibition leading to population decline (1)	AdverseOutcome
Aop:337 - DNA methyltransferase inhibition leading to population decline (2)	AdverseOutcome
Aop:338 - DNA methyltransferase inhibition leading to population decline (3)	AdverseOutcome
Aop:339 - DNA methyltransferase inhibition leading to population decline (4)	AdverseOutcome
Aop:340 - DNA methyltransferase inhibition leading to transgenerational effects (1)	AdverseOutcome
Aop:341 - DNA methyltransferase inhibition leading to transgenerational effects (2)	AdverseOutcome
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish	AdverseOutcome
Aop:297 - Inhibition of retinaldehyde dehydrogenase leads to population decline	AdverseOutcome
Aop:346 - Aromatase inhibition leads to male-biased sex ratio via impacts on gonad differentiation	AdverseOutcome
Aop:326 - Thermal stress leading to population decline (3)	AdverseOutcome
Aop:325 - Thermal stress leading to population decline (2)	AdverseOutcome
Aop:324 - Thermal stress leading to population decline (1)	AdverseOutcome
Aop:363 - Thyroperoxidase inhibition leading to altered visual function via altered retinal layer structure	AdverseOutcome
Aop:349 - Inhibition of 11β-hydroxylase leading to decresed population trajectory	AdverseOutcome
Aop:348 - Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	AdverseOutcome
Aop:376 - Androgen receptor agonism leading to male-biased sex ratio	AdverseOutcome
Aop:386 - Deposition of ionizing energy leading to population decline via inhibition of photosynthesis	AdverseOutcome
Aop:387 - Deposition of ionising energy leading to population decline via mitochondrial dysfunction	AdverseOutcome
Aop:388 - Deposition of ionising energy leading to population decline via programmed cell death	AdverseOutcome
Aop:389 - Oxygen-evolving complex damage leading to population decline via inhibition of photosynthesis	AdverseOutcome
Aop:364 - Thyroperoxidase inhibition leading to altered visual function via decreased eye size	AdverseOutcome
Aop:365 - Thyroperoxidase inhibition leading to altered visual function via altered photoreceptor patterning	AdverseOutcome
Aop:399 - Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	AdverseOutcome
Aop:410 - GSK3beta inactivation leading to increased mortality via defects in developing inner ear	AdverseOutcome

AOP ID and Name	Event Type
Aop:216 - Deposition of energy leading to population decline via DNA strand breaks and follicular atresia	AdverseOutcome
Aop:238 - Deposition of energy leading to population decline via DNA strand breaks and oocyte apoptosis	AdverseOutcome
Aop:299 - Deposition of energy leading to population decline via DNA oxidation and follicular atresia	AdverseOutcome
Aop:311 - Deposition of energy leading to population decline via DNA oxidation and oocyte apoptosis	AdverseOutcome
Aop:444 - Ionizing radiation leads to reduced reproduction in Eisenia fetida via reduced spermatogenesis and cocoon hatchability	AdverseOutcome
Aop:138 - Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:177 - Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:97 - 5-hydroxytryptamine transporter (5-HTT; SERT) inhibition leading to population decline	AdverseOutcome
Aop:203 - 5-hydroxytryptamine transporter inhibition leading to decreased reproductive success and population decline	AdverseOutcome
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity	AdverseOutcome
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior	AdverseOutcome
Aop:323 - PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	AdverseOutcome

Biological Context

Level of Biological Organization

Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Consideration of population size and changes in population size over time is potentially relevant to all living organisms.

Key Event Description

A population can be defined as a group of interbreeding organisms, all of the same species, occupying a specific space during a specific time (Vandermeer and Goldberg 2003, Gotelli 2008). As the population is the biological level of organization that is often the focus of ecological risk assessments, population growth rate (and hence population size over time) is important to consider within the context of applied conservation practices.

If N is the size of the population and t is time, then the population growth rate (dN/dt) is proportional to the instantaneous rate of increase, r, which measures the per capita rate of population increase over a short time interval. Therefore, r, is a difference between the instantaneous birth rate (number of births per individual per unit of time; b) and the instantaneous death rate (number of deaths per individual per unit of time; d) [Equation 1]. Because r is an instantaneous rate, its units can be changed via division. For example, as there are 24 hours in a day, an r of 24 individuals/(individual x day) is equal to an r of 1 individual/(individual/hour) (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020).

Equation 1: $r = b - d$

This key event refers to scenarios where $r < 0$ (instantaneous death rate exceeds instantaneous birth rate).

Examining r in the context of population growth rate:

- A population will decrease to extinction when the instantaneous death rate exceeds the instantaneous birth rate ($r < 0$).
- The smaller the value of r below 1, the faster the population will decrease to zero.
- A population will increase when resources are available and the instantaneous birth rate exceeds the instantaneous death rate ($r > 0$)
- The larger the value that r exceeds 1, the faster the population can increase over time
- A population will neither increase or decrease when the population growth rate equals 0 (either due to $N = 0$, or if the per capita birth and death rates are exactly balanced). For example, the per capita birth and death rates could become exactly balanced due to density dependence and/or to the effect of a stressor that reduces survival and/or reproduction (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020).

Effects incurred on a population from a chemical or non-chemical stressor could have an impact directly upon birth rate (reproduction) and/or death rate (survival), thereby causing a decline in population growth rate.

- Example of direct effect on r: Exposure to 17b-trenbolone reduced reproduction (i.e., reduced b) in the fathead minnow over 21 days at water concentrations ranging from 0.0015 to about 41 mg/L (Ankley et al. 2001; Miller and Ankley 2004).

Alternatively, a stressor could indirectly impact survival and/or reproduction.

- Example of indirect effect on r: Exposure of non-sexually differentiated early life stage fathead minnow to the fungicide prochloraz has been shown to produce male-biased sex ratios based on gonad differentiation, and resulted in projected change in population growth rate (decrease in reproduction due to a decrease in females and thus recruitment) using a population model. (Holbech et al., 2012; Miller et al. 2022)

Density dependence can be an important consideration:

- The effect of density dependence depends upon the quantity of resources present within a landscape. A change in available resources could increase or decrease the effect of density dependence and therefore cause a change in population growth rate via indirectly impacting survival and/or reproduction.
- This concept could be thought of in terms of community level interactions whereby one species is not impacted but a competitor species is impacted by a chemical stressor resulting in a greater availability of resources for the unimpacted species. In this scenario, the impacted species would experience a decline in population growth rate. The unimpacted species would experience an increase in population growth rate (due to a smaller density dependent effect upon population growth rate for that species).

Closed versus open systems:

- The above discussion relates to closed systems (there is no movement of individuals between population sites) and thus a declining population growth rate cannot be augmented by immigration.
- When individuals depart (emigrate out of a population) the loss will diminish population growth rate.

Population growth rate applies to all organisms, both sexes, and all life stages.

How it is Measured or Detected

Population growth rate (instantaneous growth rate) can be measured by sampling a population over an interval of time (i.e. from time t = 0 to time t = 1). The interval of time should be selected to correspond to the life history of the species of interest (i.e. will be different for rapidly growing versus slow growing populations). The population growth rate, r, can be determined by taking the difference (subtracting) between the initial population size, $N_{t=0}$ (population size at time t=0), and the population size at the end of the interval, $N_{t=1}$ (population size at time t = 1), and then subsequently dividing by the initial population size.

Equation 2: $r = (N_{t=1} - N_{t=0}) / N_{t=0}$

The diversity of forms, sizes, and life histories among species has led to the development of a vast number of field techniques for estimation of population size and thus population growth over time (Bookhout 1994, McComb et al. 2021).

- For stationary species an observational strategy may involve dividing a habitat into units. After setting up the units, samples are performed throughout the habitat at a select number of units (determined using a statistical sampling design) over a time interval (at time t = 0 and again at time t = 1), and the total number of organisms within each unit are counted. The numbers recorded are assumed to be representative for the habitat overall, and can be used to estimate the population growth rate within the entire habitat over the time interval.
- For species that are mobile throughout a large range, a strategy such as using a mark-recapture method may be employed (i.e. tags, bands, transmitters) to determine a count over a time interval (at time = 0 and again at time =1).

Population growth rate can also be estimated using mathematical model constructs (for example, ranging from simple differential equations to complex age or stage structured matrix projection models and individual based modeling approaches), and may assume a linear or nonlinear population increase over time (Caswell 2001, Vandermeer and Goldberg 2003, Gotelli 2008, Murray and Sandercock 2020). The AOP framework can be used to support the translation of pathway-specific mechanistic data into responses relevant to population models and output from the population models, such as changing (declining) population growth rate, can be used to assess and manage risks of chemicals (Kramer et al. 2011). As such, this translational capability can increase the capacity and efficiency of safety assessments both for single chemicals and chemical mixtures (Kramer et al. 2011).

Some examples of modeling constructs used to investigate population growth rate:

- A modeling construct could be based upon laboratory toxicity tests to determine effect(s) that are then linked to the population model and used to estimate decline in population growth rate. Miller et al. (2007) used concentration–response data from short term reproductive assays with fathead minnow (*Pimephales promelas*) exposed to endocrine disrupting chemicals in combination with a population model to examine projected alterations in population growth rate.

- A model construct could be based upon a combination of effects-based monitoring at field sites (informed by an AOP) and a population model. Miller et al. (2015) applied a population model informed by an AOP to project declines in population growth rate for white suckers (Catostomus commersoni) using observed changes in sex steroid synthesis in fish exposed to a complex pulp and paper mill effluent in Jackfish Bay, Ontario, Canada. Furthermore, a model construct could be comprised of a series of quantitative models using KERs that culminates in the estimation of change (decline) in population growth rate.

- A quantitative adverse outcome pathway (qAOP) has been defined as a mathematical construct that models the dose–response or response–response relationships of all KERs described in an AOP (Conolly et al. 2017, Perkins et al. 2019). Conolly et al. (2017) developed a qAOP using data generated with the aromatase inhibitor fadrozole as a stressor and then used it to predict potential population-level impacts (including decline in population growth rate). The qAOP modeled aromatase inhibition (the molecular initiating event) leading to reproductive dysfunction in fathead minnow (Pimephales promelas) using 3 computational models: a hypothalamus–pituitary–gonadal axis model (based on ordinary differential equations) of aromatase inhibition leading to decreased vitellogenin production (Cheng et al. 2016), a stochastic model of oocyte growth dynamics relating vitellogenin levels to clutch size and spawning intervals (Watanabe et al. 2016), and a population model (Miller et al. 2007).

- Dynamic energy budget (DEB) models offer a methodology that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of energy resources (Nisbet et al. 2000, Nisbet et al. 2011). Murphy et al. (2018) developed a conceptual model to link DEB and AOP models by interpreting AOP key events as measures of damage-inducing processes affecting DEB variables and rates.

- Endogenous Lifecycle Models (ELMs), capture the endogenous lifecycle processes of growth, development, survival, and reproduction and integrate these to estimate and predict expected fitness (Etterson and Ankley, 2021). AOPs can be used to inform ELMs of effects of chemical stressors on the vital rates that determine fitness, and to decide what hierarchical models of endogenous systems should be included within an ELM (Etterson and Ankley, 2021).

Regulatory Significance of the AO

Maintenance of sustainable fish and wildlife populations (i.e., adequate to ensure long-term delivery of valued ecosystem services) is a widely accepted regulatory goal upon which risk assessments and risk management decisions are based.

References

- Ankley GT, Jensen KM, Makynen EA, Kahl MD, Korte JJ, Hornung MW, Henry TR, Denny JS, Leino RL, Wilson VS, Cardon MD, Hartig PC, Gray LE. 2003. Effects of the androgenic growth promoter 17b-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. Environ. Toxicol. Chem. 22: 1350–1360.
- Bookhout TA. 1994. Research and management techniques for wildlife and habitats. The Wildlife Society, Bethesda, Maryland. 740 pp.
- Caswell H. 2001. Matrix Population Models. Sinauer Associates, Inc., Sunderland, MA, USA
- Cheng WY, Zhang Q, Schroeder A, Villeneuve DL, Ankley GT, Conolly R. 2016. Computational modeling of plasma vitellogenin alterations in response to aromatase inhibition in fathead minnows. Toxicol Sci 154: 78–89.
- Conolly RB, Ankley GT, Cheng W-Y, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. 2017. Quantitative adverse outcome pathways and their application to predictive toxicology. Environ. Sci. Technol. 51: 4661-4672.
- Etterson MA, Ankley GT. 2021. Endogenous Lifecycle Models for Chemical Risk Assessment. Environ. Sci. Technol. 55: 15596-15608.
- Gotelli NJ, 2008. A Primer of Ecology. Sinauer Associates, Inc., Sunderland, MA, USA.
- Holbech H, Kinnberg KL, Brande-Lavridsen N, Bjerregaard P, Petersen GI, Norrgren L, Orn S, Braunbeck T, Baumann L, Bomke C, Dorgerloh M, Bruns E, Ruehl-Fehlert C, Green JW, Springer TA, Gourmelon A. 2012 Comparison of zebrafish (*Danio rerio*) and fathead minnow *(Pimephales promelas*) as test species in the Fish Sexual Development Test (FSDT). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 155: 407–415.
- Kramer VJ, Etterson MA, Hecker M, Murphy CA, Roesijadi G, Spade DJ, Stromberg JA, Wang M, Ankley GT. 2011. Adverse

outcome pathways and risk assessment: Bridging to population level effects. Environ. Toxicol. Chem. 30, 64-76.
- McComb B, Zuckerberg B, Vesely D, Jordan C. 2021. Monitoring Animal Populations and their Habitats: A Practitioner's Guide. Pressbooks, Oregon State University, Corvallis, OR Version 1.13, 296 pp.
- Miller DH, Villeneuve DL, Santana Rodriguez KJ, Ankley GT. 2022. A multidimensional matrix model for predicting the effect of male biased sex ratios on fish populations. Environmental Toxicology and Chemistry 41(4): 1066-1077.
- Miller DH, Tietge JE, McMaster ME, Munkittrick KR, Xia X, Griesmer DA, Ankley GT. 2015. Linking mechanistic toxicology to population models in forecasting recovery from chemical stress: A case study from Jackfish Bay, Ontario, Canada. Environmental Toxicology and Chemistry 34(7): 1623-1633.
- Miller DH, Jensen KM, Villeneuve DE, Kahl MD, Makynen EA, Durhan EJ, Ankley GT. 2007. Linkage of biochemical responses to population-level effects: A case study with vitellogenin in the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 26: 521–527.
- Miller DH, Ankley GT. 2004. Modeling impacts on populations: Fathead minnow (*Pimephales promelas*) exposure to the endocrine disruptor 17b-trenbolone as a case study. Ecotox Environ Saf 59: 1–9.
- Murphy CA, Nisbet RM, Antczak P, Garcia-Reyero N, Gergs A, Lika K, Mathews T, Muller EB, Nacci D, Peace A, Remien CH, Schultz IR, Stevenson LM, Watanabe KH. 2018. Incorporating suborganismal processes into dynamic energy budget models for ecological risk assessment. Integrated Environmental Assessment and Management 14(5): 615–624.
- Murray DL, Sandercock BK (editors). 2020. Population ecology in practice. Wiley-Blackwell, Oxford UK, 448 pp.
- Nisbet RM, Jusup M, Klanjscek T, Pecquerie L. 2011. Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. The Journal of Experimental Biology 215: 892-902.
- Nisbet RM, Muller EB, Lika K, Kooijman SALM. 2000. From molecules to ecosystems through dynamic energy budgets. J Anim Ecol 69: 913–926.
- Perkins EJ, Ashauer R, Burgoon L, Conolly R, Landesmann B,, Mackay C, Murphy CA, Pollesch N, Wheeler JR, Zupanic A, Scholzk S. 2019. Building and applying quantitative adverse outcome pathway models for chemical hazard and risk assessment. Environmental Toxicology and Chemistry 38(9): 1850–1865.
- Vandermeer JH, Goldberg DE. 2003. Population ecology: first principles. Princeton University Press, Princeton NJ, 304 pp.
- Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lattieri T, Munn S, Nepelska M, Ottinger MA, Vergauwen L, Whelan M. Adverse outcome pathway (AOP) development 1: Strategies and principles. Toxicol Sci. 2014: 142:312–320
- Watanabe KH, Mayo M, Jensen KM, Villeneuve DL, Ankley GT, Perkins EJ. 2016. Predicting fecundity of fathead minnows (*Pimephales promelas*) exposed to endocrine-disrupting chemicals using a MATLAB(R)-based model of oocyte growth dynamics. PLoS One 11: e0146594.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2073: Activation, PPARα leads to Decreased, cholesterol

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI
mice	Mus sp.	High	NCBI
mammals	mammals	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	High

Sex Applicability

Sex	Evidence
Male	High
Female	Moderate

TAXONOMIC APPLICABILITY

The understanding of the effects of PPARα agonists on cholesterol primarily comes from studies on mice and humans to develop pharmaceuticals. However, lowered cholesterol in response to a PPARα agonist occurs in other mammals including rats, dogs, and guinea pigs at low, non-toxic doses (Meyer et al., 1999).

There are several studies showing that in fish PPARα agonism decreases cholesterol via the same mechanisms as in humans:

1. LPL is conserved in zebrafish (NCBI). It is increased in several fish species exposed to PPARα agonists (Prindiville et al., 2011; Teles et al., 2016; Guo et al., 2015)
2. LDL is decreased in several fish species exposed to PPARα agonists (see Table 1)
3. CETP is conserved in zebrafish (NCBI)
4. APOA1 is conserved in zebrafish (NCBI). However, results are mixed on the effects of PPARα agonists on APOA1 (Corcoran et al., 2015; Teles et al., 2016) and HDL (see table 1) . In mice APOA1 is not regulated by PPARα (Staels & Auwerx, 1998), so this may be the case in fish.

SEX APPLICABILITY

Male and female mice show different effects in several endpoints, including total cholesterol, in response to fibrate administration. This is likely due to estrogen partially and indirectly inhibiting PPARα (Yoon, 2010; Jeong & Yoon, 2012). In fish, males and females often show differing effects on cholesterol (Lee et al., 2019; Runnalls et al., 2007).

Key Event Relationship Description

PPARα is a nuclear receptor. With an agonist it promotes transcription of many genes, several of which are involved in cholesterol transport and metabolism (reviewed in Rakhshandehroo et al., 2010).

Hydrophobic lipid molecules (such as cholesterol, cholesteryl ester, and triglycerides) are transported in the aqueous plasma of organisms by forming lipoprotein complexes with apolipoproteins. There are different groups of lipoproteins which use different apolipoproteins and ratios of lipids: low-density (LDL), very low-density (VLDL), and high density (HDL).

Fibrates are a class of drug that agonize PPARα to lower LDL and VLDL while slightly increasing HDL in humans (Singh & Correa, 2020).

Evidence Supporting this KER

See below.

Biological Plausibility

There are 4 proposed mechanisms through which PPARα agonists [fibrates] lower cholesterol in humans (Staels et al., 1998; Chruściel et al., 2015):

1. Increasing lipoprotein lipase (LPL) and decreasing its inhibitor, APOC3. LPL catabolizes triglycerides in VLDL which lowers the amount VLDL.
2. Formation of LDL with a higher affinity for the LDL receptor resulting in increased cellular uptake and breakdown of LDL.
3. Reduced cholesterol ester transfer protein (CEPT) expression. CEPT transfers cholesteryl ester and triglycerides between HDL and VLDL
4. Increased APOA1 and APOA2, the protein components of HDL, in the liver causing increased production of HDL.

Empirical Evidence

Speies	PPARα Agonist	Total CHL	HDL	LDL	VLDL	Citation
Adult Nile tilapia (*O. niloticus*)	200 mg fenofibrate/kg BW for 4 weeks	decreased	increased	n.s.	--	Ning et al. 2017
Juvenile female rainbow trout (*O. mykiss*)	100 mg gemfibrozil/kg BW every 3 days for 15 days	-22%	-27%	-34%	-58%	Prindiville et al. 2011
Medaka (*O. latipes*) embryos	0.04 – 3.7 mg gemfibrozil /L for 155 days	n.s.	--	--	--	Lee et al. 2019
Medaka (*O.*	0.04 – 3.7 mg gemfibrozil /L for 21	n.s. (females) decreased	--	--	--	

latipes) adults	days	(males)				
Adult zebrafish (*D. rerio*)	16 mg gemfibrozil/kg BW per day for 30 days	-15% (females) -19% (males)	--	--	--	Al-Habsi et al. 2016
Adult Male Zebrafish (*D. rerio*)	35, 667, & 1428 mg bezafibrate/kg BW for 48 hours, 7 days, & 21 days	-30% by 21 days, all doses	--	--	--	Velasco-Santamaría et al. 2011
Juvenile grass carp (*C. idella*) fed HFD	100 mg fenofibrate/kg BW per day for 2 weeks	-22%	n.s.	-45%	--	Du et al. 2008
Adult grass carp (*C. idella*) fed HFD or HCD	50 mg clofibrate/kg BW per day for 4 weeks	-28% (both)	-9% (HCD) -16% (HFD)	-23% (HCD) - 34% (HFD)	--	Guo et al. 2015
Adult fathead minnow (*P. promelas*)	1 mg/L clofibric acid for 21 days	Decreased (females) n.s. (males)	n.s.	n.s.	--	Runnalls et al. 2007
Juvenile Turbot (*S. maximus*)	5 or 50 mg WY-14,643/kg BW for 7 or 21 days	decreased	decreased	--	--	Urbatzka et al. 2015

Table 1: Concordance Table for Teleost Fish. Body Weight (BW), Not Significant (n.s.), Cholesterol (CHL), High Fat Diet (HFD), High Carbohydrate Diet (HCD)

Uncertainties and Inconsistencies

Although humans taking fibrate medications show lowed LDL and VLDL but slightly increased HDL, this pattern is not seen in fish (Prindiville et al., 2011). The exact reason(s) why is not well understood.

Quantitative Understanding of the Linkage

See below

Response-response relationship

After a 7 day exposure to bezafibrate (BZF), male zebrafish exposed to 1.7 mg BZF/g food showed no significant decrease in plasma cholesterol ($p>0.05$). However, those exposed to 33 and 70 mg BZF/g food showed a 25 and 48% reduction, respectively, in plasma cholesterol ($p=0.04$ and $p<0.001$, respectively) (Velasco-Santamaría et al., 2011).

Time-scale

Lowered cholesterol in adult male zebrafish due to bezafibrate exposure can be seen after 7 days, but not after just 48 hours (Velasco-Santamaría et al., 2011).

Known modulating factors

Modulating factors haven't been evaluated yet.

Known Feedforward/Feedback loops influencing this KER

Feedback/feedforward loops haven't been evaluated yet.

References

Al-Habsi, A.A., A. Massarsky, T.W. Moon (2016) “Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (*Danio rerio*)”, *Comparative Biochemistry and Physiology, Part B,* Vol. 199, Elsevier, pp. 87-96. http://dx.doi.org/10.1016/j.cbpb.2015.11.009

Chruściel, P. et al. (2015) “Statins and fibrates: Should they still be recommended?”, in *Combination Therapy in Dyslipidemia,* Springer, pp. 11-23. doi: 10.1007/978-3-319-20433-8_2

Corcoran, J. et al. (2015) “Effects of the lipid regulating drug clofibric acid on the PPARα-regulated gene transcript levels in common carp (*Cyprinus carpio*) at pharmacological and environmental exposure levels”, *Aquatic Toxicology,* Vol. 161, Elsevier, pp. 127-137. http://dx.doi.org/10.1016/j.aquatox.2015.01.033

Du, Z. et al. (2008) “Hypolipidaemic effects of fenofibrate and fasting in the herbivorous grass carp (*Ctenopharyngodon Idella*) fed a high-fat diet”, *British Journal of Nutrition*, Vol. 100, Cambridge University Press, pp. 1200-1212. doi:10.1017/S0007114508986840

Guo, X. et al. (2015) “Effects of lipid-lowering pharmaceutical clofibrate on lipid and lipoprotein metabolism of grass carp

(*Ctenopharyngodon idellal* Val.) fed with the high non-protein energy diets", *Fish Physiology and Biochemistry,* Vol. 41, Springer, pp. 331-343. doi: 10.1007/s10695-014-9986-8

Jeong, S. & M. Yoon (2012) "Inhibition of the actions of peroxisome proliferator-activated receptor α on obesity by estrogen", *Obesity*, Vol. 15(6), Wiley, pp. 1430-1440. https://doi.org/10.1038/oby.2007.171

Lee, G. et al. (2019) "Effects of gemfibrozil on sex hormones and reproduction related performances of *Oryzias latipes* following long-term (155 d) and short-term (21 d) exposure", *Ecotoxicology and Environmental Safety*, Vol. 173, Elsevier, pp. 174-181. https://doi.org/10.1016/j.ecoenv.2019.02.015

Meyer, K. et al. (1999) "Species difference in induction of hepatic enzymes by BM17.0744, an activator of peroxisome proliferator-activated receptor alpha (PPARα)", *Molecular Toxicology,* Vol. 73, Springer-Verlag, pp. 440-450. https://doi.org/10.1007/s002040050633

Ning, L. et al. (2017) "Nutritional background changes the hypolipidemic effects of fenofibrate in Nile tilapia (*Oreochromis niloticus*)", *Scientific Reports,* Vol. 7(41706), Nature. https://doi.org/10.1038/srep41706

Prindiville, J.S. et al. (2011) "The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout", *Toxicology and Applied Pharmacology,* Vol. 251, Elsevier, pp. 201-238. doi:10.1016/j.taap.2010.12.013

Rakhshandehroo, M. et al. (2010) "Peroxisome Proliferator-Activated Receptor Alpha Target Genes", *PPAR Research,* Vol. 2010, Hindawi, https://doi.org/10.1155/2010/612089

Runnalls, T. J., D. N. Hala, J. S. Sumpter (2007) "Preliminary studies into the effects of the human pharmaceutical clofibric acid on sperm parameters in adult fathead minnow", *Aquatic Toxicology,* Vol. 84, Elsevier, pp. 111-118. doi:10.1016/j.aquatox.2007.06.005

Singh, G. and R. Correa (2020) "Fibrate Medications", in *StatPearls.* StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK547756/

Staels, B. & J. Auwerx (1998) "Regulation of apo A-I gene expression by fibrates", *Atherosclerosis,* Vol. 137, Elsevier, pp. s19-23. https://doi.org/10.1016/S0021-9150(97)00313-4

Staels, B. et al. (1998) "Mechanism of action of fibrates on lipid and lipoprotein metabolism", *Cardiovascular Drugs,* Vol. 98(19), American Heart Association, pp. 2088-2093.

Teles, M. et al. (2016) "Evaluation of gemfibrozil effects on a marine fish (*Sparus aurata*) combining gene expression with conventional endocrine and biochemical endpoints", *Journal of Hazardous Materials,* Vol. 318, Elsevier, pp. 600-607. http://dx.doi.org/10.1016/j.jhazmat.2016.07.044

Urbatzka, R. et al. (2015) "Effects of the PPARα agonist WY-14,643 on plasma lipids, enzymatic activities and mRNA expression of lipid metabolism genes in a marine flatfish, *Scophthalmus maximus", Aquatic Toxicology*, Vol. 164, Elsevier, pp. 155-162. http://dx.doi.org/10.1016/j.aquatox.2015.05.004

Velasco-Santamaría, Y.M. et al. (2011) "Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (*Danio rerio*)", *Aquatic Toxicology,* Vol. 105, Elsevier, pp. 107-118. doi:10.1016/j.aquatox.2011.05.018

Yoon, M (2010) "PPARα in Obesity: Sex Differences and Estrogen Involvement", *PPAR Research,* Vol. 2010, Hindawi, https://doi.org/10.1155/2010/584296

Relationship: 2072: Decreased, cholesterol leads to Decreased, 11KT

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI
Vertebrates	Vertebrates	Low	NCBI

Sex Applicability

Sex	Evidence

Sex	Evidence
Male	High
Female	Low

Taxanomic Applicability: The understanding of steroid hormone biosynthesis is developed from human and rodent studies but is generally conserved among vertebrates. Cyp11a1, which performs the first step of converting cholesterol to steroid hormones, is only found in vertebrates (Slominski et al., 2015). However, the relationship may not be relevant or studied in organisms in which 11KT isn't a primary androgen. 11KT is particularly relevant teleost fish as it is the dominant androgen and involved in testicular development and courtship behavior (Brantley et al., 1993; Barannikova et al., 2004; Gemmell et al., 2019). Evidence supporting this KER comes from a few fish species, including zebrafish and medaka, but is biologically plausible for all teleost fish.

Sex Applicability: Male and female fish use the same biological processes to produce steroids and express the necessary enzymes. In most fish species 11KT is significantly lower in females versus males, however a a few species of the order Perciformes show no sexual dimorphism (Lokman et al. 2002). In species with sexual dimorphism, males could show more significant effects resulting from lowered 11-KT than females. Decreased production of 11-KT in females may not be detectable due to low baseline production, however there are few studies available showing the relationship between cholesterol and 11KT in female fish.

Life-Stage Applicability:

Key Event Relationship Description

The cholesterol molecule is the precursor for all steroid hormone synthesis. Cholesterol is obtained from *de novo* synthesis within cells or uptake of extracellular cholesterol (Eacker et al., 2008), however the dependence on either source varies by species (Klinefelter et al., 2014). Cholesterol is then transported into the inner mitochondrial membrane via the steroidogenic acute regulatory protein (StAR). Cholesterol is then converted to pregnenolone via the enzyme cytochrome P450 side-chain cleavage (cyp11a1). This is the rate-limiting step of steroidogenesis (Arukwe, 2008). Pregnenolone is then used to produce all other steroid hormones. 11-KT is synthesized from testosterone primarily using the enzymes CYP11β1 and HSD11β2 (Yazawa et al., 2008).

Evidence Supporting this KER

Time	Dose	Decreased Cholesterol?	Decreased 11-KT?	Citation	Species
48 hours	1.7, 33, & 70 mg/g Bezafibrate	No	No	Velasco-Santamaría et al. 2011	*Danio Rerio*
7 days	33 & 70 mg/g Bezafibrate	Yes	No		
21 days	1.7 & 33 mg/g Bezafibrate	Yes	No		
21 days	70 mg/g Bezafibrate	Yes	Yes		
67 days	10 ug/L Gemfibrozil	Decreased ex vivo 11-KT production unless supplemented with 25OH-cholesterol		Fraz et al. 2018	*Danio Rerio*
21 days	0.04 mg/L Gemfibrozil	Yes	No	Lee et al. 2019	*Oryzias latipes*
21 days	0.4 & 3.7 mg/L Gemfibrozil	Yes	Yes		

Biological Plausibility

The process of steroid hormone biosynthesis is well understood, and cholesterol is the precursor for all steroid hormones.

Empirical Evidence

Dose Concordance

In male zebrafish bezafibrate lowers cholesterol in lower doses than 11KT (Velasco-Santamaría et al. 2011).

In male medaka gemfibrozil lowers cholesterol in a lower dose than 11KT (Lee et al. 2019)

Temporal Concordance

Male zebrafish fed bezafibrate show lowered cholesterol days before lowered 11KT (Velasco-Santamaría et al. 2011).

Incidence Concordance

Fraz et al. (2018) show reduced ex vivo production of 11KT in male Zebrafish, due to gemfibrozil exposure, is corrected by addition of 25-hydroxycholesterol. This means the decreased steroid synthesis is due to decreased cholesterol availability. Addition of human chorionic gonadotropin, which binds to the LHCG receptor to promote 11KT synthesis, does not correct the decrease in 11KT.

Uncertainties and Inconsistencies

Although Al-Habsi et al. (2016) show female zebrafish exposed to gemfibrozil and/or atorvastatin have decreased cholesterol and testosterone, decreased testosterone was not seen in males. Although several papers show 11KT is generally correlated with testosterone concentrations (Spanò et al., 2004; Maclatchy & Vanderkraak 1995; Lorenzi et al., 2008), it's uncertain if 11KT was actually affected.

11KT levels can have high variability between fish. Although Lee et al. (2019) shows a decrease in testosterone and 11KT in a 21-day study, steroid measurements from the 155-day study showed no significant effects. This is possibly due to limited samples size (n=3-5).

Quantitative Understanding of the Linkage

Response-response relationship

Velasco-Santamaría et al. (2011) sampled male zebrafish fed several doses of bezafibrate (1.7, 33, & 70 mg BZF/g food) at several timepoints (48 hours, 7 days, and 21 days). Decreased plasma cholesterol is observed after 7 days to 33 mg/g. However, 11-KT isn't significantly decreased until 21 days to 70 mg/g. There is a positive linear correlation between cholesterol and 11KT ($r=0.291$, $p=0.0004$). These decreases are observed without significant changes to cyp11a1 or StAR.

Male medaka exposed to gemfibrozil for 21 days show decreased cholesterol with doses of 0.03, 0.3, and 3.0 mg/L. However, decreases in 11KT is only significant at doses of 0.3 and 3.0 mg/L (Lee et al. 2019).

Time-scale

Decreases in cholesterol in Zebrafish due to bezafibrate exposure can be seen after 7 days, however, decreases in plasma 11-KT aren't significant until 14 days later (Velasco-Santamaría et al. 2011).

A six-week exposure to gemfibrozil, a cholesterol-lowering pharmaceutical, is sufficient to lower 11-KT levels in the plasma, testes, and whole-body samples of male Zebrafish (Fraz et al. 2018). A 21-day exposure to gemfibrozil is sufficient to lower plasma cholesterol and 11-KT levels in male Japanese Medaka (Lee et al. 2019).

Known Feedforward/Feedback loops influencing this KER

Decreases in plasma cholesterol are correlated with a slight increase in StAR in zebrafish (Velasco-Santamaría et al. 2011). This is a possible compensatory mechanism to increase the amount of cholesterol in the mitochondria.

References

Al-Habsi, A.A., A. Massarsky, T.W. Moon (2016) "Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (*Danio rerio*)", *Comparative Biochemistry and Physiology, Part B,* Vol. 199, Elsevier, pp. 87-96. http://dx.doi.org/10.1016/j.cbpb.2015.11.009

Arukwe, A. (2008) "Steroidogenic acute regulatory (StAR) protein and cholesterol side-chain cleavage (P450*scc*)-regulated steroidogenesis as an organ-specific molecular and cellular target for endocrine disrupting chemical in fish", *Cell Biology and Toxicology,* Vol. 24, Springer, pp. 527-540. https://doi.org/10.1007/s10565-008-9069-7

Barannikova, I.A., L.V. Bayunova, T.B. Semenkova (2004) "Serum levels of testosterone, 11-ketotestosterone and oestradiol-17β in three species of sturgeon during gonadal development and final maturation induced by hormonal treatment", *Journal of Fish Biology,* Vol. 64(5), Wiley-Blackwell, pp. 1330-1338. https://doi.org/10.1111/j.0022-1112.2004.00395.x

Brantley, R.K., J.C. Wingfield, A.H. Bass (1993) "Sex steroid levels in *Porichthys notatus,* a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes", *Hormones and Behavior*, Vol. 27(3), Elsevier, pp. 332-347. https://doi.org/10.1006/hbeh.1993.1025

Eacker, S. M. et al. (2008) "Hormonal regulation of testicular steroid and cholesterol homeostasis", *Molecular Endocrinology*, Vol. 22(3), pp. 623-635. https://doi.org/10.1210/me.2006-0534

Fraz, S., A.H. Lee, J.Y. Wilson (2018) "Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (*Danio rerio)*", *Aquatic Toxicology*, Vol. 198, Elsevier, pp. 1-9. https://doi.org/10.1016/j.aquatox.2018.02.006

Gemmell, N.J. et al. (2019) “Natural sex change in fish”, in *Sex Determination in Vertebrates*, Vol. 134, Academic Press, pp. 71-117. doi: 10.1016/bs.ctdb.2018.12.014.

Klinefelter, G.R., J.W. Laskey, R.P. Amann (2014) “Statin drugs markedly inhibit testosterone production by rat Leydig cells in vitro: Implications for men”, *Reproductive Toxicology,* Vol. 45, Elsevier, pp. 52-58. https://doi.org/10.1016/j.reprotox.2013.12.010

Lee, G. et al. (2019) “Effects of gemfibrozil on sex hormones and reproduction related performances of *Oryzias latipes* following long-term (155 d) and short-term (21 d) exposure”, *Ecotoxicology and Environmental Safety,* Vol. 173, Elsevier, pp. 174-181. https://doi.org/10.1016/j.ecoenv.2019.02.015

Lokman, P.M. et al. (2002) “11-Oxygenated androgens in female teleosts: prevalence, abundance, and life history implications”, *General and Comparative Endocrinology,* Vol. 129, Academic Press, pp. 1-12. doi: 10.1016/s0016-6480(02)00562-2

Lorenzi, V. et al. (2008) “Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone, and 17β-estradiol in the bluebanded goby (*Lythrypnus dalli)*”, *General and Comparative Endocrinology,* Vol. 155(2)., Elsevier, pp. 438-446. https://doi.org/10.1016/j.ygcen.2007.07.010

MacLatchy, D.L., G.J. Vanderkraak (1995) “The phytoestrogen β-sitosterol alters the reproductive endocrine status of goldfish”, *Toxicology and Applied Pharmacology,* Vol. 134(2), Elsevier, pp. 305-312. https://doi.org/10.1006/taap.1995.1196

Slominski, A.T. et al. (2015) “Novel activities of CYP11A1 and their potential physiological significance”, *The Journal of Steroid Biochemistry and Molecular Biology,* Vol. 151, Elsevier, pp. 25-37. https://doi.org/10.1016/j.jsbmb.2014.11.010

Spanó, L. et al. (2004) “Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*)”, *Aquatic Toxicology,* Vol. 66(4), Elsevier, pp. 369-379. https://doi.org/10.1016/j.aquatox.2003.10.009

Velasco-Santamaría, Y.M. et al. (2011) “Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (*Danio rerio*)”, *Aquatic Toxicology,* Vol. 105, Elsevier, pp. 107-118. doi:10.1016/j.aquatox.2011.05.018

Yazawa, T. (2008) “Cyp11b1 is induced in the murine gonad by luteinizing hormone/human chorionic gonadotropin and involved in the production of 11-ketotestosterone, a major fish androgen: Conservation and evolution of the androgen metabolic pathway”, *Endocrinology,* Vol. 149(4), Oxford Academy, pp. 1786-1792. https://doi.org/10.1210/en.2007-1015

Relationship: 2076: Decreased, 11KT leads to Impaired, Spermatogenesis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	adjacent	High	Low
Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Male	High

Taxonomic:

11-KT is the main androgen in teleost fish (Borg, B. 1994).

Sex Applicability:

11-KT is present in both male and female fish; however, spermatogenesis is a male-specific process.

Life Stage Applicability:

Spermatogenesis is observable in male fish that have reached the reproductive stage.

Key Event Relationship Description

Androgens are critical for maintaining the normal male reproductive system (Tang, H., et al. 2018). Of these androgens, 11-KT has been identified as the most important in teleost fish (Borg, B. 1994). 11-KT is produced by the cyp11c1 encoded enzyme, 11ß-hydroxylase (Zheng, et al. 2020). 11-KT has been shown to bind to the androgen receptor with similar affinity as testosterone in zebrafish (Jorgensen, et al. 2007). It is well documented that 11-KT is involved in spermatogenesis, spermiation, male secondary sexual characteristics, and breeding behaviors (Geraudie, P. et al. 2010; Amer, M.A. et al. 2001). 11-KT is needed for the inducement of spermatogenesis and sperm production in teleost fish, with 10 ng/ml 11-KT being sufficient to induce full spermatogenesis in the Japanese eel (Miura, C. and T. Miura 2011). The mechanism through which 11-KT induces spermatogenesis is believed to be via activation of Sertoli cells and activin B (Miura et al. 2011; Miura et al. 2001; Sales, C.F., et al. 2020; Cavaco J.E.B., et al. 1998). 11-KT is not responsible for the acquisition of sperm motility in salmonids (Miura, et al. 1992).

Evidence Supporting this KER

Table 2. Effect of either 11-ketotestosterone (11-KT) treatment or increased testicular production/plasma concentrations of 11-KT on spermatogenesis.

Species	Experimental design	11-KT treatment or response	Spermatogenesis effect	11-KT (+) [1]	Spermatogenesis (+) [1]	Citation
Senegalese sole (*Solea senegalensis*)	Treated with saline (control) or with 50 µg/kg GnRHa, with or without another implant containing 2 or 7 mg/kg 11-ketoandrostenedione for 28 days	Fish treated with GnRHa + OA saw increased 11-KT levels compared to control and GnRHa alone	Fish treated with GnRHa + OA saw lower number of spermatogonia and spermatocytes and a higher number of spermatids than those of GnRHa or control	Yes	Yes	Agulleiro, M.J., et al. 2007
Japanese huchen (*Hucho perryi*)	Incubated immature testis fragments	10 ng/ml for 15 days	BrdU (proliferation marker) index reached 34.5% ± 1.7%; percentage of late type B spermatogonia reached about 7.5% compared to 0% in control	Yes	Yes	Amer, M.A. et al. 2001
African catfish (*Clarias gariepinus*)	Juvenile male catfish implanted with pellets containing 30 µg/g body weight of 11-KT	30 µg/g body weight of 11-KT; plasma 11-KT levels reached 8.3 ± 0.6 ng/ml after 2 weeks	GSI increased compared to control; testicular stage 1 (contain spermatogonia only) and 2 (contain spermatogonia and spermatocytes) increased from about 90% stage 1 and 10% stage 2 in end control to about 25% stage 1 and 75% stage 2	Yes	Yes	Cavaco, J.E.B. et al. 2001
African catfish (*Clarias gariepinus*)	Male catfish at beginning of spermatogenesis implanted with pellets containing 30 µg/g body weight of 11-KT	Plasma 11-KT levels reached 6.1 ± 0.8 ng/ml after 2 weeks	Testicular stages changed from about 65% stage 1 and 35% stage 2 in the end control to about 65% stage 2 and 35% stage 3 (contain spermatogonia, spermatocytes and	Yes	Yes	Cavaco J.E.B., et al. 1998

			spermatids)			
	Male catfish at beginning of spermatogenesis implanted with pellets containing 30 µg/g body weight of 11β-hydroxyandrostenedione	Plasma 11-KT levels reached 7.3 ± 0.7 ng/ml after 2 weeks	Testicular stages changed from about 65% stage 1 and 35% stage 2 in the end control to about 55% stage 2 and 40% stage 3	Yes	Yes	
	Male catfish at beginning of spermatogenesis implanted with pellets containing 30 µg/g body weight of androstenetrione	Plasma 11-KT levels reached 2.4 ± 0.3 ng/ml after 2 weeks	Testicular stages changed from about 65% stage 1 and 35% stage 2 in the end control to about 50% stage 2 and 50% stage 3	Yes	Yes	
Atlantic salmon (*Salmo salar*)	Immature fish injected with 25 µg adrenosterone/g of body weight	After 7 and 14 days, 11-KT plasma levels significantly increased compared to control (7 days post-treatment were higher)	5-fold higher number of type A differentiated spermatogonia than control fish after 14 days (7-day samples lost - no data)	Yes	Yes	Melo, M.C. et al. 2015
Japanese eel (*Anguilla japonica*)	Immature testes were removed and cultured in medium with varying levels of 11-KT	0.01 ng/ml 11-KT for 15 days	No effect	Yes	No	Miura, T., et al. 1991
		0.1 ng/ml 11-KT for 15 days	No effect	Yes	No	
		1 ng/ml 11-KT for 15 days	No effect	Yes	No	
		10 ng/ml 11-KT for 15 days	Mitosis occurred in 50-60% of cysts (as effective as 100 ng/ml 11-KT treatment)	Yes	Yes	
		100 ng/ml 11-KT for 15 days	Mitosis occurred in 50-60% of cysts (as effective as 10 ng/ml 11-KT treatment)	Yes	Yes	
Japanese eel (*Anguilla japonica*)	Immature testis fragments cultured in media with 11-KT for up to 36 days	10 ng/ml of 11-KT for 9 days	Began mitotic division; produced late-type B spermatogonia	Yes	Yes	Miura, T., et al. 1991
		10 ng/ml of 11-KT for 18 days	Produced zygotene spermatocytes from meiotic prophase	Yes	Yes	
		10 ng/ml of 11-KT for 21 days	Spermatids and spermatozoa observed	Yes	Yes	
		10 ng/ml of 11-KT for 36 days	All stages of germ cells present	Yes	Yes	
Chub mackerel (*Scomber japonicus*)	Peptide mix containing synthetic peptides corresponding to chub mackerel Kiss1-15 at a final concentration of 250 ng/g fish were injected 3 times at 2-week interval (immature adult)	Treated fish showed significantly higher 11-KT levels	Significantly higher levels of spermatids and spermatozoa	Yes	Yes	Selvaraj, S., et al. 2013
Japanese eel (*Anguilla japonica*)	Testicular fragment treated with 0.01 ng/ml cortisol	No significant change in 11-KT production	Nonsignificant increase in BrdU Index	No	No	Ozaki, Y., et al. 2006

		compared to control	compared to control			
	Testicular fragment treated with 0.1 ng/ml cortisol	No significant change in 11-KT production compared to control	Significant increase in BrdU Index compared to control	No	Yes	
	Testicular fragment t treated with 1 ng/ml cortisol	Nonsignificant, slight increase in 11-KT production compared to control	Significant increase in BrdU Index compared to control	No	Yes	
	Testicular fragment treated with 10 ng/ml cortisol	Nonsignificant increase in 11-KT production compared to control	Significant increase in BrdU Index compared to control	No	Yes	
	Testicular fragment treated with 100 ng/ml cortisol	Significant increase in 11-KT production compared to control	Significant increase in BrdU Index compared to control	Yes	Yes	
Zebrafish (*Danio rerio)*	*cyp11c1* knockout rescue via 11-ketoandrostenedione (11-KA) treatment	100 nM 11-KA for 4 hours per day for 10 days	Promoted the juvenile ovary-to-testis transition; genes associated with Leydig cell development/function restored; increased sperm volume	Yes	Yes	Zhang, Q., et al. 2020

[1] (+) represents an effect on the key event has been established.

It is well known that 11-KT is a critical androgen for proper male reproduction in teleost fish. The males' primary reproductive role is to fertilize the oocytes.

Seasonal changes in 11-KT are correlated with cyclic spermatogenesis events in teleosts (Basak, R., et al. 2016). In many teleost fish, 11-KT levels peak at spawning (see Table 1 below).

11-KT is proposed to induce spermatogenesis via the activation of Sertoli cells, which in turn regulates factors including activin B (Miura et al. 2011; Miura et al. 2001). Activin B stimulates spermatogonial proliferation (Sales, C.F., et al. 2020; Cavaco J.E.B., et al. 1998).

Zhang et al. (2020) showed that zebrafish with *cyp11c1* knockout have reduced 11-KT levels, smaller genitalia, inability naturally mate, defective Leydig and Sertoli cells, and insufficient spermatogenesis. This is corrected by treatment of 100 nM 11-KA (which is converted to 11-KT *in vivo*) for 4 hours per day for 10 days. This shows that spermatogenesis was arrested due to insufficient 11-KT levels.

Table 1. Plasma concentrations of 11-KT peak during spawning and decline shortly after in a variety of species.

Species	Scientific name	Reproductive strategy [1]	Citation
Japanese huchen	*Hucho perryi*	Single	Amer et al., 2001
Bester	*Huso huso L. female x Acipenser ruthenus L. male*	Single	Amiri et al., 1996
Spotted snakehead	*Channa punctatus*	Multiple	Basak et al., 2016
Chanchita	*Cichlasoma dimerus*	Multiple	Birba et al., 2015
Largemouth bass	*Micropterus salmoides salmoides*	Multiple	Brown et al.,

Biological Plausibility

Empirical Evidence

In African catfish, 11-ketotestosterone, but not testosterone, stimulated spermatogenesis (Cavaco et al., 2001)

Juvenile atlantic salmon injected with adrenosterone, which is converted to 11KT, show increased 11KT in their plasma and increased differentiation of spermatogonia (Melo et al., 2015)

Nile tilapia lacking cyp11c1 show dramatically reduced 11KT levels and delayed spermatogenesis. Spermatogenesis is rescued by 11KT supplementation. Without 11KT supplementation, spermatogenesis occurred later with fewer viable sperm (Zheng et al., 2020)

Injection of female honeycomb grouper, a protogynous hermaphroditic fish, with 11KT induces a female-to-male sex change and stimulates spermatogenesis (Bhandari et al., 2006)

In nile tilapia the absence of functional eukaryotic elongation factor 1 alpha (eEF1A) causes infertility and arrest of spermatogenesis. Heterozygous mutation causes significantly reduced 11KT and abnormal spermiogenesis

Largemouth bass	*Micropterus salmoides salmoides*	Multiple	Brown et al., 2019
Chinook salmon	*Oncorhynchus tshawytscha*	Single	Campbell et al., 2003
Gilthead seabream	*Sparus aurata L.*	Multiple	Chaves-Pozo et al., 2008
Mummichog	*Fundulus heteroclitus*	Multiple	Cochran, 1987
Eastern Mosquitofish	*Gambusia holbrooki*	Multiple	Edwards et al., 2013
Rainbow trout	*Salmo gairdneri*	Single	Fostier et al., 1984
Senegalese sole	*Solea senegalensis*	Multiple	García-López et al., 2006
Roach	*Rutilus rutilus*	Multiple	Geraudine et al., 2010
Sterlet	*Acipenser ruthenus*	Single	Golpour et al., 2017
Sablefish	*Anoplopoma fimbria*	Multiple	Guzmán et al., 2018
Brook trout	*Salvelinus fontinalis*	Single	de Montgolfier et al., 2009
Brill	*Scophthalmus rhombus L.*	Multiple	Hachero-Cruzado et al., 2012
Three-spined stickleback	*Gasterosteus aculeatus*	Multiple	Hellqvist et al., 2006
Red-spotted grouper	*Epinephelus akaara*	Multiple	Li et al., 2007
Japanese dace	*Tribolodon hakonesis*	Multiple	Ma et al., 2005
Walleye	*Stizostedion vitreum*	Single	Malison et al., 1994
Florida gar	*Lepisosteus platyrhincus*	Multiple	Orlando et al., 2003
Chum Salmon	*Oncorhynchus keta*	Single	Onuma et al., 2009
Hornyhead Turbot	*Pleuronichthys verticalis*	Multiple	Reyes et al., 2012
Golden mahseer	*Tor putitora*	Multiple	Shahi et al., 2015
Plainfin midshipman	*Porichthys notatus*	Single	Sisneros et al., 2004
Amago salmon	*Oncorhynchus rhodurus*	Single	Ueda et al., 1983; Sakai et al., 1989
Atlantic halibut	*Hippoglossus hippoglossus L.*	Multiple	Weltzien et al., 200

[1] Defined as single spawning species (spawn once/year) or multiple spawning species (spawn multiple clutches of eggs per reproductive period).

(Chen et al., 2017)

Dose concordance

Increases in 11-KT levels correspond with increases in spermatogenesis in multiple studies (see Table 2 above). Melo et al. (2015) showed that treatment of adrenosterone - or OA - (which is converted to 11-KT *in vivo*) increases 11-KT levels, and this sustained increase induces spermatogonial differentiation.

Decreases in 11-KT levels correspond with decreases in spermatogenesis in multiple studies (see Table 3 above). Liu, Z.H., et al. (2018) showed that exposure to 10 ng/L DES for 28 days significantly decreases 11-KT levels and disrupts spermatogenesis. Additionally, exposure to 100 ng/L DES for 28 days has further negative effects on 11-KT levels and spermatogenesis.

Temporal concordance

11-KT peaks at spawning in a number of teleost fish (see Table 1 above).

Melo et a. (2015) showed treatment with adrenosterone (OA) caused an increase in 11-KT levels, which sustained through 7 days after treatment and (to a lesser extent) 14 days after treatment. Type A differentiated spermatogonial numbers also increased 14 days after treatment. There was no spermatogenesis data for 7 days after treatment, due to the samples being lost.

A study by de Waal et al. (2009) showed treatment with 10 nM E2 for 6 and 21 days resulted in decreased 11-KT levels and decreased spermatogonial proliferation. The 21 day treatment saw more spermatogonial arrest than the 6 day treatment.

Table 3. Effect of either decreased plasma concentration or testicular production of 11-ketotestosterone (11-KT) on spermatogenesis.

Species	Experimental design	11-KT treatment or response	Spermatogenesis effect	11-KT	Spermatogenesis	Citation

				( ) [1]	( ) [1]	
Guinean tilapia (*Tilapia guineensis*)	Fish from multiple sites contaminated with pesticides were studied	Levels significantly lower in contaminated sites	Amounts of spermatids and spermatozoa were decreased in contaminated sites	Yes	Yes	Agbohessi, P.T., et al. 2015
African catfish (*Clarias gariepinus*)		Levels significantly lower in contaminated sites; larger change than in Guinean tilapia	Amounts of spermatozoa were decreased in contaminated sites	Yes	Yes	
Nile tilapia (*Oreochromis niloticus*)	Heterozygous mutation of eEF1A1b (eEF1A1b$^{+/-}$) via CRISPR/Cas9	Significantly decreased serum 11-KT at 90 and 180 days after hatch (dah)	Absence of spermatocytes at 90 dah, and decreased number of spermatocytes, spermatids and spermatozoa at 180 dah	Yes	Yes	Chen, J. et al. 2017
Zebrafish (*Danio rerio*)	Adult fish exposed to 10 nM 17β-estradiol (E2) via water for 6 days	Significantly decreased ex vivo testicular production; 6 day exposure to 10 nM E2	Type B spermatogonia, primary spermatocytes, and secondary spermatocytes decreased to 54-60% of control levels	Yes	Yes	de Waal et al. 2009
	Adult fish exposed to 10 nM E2 via water for 21 days	Significantly decreased ex vivo testicular production; 6 day exposure to 10 nM E2	Type B spermatogonia, primary and secondary spermatocytes, and spermatids significantly decreased further (e.g, spermatids to 19% of control)	Yes	Yes	
Goldfish (*Carassius auratus*)	Mature fish exposed for 30 days to 100 µg/L anti-androgen vinclozolin (VZ) water	Increase in 11-KT level (compared to control)	Nonsignificant decrease (compared to control) in sperm volume, motility, and velocity	No	No	Hatef, A. et al. 2012
	Mature fish exposed for 30 days to 400 µg/L anti-androgen vinclozolin (VZ) water	No significant change in 11-KT level (compared to control)	Nonsignificant decrease (compared to control) in sperm volume, motility and velocity; spermatozoa without flagella or with damaged flagella were observed	No	Yes	
	Mature fish exposed for 30 days to 800 µg/L anti-androgen vinclozolin (VZ) water	Decrease in 11-KT level (compared to control); similar level to E2 negative control	Significant decrease (compared to control) in sperm volume, motility, and velocity; spermatozoa without flagella or with damaged flagella were observed	Yes	Yes	
Yellow catfish (*Pelteobagrus fulvidraco*)	Juvenile fish exposed to 10 ng/L DES for 28 days via water	Plasma levels lightly (but significantly) decreased compared to control	Loss of spermatids; presence of several lacunas	Yes	Yes	Liu, Z.H., et al. 2018
	Juvenile fish exposed to 100 ng/L	Plasma levels lightly (but	Loss of spermatids; more lacunae than 10	Yes	Yes	

	DES for 28 days via water	significantly) decreased compared to control	ng/L exposure			
Nile tilapia (*Oreochromis niloticus*)	Sexually mature males exposed via water to 200 ng/L diuron for 25 days	No significant change compared to control	No change to seminiferous tubules, and no change to spermatid or spermatozoa numbers	No	No	Pereira, T.S., et al. 2015
	Sexually mature males exposed to 200 ng/L DCA (diuron metabolite) for 25 days	Significant decrease of 11% compared to control	Seminiferous tubules reduced about 60% and spermatid and spermatozoa amounts decreased by about 10% compared to control	Yes	Yes	
	Sexually mature males exposed to 200 ng/L DCPU (diuron metabolite) for 25 days	Significant decrease of 11% compared to control	Seminiferous tubules reduced about 60% and spermatid and spermatozoa amounts decreased by about 10% compared to control	Yes	Yes	
	Sexually mature males exposed to 200 ng/L DCPMU (diuron metabolite) for 25 days	Significant decrease of 11% compared to control	Seminiferous tubules reduced about 60% and spermatid and spermatozoa amounts decreased by about 10% compared to control	Yes	Yes	
Nile tilapia (*Oreochromis niloticus*)	Adult males; starvation for 7 days	Significant reduction in plasma 11-KTcompared to control	Significant decrease in number of spermatocytes and spermatozoa	Yes	Yes	Sales, C.F., et al. 2020
	Adult males; starvation for 14 days	Significant reduction in plasma 11-KT compared to control	Significant decrease in number of spermatocytes and spermatozoa	Yes	Yes	
	Adult males; starvation for 21 days	Significant reduction in plasma 11-KT compared to control	Significant decrease in number of spermatocytes and spermatozoa; significant decrease type A undifferentiated and differentiated spermatogonia	Yes	Yes	
	Adult males; starvation for 28 days	Significant reduction in plasma 11-KT compared to other starvation durations	Significant decrease in number of spermatocytes and spermatozoa; significant decrease type A undifferentiated and differentiated spermatogonia	Yes	Yes	
Zebrafish (*Danio rerio)*	Androgen receptor (*ar*) knockout	Significantly decreased in adult whole-body homogenate	Significant decrease in number of germ cells, most of which were stopped at early stages of development; some spermatozoon found	Yes	Yes	Tang, H., et al. 2018
Zebrafish	Bezafibrate (BZF) administered orally	Non-significant decrease	Did not report results	No	n/a	Velasco-Santamaría,

(*Danio rerio*)	to adult males at 1.7 mg BZF/g food for 21 days	compared to control				Y.M., et al. 2011
	Bezafibrate (BZF) administered orally to adult males at 33 mg BZF/g food for 21 days	Non-significant decrease compared to control	Did not report results	No	n/a	
	Bezafibrate (BZF) administered orally to adult males at 70 mg BZF/g food for 21 days	Significant decrease compared to control	Testicular degeneration; increased syncytia and spermatocytes	Yes	Yes	
Zebrafish (*Danio rerio*)	Adult males exposed for 30 days to 100 ng/L DES (estrogen) via water	Plasma levels decreased 3-fold	Adverse effect on testicular development and spermatogenesis; sperm concentration decreased 3-fold	Yes	Yes	Yin, P. et al. 2017
	Adult males exposed for 30 days to 300 µg/L FLU (anti-androgen)	Plasma levels decreased 2-fold	Adverse effect on testicular development and spermatogenesis; sperm concentration decreased 3-fold	Yes	Yes	
	Adult males exposed for 30 days to combo of 100 ng/L DES and 300 µg/L FLU	Plasma levels decreased 6-fold	Adverse effect on testicular development and spermatogenesis; sperm concentration decreased 4-fold	Yes	Yes	
Zebrafish (*Danio rerio*)	*Mettl3* mutation	Serum concentration significantly decreased	Little or no mature sperm; 24.4% spermatogonia, 56.1% spermatocytes, and 10.4% spermatozoa (compared to 7.5%, 26.7%, and 50.1% in wild type)	Yes	Yes	Xia, H. et al. 2018
Zebrafish (*Danio rerio*)	*cyp11c1* knockout via CRISPR/Cas9 (homozygous mutation)	Significantly decreased levels	Insufficient spermatogenesis, but not completely blocked; sperm volume significantly decreased	Yes	Yes	Zhang, Q., et al. 2020

[1] (–) represents an effect on the key event has been established.

Uncertainties and Inconsistencies

In a study by Hatef, A. et al. (2012), treatment with the anti-androgen vinclozolin at 100 µg/L saw an increase in 11-KT levels with no significant change to spermatogenesis. This is consistent with other studies provided. Additionally, treatment at 400 µg/L saw no significant change in 11-KT levels with a decrease in spermatogenesis (although this decrease may not be statistically significant). The reason for these increases in 11-KT remains unknown; however, it is hypothesized that it is due to competitive androgen receptor binding.

Ozaki et al. (2006) showed that treatment with 100 ng/ml of cortisol significantly increased 11-KT levels. However, the less concentrated doses only saw non-significant increases in 11-KT with significant increases in spermatogenesis observed in all but the lowest dose. Despite this, Ozaki et al. make the generalization that cortisol treatment increased 11-KT and, in turn, spermatogenesis.

The study by Runnalls et al. (2007) saw treatment with Clofibric acid caused no significant changes to 11-KT levels, but that the levels did appear lower. Additionally, these treatments saw no significant effect on sperm number, but did see a significant increase in the number of non-viable sperm.

In a study by Zhang, Q., et al. (2020), *cyp11c1* knockout did not completely block spermatogenesis. Zhang et al. explain this could be due to other androgens (11β-hydroxyandrostenedione and testosterone) compensating for the reduction in 11-KT, as they can both bind to the androgen receptor to influence downstream signaling.

Quantitative Understanding of the Linkage

Response-response relationship

Decreases in 11-KT levels were also seen with decreases in spermatogenesis in several studies (see table above).

10 ng/ml of 11-KT has been shown to be needed to induce full spermatogenesis in Japanese eel (Amer, M.A. et al. 2001; Miura, C. et al. 2011).

References

Agbohessi, P.T., Imorou Toko I., Ouédraogo, A., Jauniaux, T., Mandiki, S.N., & Kestemont, P. (2014). Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in Benin (West Africa). *Science of the Total Environment*, *506-507*, 567-584. https://doi.org/10.1016/j.scitotenv.2014.11.047

Agulleiro, M.J., Scott, A.P., Duncan, N., Mylonas, C.C., & Cerdà, J. (2007). Treatment of GnRHa-implanted Senegalese sole (Solea senegalensis) with 11-ketoandrostenedione stimulates spermatogenesis and increases sperm motility. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *147*(4), 885-92. https://doi.org/10.1016/j.cbpa.2007.02.008

Amer, M.A., Miura, T., Miura, C., & Yamauchi, K. (2001). Involvement of Sex Steroid Hormones in the Early Stages of Spermatogenesis in Japanese Huchen (Hucho perryi). *Biology of Reproduction, 65*(4), 1057–1066. https://doi.org/10.1095/biolreprod65.4.1057

Amiri, B.M., Maebayashi, M., Adachi, S., & Yamauchi, K. (1996). Testicular development and serum sex steroid profiles during the annual sexual cycle of the male sturgeon hybrid the bester. *Journal of Fish Biology*, *48*(6), 1039-1050. https://doi.org/10.1111/j.1095-8649.1996.tb01802.x

Aoki, K.A., Harris, C.A., Katsiadaki, I., & Sumpter, J.P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. *Environmental Toxicology and Chemistry*, *30*(6), 1338-1345. https://doi.org/10.1002/etc.502

Baatrup, E. & Junge, M. (2001). Antiandrogenic pesticides disrupt sexual characteristics in the adult male guppy Poecilia reticulata. *Environmental Health Perspectives*, *109*(10), 1063-70. DOI: 10.1289/ehp.011091063

Basak, R., Roy, A. & Rai, U. (2016). Seasonality of reproduction in male spotted murrel Channa punctatus: correlation of environmental variables and plasma sex steroids with histological changes in testis. *Fish Physiology and Biochemistry*, *42*(5), 1249-1258. https://doi.org/10.1007/s10695-016-0214-6

Berglund, I., Antonopoulou, E., Mayer, I., & Borg, B. (1995). Stimulatory and inhibitory effects of testosterone on testes in Atlantic salmon male parr. *Journal of Fish Biology*, *47*(4), 586-598. https://doi.org/10.1111/j.1095-8649.1995.tb01925.x

Bhandari, R.K., Alam, M.A., Soyano, K., & Nakamura, M. (2006). Induction of female-to-male sex change in the honeycomb grouper (Epinephelus merra) by 11-ketotestosterone treatments. *Zoological Science*, *23*(1), 65-69. https://doi.org/10.2108/zsj.23.65

Birba, A., Ramallo, M.R., Nostro, F.L., Moreira, R.G., & Pandolfi, M. (2015). Reproductive and parental care physiology of Cichlasoma dimerus males. *General and Comparative Endocrinology*, *15*, 193-200. https://doi.org/10.1016/j.ygcen.2015.02.004

Borg, B. (1994). Androgens in teleost fishes. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology, and Endocrinology*, *109*(3), 219-245. https://doi.org/10.1016/0742-8413(94)00063-G

Brown, M.L., Kasiga, T., Spengler, D.E., & Clapper, J.A. (2019). Reproductive cycle of northern largemouth bass Micropterus salmoides salmoides. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *331*(10), 540-551. https://doi.org/10.1002/jez.2323

Campbell, B., Dickey, J.T., & Swanson, P. (2003). Endocrine changes during onset of puberty in male spring Chinook salmon, Oncorhynchus tshawytscha. *Biology of Reproduction*, *69*(6), 2109-2117. https://doi.org/10.1095/biolreprod.103.020560

Cavaco, J.E.B., Bogerd, J., Goos, H., & Schulz, R.W. (2001). Testosterone inhibits 11-ketotestosterone-induced spermatogenesis in African catfish (Clarias gariepinus). *Biology of Reproduction*, *65*(6), 1807-1812. https://doi.org/10.1095/biolreprod65.6.1807

Cavaco, J.E.B., Vilrokx, C., Trudeau, V.L., Schulz, R.W., & Goos, H.J.T. (1998). Sex steroids and the initiation of puberty in male African catfish, Clarias gariepinus. *American Journal of Physiology*, *275*(6), 1793-1802. https://doi.org/10.1152/ajpregu.1998.275.6.R1793

Chauvigné, F., Parhi, J., Ollé, J., & Cerdà, J. (2017). Dual estrogenic regulation of the nuclear progestin receptor and spermatogonial renewal during gilthead seabream (*Sparus aurata*) spermatogenesis. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *206*, 36-46. https://doi.org/10.1016/j.cbpa.2017.01.008

Chaves-Pozo, E., Arjona, F.J., García-López, A., García-Alcázar, A., Meseguer, J., & García-Ayala A. (2008). Sex steroids and metabolic parameter levels in a seasonal breeding fish (Sparus aurata L.). *General and Comparative Endocrinology*, *156*(3), 531-536. https://doi.org/10.1016/j.ygcen.2008.03.004

Chen, J., Jiang, D., Tan, D., Fan, Z., Wei, Y., Li, M., & Wang, D. (2017). Heterozygous mutation of eEF1A1b resulted in spermatogenesis arrest and infertility in male tilapia, Oreochromis niloticus. *Scientific Reports*, *7,* 43733. https://doi.org/10.1038/srep43733

Cochran, R.C. (1987). Serum androgens during the annual reproductive cycle of the male mummichog, Fundulus heteroclitus. *General and Comparative Endocrinology*, *65*(1), 141-148. https://doi.org/10.1016/0016-6480(87)90233-4

de Montgolfier, B., Faye, A., Audet, C., & Cyr, D.G. (2009). Seasonal variations in testicular connexin levels and their regulation in the brook trout, Salvelinus fontinalis. *General and Comparative Endocrinology*, *162*(3), 276-285. https://doi.org/10.1016/j.ygcen.2009.03.025

de Waal, P.P., Leal, M.C., García-López, A., Liarte, S., de Jonge, H., Hinfray, N., Brion, F., Schulz, R.W., & Bogerd, J. (2009). Oestrogen-induced androgen insufficiency results in a reduction of proliferation and differentiation of spermatogonia in the zebrafish testis. *Journal of Endocrinology*, *202*(2), 287-97. https://doi.org/10.1677/JOE-09-0050

Delvin, R.H. & Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, *208*(3-4), 191-364. https://doi.org/10.1016/S0044-8486(02)00057-1

Edwards, T.M., Miller, H.D., Toft, G., & Guillette, L.J. Jr. (2013). Seasonal reproduction of male Gambusia holbrooki (eastern mosquitofish) from two Florida lakes. *Fish Physiology and Biochemistry*, *39*(5), 1165-1180. https://doi.org/10.1007/s10695-013-9772-z

Fostier, A., Billard, R., & Breton, B. (1984). Plasma 11-oxotestosterone and gonadotrophin in relation to the arrest of spermiation in rainbow trout (Salmo gairdneri). *General and Comparative Endocrinology*, *54*(3), 378–381. DOI: 10.1016/0016-6480(84)90150-3

Fostier, A., Billard, R., & Breton, B. (1984). Plasma 11-oxotestosterone and gonadotrophin in relation to the arrest of spermiation in rainbow trout (Salmo gairdneri). G*eneral and Comparative Endocrinology*, *54*(3), 378-381. DOI: 10.1016/0016-6480(84)90150-3

García-López, A., Fernández-Pasquier, V., Couto, E., Canario, A.V., Sarasquete, C., & Martínez-Rodríguez, G. (2006). Testicular development and plasma sex steroid levels in cultured male Senegalese sole Solea senegalensis Kaup. *General and Comparative Endocrinology*, *147*(3), 343-351. https://doi.org/10.1016/j.ygcen.2006.02.003

Geraudie, P., Gerbron, M., & Minier, C. (2010). Seasonal variations and alterations of sex steroid levels during the reproductive cycle of male roach (Rutilus rutilus). *Marine Environmental Research*, *69*(S1), S53-S55. https://doi.org/10.1016/j.marenvres.2009.11.008

Golpour, A., Broquard, C., Milla, S., Dadras, H., Baloch, A.R., Saito, T., & Pšenička, M. (2017). Gonad histology and serum 11-KT profile during the annual reproductive cycle in sterlet sturgeon adult males, Acipenser ruthenus. *Reproduction in Domestic Animals*, *52*(2), 319-326. https://doi.org/10.1111/rda.12911

Golshan, M. & Alavi, S.M.H. (2019). Androgen signaling in male fishes: Examples of anti-androgenic chemicals that cause reproductive disorders. *Theriogenology*, *139*, 58-71. https://doi.org/10.1016/j.theriogenology.2019.07.020

Golshan, M. & Alvai S.M.H. (2019). Androgen signaling in male fishes: Examples of anti-androgenic chemicals that cause reproductive disorders. *Theriogenology*, *139*, 58-71. https://doi.org/10.1016/j.theriogenology.2019.07.020

Guzmán, J.M., Luckenbach, J.A., da Silva, D.A.M., Hayman, E.S., Ylitalo, G.M., Goetz, F.W., & Swanson, P. (2018). Seasonal variation of pituitary gonadotropin subunit, brain-type aromatase and sex steroid receptor mRNAs, and plasma steroids during gametogenesis in wild sablefish. *Comparative Biochemistry and Physiology Part A: Molecular Integrated Physiology*, *219-220*, 48-57. https://doi.org/10.1016/j.cbpa.2018.02.010.

Hachero-Cruzado, I., Forniés, A., Herrera, M., Mancera, J.M., & Martínez-Rodríguez, G. (2012). Sperm production and quality in Brill scophthalmus rhombus L.: Relation to circulating sex steroid levels. *Fish Physiology and Biochemistry*, *39*(2), 215-220. https://doi.org/10.1007/s10695-012-9692-3

Hatef, A., Alavi, S.M.H., Milla, S., Křišťan, J., Golshan, M., Fontaine, P., & Linhart, O. (2012). Anti-androgen vinclozolin impairs sperm quality and steroidogenesis in goldfish. *Aquatic Toxicology*, *122-123*, 181-187. https://doi.org/10.1016/j.aquatox.2012.06.009.

Hellqvist, A., Schmitz, M., Mayer, I., & Borg, B. (2006). Seasonal changes in expression of LH-beta and FSH-beta in male and female three-spined stickleback, Gasterosteus aculeatus. *General and Comparative Endocrinology*, *145*(3), 263-269. https://doi.org/10.1016/j.ygcen.2005.09.012

Idler, D.R., Bitners, I.I., & Schmidt, P.J. (1961). 11-KETOTESTOSTERONE: AN ANDROGEN FOR SOCKEYE SALMON. *Canadian Journal of Biochemistry and Physiology*, *39*(11), 1737-1742. https://doi.org/10.1139/o61-191

Jensen, K.M., Kahl, M.D., Makynen, E.A., Korte, J.J., Leino, R.L., Butterworth, B.C., & Ankley, G.T. (2004). Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow. *Aquatic Toxicology*, *70*(2), 99-110. https://doi.org/10.1016/j.aquatox.2004.06.012

Jørgensen, A., Andersen, O., Bjerregaard, P., & Rasmussen, L.J. (2007). Identification and characterization of an androgen receptor from zebrafish Danio rerio. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *146*(4), 561-568. https://doi.org/10.1016/j.cbpc.2007.07.002

Kobayashi, Y., Nozu, R., & Nakamura, M. (2011). Role of estrogen in spermatogenesis in initial phase males of the three-spot wrasse (Halichoeres trimaculatus): effect of aromatase inhibitor on the testis. *Developmental Dynamics*, *240*(1), 116-121. https://doi.org/10.1002/dvdy.22507

Li, G.L., Liu, X.C., & Lin, H.R. (2007). Seasonal changes of serum sex steroids concentration and aromatase activity of gonad and brain in red-spotted grouper (Epinephelus akaara). *Animal Reproduction Science*, *99*(1-2), 156-166. https://doi.org/10.1016/j.anireprosci.2006.05.015

Li, M., Liu, X., Dai, S., Xiao, H., Qi, S., Li, Y., Zheng, Q., Jie, M., Cheng, C.H.K., & Wang, D. (2020). Regulation of spermatogenesis and reproductive capacity by Igf3 in tilapia. *Cellular and Molecular Life Sciences*, *77*(23), 4921-4938. https://doi.org/10.1007/s00018-019-03439-0

Liu, Z.H., Chen, Q.L., Chen, Q., Li, F., & Li, Y.W. (2018). Diethylstilbestrol arrested spermatogenesis and somatic growth in the juveniles of yellow catfish (*Pelteobagrus fulvidraco*), a fish with sexual dimorphic growth. *Fish Physiology and Biochemistry*, *44*(3), 789-803. https://doi.org/10.1007/s10695-018-0469-1

Ma, Y.X., Matsuda, K. & Uchiyama, M. (2005). Seasonal variations in plasma concentrations of sex steroid hormones and vitellogenin in wild male Japanese dace (Triboldon hakonesis) collected from different sites of the Jinzu river basin. *Zoological Science*, *22*(8), 861-868.

https://doi.org/10.2108/zsj.22.861

Malison, J.A., Procarione, L.S., Barry, T.P., Kapuscinski, A.R., & Kayes, T.B. (1994). Endocrine and gonadal changes during the annual reproductive cycle of the freshwater teleost,Stizostedion vitreum. *Fish Physiology and Biochemistry*, *13*(6), 473-484. DOI: 10.1007/BF00004330

Manire, C.A., Rasmussen, L.E., & Gross, T.S. (1999). Serum steroid hormones including 11-ketotestosterone, 11-ketoandrostenedione, and dihydroprogesterone in juvenile and adult bonnethead sharks, Sphyrna tiburo. *Journal of Experimental Zoology*, *284*(5), 595-603. https://doi.org/10.1002/(SICI)1097-010X(19991001)284:5<595::AID-JEZ15>3.0.CO;2-6

Melo, M.C., van Dijk, P., Andersson, E., Nilsen, T.O., Fjelldal, P.G., Male, R., Nijenhuis, W., Bogerd, J., de França, L.R., Taranger, G.L., & Schulz R.W. (2015). Androgens directly stimulate spermatogonial differentiation in juvenile Atlantic salmon (Salmo sala). *General and Comparative Endocrinology*, 211, 52-61. https://doi.org/10.1016/j.ygcen.2014.11.015.

Miura, C. & Miura, T. (2011). Analysis of spermatogenesis using an eel model. *Aqua-BioScience Monographs*, *4*(4), 105-129. doi:10.5047/absm.2011.00404.0105

Miura, C., Kuwahara, R., & Miura, T. (2007). Transfer of spermatogenesis-related cDNAs into eel testis germ-somatic cell coculture pellets by electroporation: methods for analysis of gene function. *Molecular Reproduction and Development*, *74*(4), 420-427. https://doi.org/10.1002/mrd.20653

Miura, C., Takahashi, N., Michino, F., & Miura, T. (2005). The effect of para-nonylphenol on Japanese eel (Anguilla japonica) spermatogenesis in vitro. *Aquatic Toxicology*, *71*(2), 133-141. https://doi.org/10.1016/j.aquatox.2004.10.015

Miura, S., Horiguchi, R., & Nakamura, M. (2008). Immunohistochemical evidence for 11beta-hydroxylase (P45011beta) and androgen production in the gonad during sex differentiation and in adults in the protandrous anemonefish Amphiprion clarkii. *Zoological Science*, *25*(2), 212-219. https://doi.org/10.2108/zsj.25.212

Miura, T. & Miura, C. (2001). Japanese Eel: A Model for Analysis of Spermatogenesis. *Zoological Science*, *18*(8), 1055-1063. https://doi.org/10.2108/zsj.18.1055

Miura, T., Ando, N., Miura, C., & Yamauchi, K. (2002). Comparative studies between in vivo and in vitro spermatogenesis of Japanese eel (*Anguilla japonica*). *Zoological Science*, *19*(3), 321-329. https://doi.org/10.2108/zsj.19.321

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. (1991). Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). *Proceedings of the National Academy of Sciences of the United States of America*, *88*(13), 5774–5778. https://doi.org/10.1073/pnas.88.13.5774

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. (1992). The role of hormones in the acquisition of sperm motility in salmonid fish. *Journal of Experimental Biology, 261*(3), 359-363. https://doi.org/10.1002/jez.1402610316

Nader, M.R., Miura, T., Ando, N., Miura, C., & Yamauchi, K. (1999). Recombinant Human Insulin-Like Growth Factor I Stimulates All Stages of 11-Ketotestosterone-Induced Spermatogenesis in the Japanese Eel, *Anguilla japonica*, In Vitro. *Biology of Reproduction*, *61*(4), 944–947. https://doi.org/10.1095/biolreprod61.4.944

Nagahama, Y., Miura, T., & Kobayashi, T. (1994). The onset of spermatogenesis in fish. *Ciba Foundation Symposium*, *182*, 255-267. DOI: 10.1002/9780470514573.ch14

Ohta, T., Miyake, H., Miura, C., Kamei, H., Aida, K., & Miura, T. (2007). Follicle-Stimulating Hormone Induces Spermatogenesis Mediated by Androgen Production in Japanese Eel, Anguilla japonica. *Biology of Reproduction*, *77*(6), 970–977. https://doi.org/10.1095/biolreprod.107.062299

Onuma, T.A., Sato, S., Katsumata, H., Makino, K., Hu, W., Jodo, A., Davis, N.D., Dickey, J.T., Ban, M., Ando, H., Fukuwaka, M., Azumaya, T., Swanson, P., Urano, A. (2009). Activity of the pituitary-gonadal axis is increased prior to the onset of spawning migration of chum salmon. *Journal of Experimental Biology, 212*(1), 56-70. doi: 10.1242/jeb.021352.

Orlando, E.F., Binczik, G.A., Thomas, P., & Guillette, L.J. Jr. (2003). Reproductive seasonality of the male Florida gar, Lepisosteus platyrhincus. *General and Comparative Endocrinology*, *131*(3), 365–371. https://doi.org/10.1016/S0016-6480(03)00036-4

Ozaki, Y., Higuchi, M., Miura, C., Yamaguchi, S., Tozawa, Y., & Miura, T. (2006). Roles of 11beta-hydroxysteroid dehydrogenase in fish spermatogenesis. *Endocrinology*, *147*(11), 5139-5146. https://doi.org/10.1210/en.2006-0391

Pereira, T.S., Boscolo, C.N., Silva, D.G., Batlouni, S.R., Schlenk, D., & Almeida, E.A. (2015). Anti-androgenic activities of diuron and its metabolites in male Nile tilapia (Oreochromis niloticus). *Aquatic Toxicology*, *164*, 10-15. https://doi.org/10.1016/j.aquatox.2015.04.013

Reyes, J.A., Vidal-Dorsch, D.E., Schlenk, D., Bay, S.M., Armstrong, J.L., Gully, J.R., Cash, C., Baker, M., Stebbins, T.D., Hardiman, G. & Kelley, K.M. (2012). Evaluation of reproductive endocrine status in hornyhead turbot sampled from southern California's urbanized costal environments. *Environmental Toxicology and Chemistry, 31*(12), 2689-2700. https://doi.org/10.1002/etc.2008

Runnalls, T.J., Hala, D.N., & Sumpter, J.P. (2007). Preliminary studies into the effects of the human pharmaceutical Clofibric acid on sperm parameters in adult Fathead minnow. *Aquatic Toxicology*, *84*(1), 111-118. https://doi.org/10.1016/j.aquatox.2007.06.005

Sakai, N., Ueda, H., Suzuki, N., & Nagahama, Y. (1989). Steroid production by amago salmon (Oncorhynchus rhodurus) testes at different development stages. *General and Comparative Endocrinology*, *75*(2), 231-240. DOI: 10.1016/0016-6480(89)90075-0

Sales, C.F., Barbosa Pinheiro, A.P., Ribeiro, Y.M., Weber, A.A., Paes-Leme, F.O., Luz, R.K., Bazzoli, N., Rizzo, E., & Melo, R.M.C. (2020). Effects of starvation and refeeding cycles on spermatogenesis and sex steroids in the Nile tilapia Oreochromis niloticus. *Molecular and Cellular Endocrinology*, *500*, 110643. https://doi.org/10.1016/j.mce.2019.110643

Schiavone, R., Zilli, L., Storelli, C., & Vilella, S. (2011). Changes in hormonal profile, gonads and sperm quality of *Argyrosomus regius* (Pices, Scianidae) during the first sexual differentiation and maturation. *Theriogenology, 77*(5), 888-898. https://doi.org/10.1016/j.theriogenology.2011.09.014

Scott, A.P., Bye, V.J., Baynes, S.M., & Springate, J.R.C. (1980). Seasonal variations in plasma concentrations of 11-ketotestosterone and testosterone in male rainbow trout, Salmo gairdnerii Richardson. *Journal of Fish Biology*, *17*(5), 495-505. https://doi.org/10.1111/j.1095-8649.1980.tb02781.x

Selvaraj, S., Ohga, H., Nyuji, M., Kitano, H., Nagano, N., Yamaguchi, A., & Matsuyama, M. (2013). Subcutaneous administration of Kiss1 pentadecapeptide accelerates spermatogenesis in prepubertal male chub mackerel (*Scomber japonicus*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *166*(2), 228-36. https://doi.org/10.1016/j.cbpa.2013.06.007

Shahi, N., Mallik, S.K., Pande, J., Das, P., & Singh, A.K. (2015). Spermatogenesis and related plasma androgen and progestin level in wild male golden mahseer, Tor putitora (Hamilton, 1822), during the spawning season. *Fish Physiology and Biochemistry*, *41*(4), 909-920. https://doi.org/10.1007/s10695-015-0057-6

Shu, T., Zhai, G., Pradhan, A., Olsson, P.E., & Yin, Z. (2020). Zebrafish cyp17a1 knockout reveals that androgen-mediated signaling is important for male brain sex differentiation. *General and Comparative Endocrinology, 295,* 113490. https://doi.org/10.1016/j.ygcen.2020.113490

Singh, P.B. & Singh, V. (2008). Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17beta and 11-ketotestosterone, and sperm motility in *Heteropneustes fossilis* (Bloch). *Chemosphere, 72*(3), 422-431. https://doi.org/10.1016/j.chemosphere.2008.02.026

Sisneros, J.A., Forlano, P.M., Knapp, M., & Bass, A.H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. *General and Comparative Endocrinology, 136*(1), 101-116. https://doi.org/10.1016/j.ygcen.2003.12.007

Takeo, J. & Yamashita, S. (2000). Rainbow trout androgen receptor-alpha fails to distinguish between any of the natural androgens tested in transactivation assay, not just 11-ketotestosterone and testosterone. *General and Comparative Endocrinology*, *117*(2), 200-206. https://doi.org/10.1006/gcen.1999.7398

Tang, H., Chen, Y., Wang, L., Yin, Y., Li, G., Guo, Y., Liu, Y., Lin, H., Cheng, C.H.K., & Liu, X. (2018). Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor. *Biology of Reproduction*, *98*(2), 227-238. https://doi.org/10.1093/biolre/iox165

Ueda, H., Nagahama, Y., Tashiro, F., & Crim, L.W. (1983). Some endocrine aspects of precocious sexual maturation in the amago salmon, *Oncorhynchus rhodurus*. *Bulletin of the Japanese Society of Scientific Fisheries*, *49*(4), 587–596. DOI: 10.2331/suisan.49.587

Velasco-Santamaría, Y.M., Korsgaard, B., Madsen, S.S., & Bjerregaard, P. (2011). Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (Danio rerio). *Aquatic Toxicology*, *105*(1-2), 107-118. https://doi.org/10.1016/j.aquatox.2011.05.018

Weil, C., & Marcuzzi, O. (1990). Cultured pituitary cell GtH response to GnRH at different stages of rainbow trout spermatogenesis and influence of steroid hormones. *General and Comparative Endocrinology*, *79*(3), 492-498. DOI: 10.1016/0016-6480(90)90080-6

Weltzien, F.A., Taranger, G.L., Karlsen, Ø., Norberg, B. (2002). Spermatogenesis and related plasma androgen levels in Atlantic halibut (Hippoglossus hippoglossus L.). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *132*(3), 567-575. https://doi.org/10.1016/S1095-6433(02)00092-2

Xia, H., Zhong, C., Wu, X., Chen, J., Tao, B., Xia, X., Shi, M., Zhu, Z., Trudeau, V. L., & Hu, W. (2018). *Mettl3* mutation disrupts gamete maturation and reduced fertility in zebrafish. *Genetics, 208*(2), 729-743. doi: 10.1534/genetics.117.300574

Yin, P., Li, Y.W., Chen, Q.L., & Liu, Z.H. (2017). Diethylstilbestrol, flutamide and their combination impaired the spermatogenesis of male adult zebrafish through disrupting HPG axis, meiosis and apoptosis. *Aquatic Toxicology*, *185*, 129-137. https://doi.org/10.1016/j.aquatox.2017.02.013

Yin, P., Li, Y.W., Chen, Q.L., & Liu, Z.H. (2017). Diethylstilbestrol, flutamide and their combination impaired the spermatogenesis of male adult zebrafish through disrupting HPG axis, meiosis and apoptosis. *Aquatic Toxicology*, *185*, 129-137. https://doi.org/10.1016/j.aquatox.2017.02.013

Zhang, Q., Ye, D., Wang, H., Wang, Y., Hu, W., Sun, Y. (2020). Zebrafish cyp11c1 Knockout Reveals the Roles of 11-ketotestosterone and Cortisol in Sexual Development and Reproduction. *Endocrinology*, *161*(6). https://doi.org/10.1210/endocr/bqaa048

Zheng, Q., Xiao, H., Shi, H., Wang, T., Sun, L., Tao, W., Kocher, T.D., Li, M., & Wang, D. (2020). Loss of cyp11c1 causes delayed spermatogenesis due to the absence of 11-ketotestosterone. *Journal of Endocrinology*, *244*(3), 487-499. https://doi.org/10.1530/JOE-19-0438

Zheng, Q., Xiao, H., Shi, H., Wang, T., Sun, L., Tao, W., Kocher, T.D., Li, M., & Wang, D. (2020). Loss of Cyp11c1 causes delayed spermatogenesis due to the absence of 11-ketotestosterone. *Journal of Endocrinology*, *244*(3), 498-499. DOI: https://doi.org/10.1530/JOE-19-0438

Relationship: 2937: Impaired, Spermatogenesis leads to Decreased, Viable Offspring

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased			

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
11-Ketotestosterone	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Male	High

Taxonomic Applicability: Spermatogenesis is one of the most conserved biological processes from *Drosophila* to humans (Wu et al., 2016). As a result, animals who utilize sexual reproduction as their way to produce offspring are heavily reliant on spermatogenesis being effective and normal. There are studies on reproduction and spermatogenesis across a multitude of taxa.

Sex Applicability: Spermatogenesis is a male-specific process (Schulz et al., 2010, Tang et al., 2018, Wu et al., 2015). Thus, the present relationship is only relevant for males.

Life Stage Applicability: Spermatogenesis and reproduction are only relevant for sexually-mature adults.

Key Event Relationship Description

Spermatogenesis is a multiphase process of cellular transformation that produces mature male gametes known as sperm for sexual reproduction. The process of spermatogenesis can be broken down into 3 phases: the mitotic proliferation of spermatogonia, meiosis, and post meiotic differentiation (spermiogenesis) (Boulanger et al., 2015). Male fertility is dependent on the quantity as well as the proper cellular morphology of the sperm formed in the testes. The fusion of sperm and oocytes is the key step for the beginning of life known as fertilization. Oocyte fertilization and the production of viable offspring from sexual reproduction are dependent on spermatogenesis and sufficient quantity and quality of sperm. When the impairment of spermatogenesis occurs, it can result in impaired reproduction with a decrease in viable offspring.

Evidence Supporting this KER

Table 1A - Concordance table [authors A-N] (full table as PDF)

Species	Experimental design	Evidence of Impaired Spermatogenesis (IS)	Evidence of Viable Offspring, Decreased (VOD)	IS observed?	VOD observed?	Citation	Notes
Zebrafish (*Danio rerio*)	Two generation exposure to 1nM BPA	• Significant decrease in sperm density of F1 and F2 males compared to control • Decreased sperm quality as measured by motility, velocity, ATP content and lipid peroxidation in F1 and F2 males	• Delayed hatching at 48hpf and increased malformation and mortality were observed in the offspring from BPA-exposed F2; paternal-specific resulting from BPA-exposed males • No significant difference in egg production and fertilization of F1 and F2 females	Yes	No: F1 and F2 Yes: offspring of F2	Chen et al., 2015	Female-biased sex ratio observed in both F1 and F2 adults

Tilapia (*Oreochromis niloticus*)	CRISPR/Cas9 mediated mutation of *eEF1A1b*; F1 sampled at 90, 120, 150 and 180 days after hatch	• Significant downregulation of key genes involving spermatogenesis • Spermatogenesis arrested; reduced number of spermatogonia and spermatocytes • Altered morphology • Delayed spermatogenesis • Reduced motility	• Reduced in vitro fertilization rate (5% vs 80% in WT) due to abnormal spermiogenesis	Yes	Yes	Chen et al., 2017	eEF1A1b - elongation factor
Zebrafish (*Danio rerio*)	Adult males exposed to two concentrations of bis-(2-ethylexhyl) phthalate (DEHP; 0.2 or 20 µg/L) for three weeks; 25 ng ethynylestradiol positive control	• Areas of spermatogonial and spermatid cysts were larger in fish exposed to 20 µg/L DEHP compared with controls • Testicular area of spermatocyte cysts was lower in males exposed to 0.2 µg/L DEHP • Testicular area occupied by spermatocytes was reduced in fish exposed to DEHP compared to controls, with a concomitant increase in the area occupied by spermatogonia	• Significant decrease in embryo production (up to 90%) observed in males treated with DEHP (0.2 and 20 µg/L) • Hatch rate of embryos significantly lower in DEHP-exposed males	Yes	Yes	Corradetti et al., 2013	Reproductive performance evaluated with untreated females in clean water
Zebrafish (*Danio rerio*)	Targeted genetic disruption of *tdrd12* through TALEN techniques	• Reduced expression of germ cell markers *vasa, dnd, piwil1* and *amh* in mutants • Deformed and apoptotic spermatogonia at 35 dpf found in mutants • Lack of spermatozoa at adult stage	• Infertile under standard breeding despite being able to induce female egg laying (0% fertilization)	Yes	Yes	Dai et al., 2017	Tudor domain-related proteins (Tdrds) have been demonstrated to be involved in spermatogenesis and Piwi-interacting RNA (piRNA) pathway
Zebrafish (*Danio rerio*)	Fish were exposed from 2 to 60 days post-hatch (dph) to nonylphenol (NP; 10, 30, or 100 µg/L nominal) or ethinylestradiol (EE2; 1, 10, or 100 ng/l	• Majority of fish exposed to 10 ng/l EE lacked differentiated gonadal tissue (undeveloped gonads) at 60 dph • One fish at NP-30 µg/l and two	• Zebrafish exposed to 10 ng/l of EE exhibited a significant reduction in the percent of viable eggs (clear vs opaque)	Yes	Yes	Hill and Janz, 2003	Due to high mortality in the 100 ng/l EE group, insufficient fish were available for analyses

	nominal); reared until adulthood (120 dph) for breeding studies	fish at NP-100 µg/l were observed to have ovatestes at 60 dph	• Significant decrease in hatch and swim-up success observed with EE2 and 100 µg NP/L				
Roach (*Rutilus rutilus*)	Mature adult roach collected from both reference and river (effluent contaminated) sites during two consecutive spawning seasons; artificially induced to spawn in laboratory	• Volume of milt released from spermiating male fish significantly lower in the intersex fish than in the reference males • Most fish that did not spermiate had testes that were clearly immature	• Fertilization rate significantly reduced when sperm from intersex males used to fertilize eggs collected from females • Both proportion of fertilized embryos reaching eyed stage and hatching success decreased with increased feminization	Yes	Yes	Jobling et al., 2002	Embryo viability was determined after 24 h (fertilization success), at eyed stage and at swim-up stage (hatching success)
Japanese medaka (*Oryzias latipes*)	Adult medaka exposed for 21 days to 29.3, 55.7, 116, 227, and 463 ng/L 17β-estradiol (E2)	• In males exposed to 463 ng/l, a few oocytes were observed in testis, and testicular tissue almost completely replaced by connective tissue • Accompanied by presence of macroscopic atrophy and degenerated spermatozoa and spermatocytes suggest a lack of spermatogenesis	• Total number of egg spawned and fertility significantly reduced at 463 ng/l E2 compared to the control	Yes	Yes	Kang et al., 2002	
Zebrafish (*Danio rerio*)	Founder fish with originally *mlh1* mutation was crossed out twice to WT fish of the TL line from which the founder was generated	• Significant decrease in weight of spermatids and spermatozoa); some spermatozoa were visible in testes of all mutant fish • Increased number and proportion of spermatogenic stages prior to spermatids compared to WT • Increase in	• Reduced fertilization rates under standard breeding conditions (0.4%) • Eggs fertilized from mutant sperm were malformed and and aneuploid	Yes	Yes	Leal et al., 2008	Mlh1 is a member of DNA mismatch repair machinery and essential for stabilization of crossovers during first meiotic division

		apoptotic cells					
Zebrafish (*Danio rerio*)	3-month-old male fish exposed to 10 ug/L of DEHP for 3 months	• No effect	• No effect	No	No	Ma et al., 2018	Semi-static exposure; half water renewed daily and whole water renewed weekly; exposed males mated with WT females
	3-month-old male fish exposed to 30 ug/L of DEHP for 3 months	• No effect	• Concentration-dependent decrease in fertilization rate	No	No		
	3-month-old male fish exposed to 100 ug/L of DEHP for 3 months	• Percent of spermatocytes increased significantly by 27.4% • Significant decrease of 32.2% in spermatids	• Significant decrease in fertilization rate by 22% compared to the control	Yes	Yes		
Zebrafish (*Danio rerio*)	Multi-generational study to 0.5, 5 and 50 ng/L ethynylestradiol (EE2) or 5 ng/L 17β-estradiol (E2)	• None of the F_1 males exposed to 5 ng/L EE2 had normal testes; 43% had gonads not fully differentiated	• Time-related decrease in egg production and egg viability 14 hpf in F_0 generation at 50 ng/L EE2 and no survival of F_1 100 hpf; no eggs produced after 10 d exposure • Exposure to 5 ng/L EE2 in the F_1 caused a 56% reduction in fecundity and no survival past 14 hpf • Proportion of nonviable eggs significantly higher for all treatments compared to control	Yes	Yes	Nash et al., 2004	

Biological Plausibility

Spermatogenesis is one of the most conserved biological processes from *Drosophila* to humans (Wu et al., 2016). The process itself is well understood and gametes produced from spermatogenesis are required for sexual reproduction.

Empirical Evidence

Dose concordance

- When exposed to 50 mg DEHP kg-1 via intraperitoneal injection for 10 days, zebrafish experienced a reduction in the proportion of spermatozoa present compared to the control group. However, at this exposure concentration there was no effect on evidence for decrease in viable offspring. Whereas when exposed to 5000 mg of DEHP kg-1, there was a significantly lower proportion of spermatozoa and a significant decrease in fertilization success (Uren-Webster et al., 2010).
- When exposed to DEHP for 3 months, zebrafish had a significant decrease in spermatids and increase in spermatocytes at the highest exposure concentration (100 ug/L) and no effect at the lowest exposure concentration (10 ug/L) (Ma et al. 2018)

Table 1B - Concordance table [authors O-Z] (full table as PDF)

Species	Experimental design	Evidence of Impaired Spermatogenesis (IS)	Evidence of Viable Offspring, Decreased (VOD)	IS observed?	VOD observed?	Citation	Notes
Zebrafish (*Danio rerio*)	Targeted genetic disruption of *fdx1b* using a TALEN approach	• Reduced sperm count compared to control (p=0.0097%) • Promale *sox9a* downregulated • Spermatogenic genes *igf3* and *insl3* downregulated	• Infertile under standard breeding despite being able to cause spawning of eggs (0% fertilization)	Yes	Yes	Oakes et al., 2019	fdx1b is an electron- providing cofactor for steroidogenic cytochrome P450
Zebrafish (*Danio rerio*)	• ENU mutagenesis screen to find mutations that lead to defects in gonadogenesis • 3 mutants focused on (*its, isa, imo*)	• Post meiotic germ cells absent at 3 months age (found aberrant germ cells instead) • Only spermatogonia and primary spermatocytes were present; no spermatids or sperm observed	• Decreased fertilization rates in cells from mutant testes (<2% vs 41.9-65.8 in WT) • Only 1 mutant embryo survived at 1 dpf compared to nearly 100% in WT	Yes	Yes	Saito et al., 2011	ENU= N-ethyl-N-nitrosourea
Zebrafish (*Danio rerio*)	*hsf5* mutants obtained by CRISPR/Cas9 technology targeting exon2	• Loss of spermatozoa along with increase in primary spermatocytes compared to WT • Decrease in sperm count and sperm motility • Altered morphology (microtubule arrangement, flagellar axoneme, sperm heads)	• No viable offspring when mutants were crossed with any types of females • Lethality of embryos via in vitro fertilization with WT females (before 1 dpf)	Yes	Yes	Saju et al., 2018	Heat shock protein 5
Medaka (*Oryzias latipes*)	Mature fish exposed to 32.6, 63.9, 116, 261, and 488 ng ethinylestradiol (EE2)/L for 21 d under flow-through conditions	• Testicular tissue composed of abnormally developed connective tissue, with only a few sper-matozoa and spermatocytes compared to control	• Significant decrease in fecundity observed at 448 ng/L	Yes	Yes	Seki et al., 2002	
Zebrafish (*Danio rerio*)	• *ar* mutant line generated using TALENs	• Upregulation of *amh* and *gsdf* • Downregulation *igf3*	• Reduced in vitro fertilization rate ≤ 20%	Yes	Yes	Tang et al., 2018	Androgen receptor

		• Reduced number of sperm • Reduction in number of germ cells observed in AR mutant fish • Increased proportion of pre-spermatids sperm cells • Small amount of mature spermatozoon still present in mutants	with WT female				
Mice	• *mPCI* deficient mice	• Morphologically abnormal sperm (lacked tails and were degenerated) • Reduced motility (12.5%) compared to control (51.5%) • Apoptotic spermatocytes likely due to destruction of Sertoli cells	• Reduced in vivo fertilization rate (0.5%) vs control (94%) with WT females	Yes	Yes	Uhrin et al., 2000	• PCI - present in seminal plasma; inhibitor of activated protein C and a variety of proteases
Zebrafish (*Danio rerio*)	Adult males exposed to 0.5 mg DEHP kg-1 (body weight) for 10 days via intraperitoneal injection	• No effect	• No effect	No	No	Uren-Webster et al., 2010	DEHP is phthalate which is a plasticizer in many mass-produced products
	Adult males exposed to 50 mg DEHP kg-1 for 10 days via intraperitoneal injection	• Significantly lower proportion of spermatozoa and a significantly greater proportion of spermatocytes	• No effect	Yes	No		
	Adult males exposed to 5000 mg DEHP kg-1 for 10 days via intraperitoneal injection	• Significantly lower proportion of spermatozoa and a significantly greater proportion of spermatocytes	• Significant decrease in fertilization success of males, especially during the second 5-day period of exposure	Yes	Yes		
Mice (C57BL/6)	BRD7-deficient mice	• Irregular head shape • Deformed acrosome • Post-meiotic development of elongating spermatids disrupted	• WT female mice coupled with homozygous mutant males did not produce any pups	Yes	Yes	Wang et al., 2016	• BRD7 is a bromodomain gene that inhibits cell growth and cell cycle progression and is a co-factor for p53

		• Abnormal morphology and degeneration of spermatids • Increased proportion of abnormal spermatids • Downregulation of various spermatogenic markers					• BRD7 has high expression in mice testes
Zebrafish (*Danio rerio*)	*mettl3* mutant fish generated using TALENs	• Significantly increased proportions of spermatogonia (24.4% vs 7.5% in WT) and spermatocytes (56.1% vs 26.7% in WT) • Significantly decreased proportion of spermatozoa (10.4% vs 50.1% in WT) • Very little or no mature sperm • Sperm motility significantly reduced (average path velocity, curvilinear velocity, and straight-line velocity)	• Decreased fertilization rate (48.8.% vs 91.4% in WT) • 8.1% of mutant male x WT female spawned successfully vs 94.4% in WT	Yes	Yes	Xia et al., 2018	MEttl3 - multicomponent methyltransferase complex
Zebrafish (*Danio rerio*)	CRISPR/Cas9 gene targeting of E2f5	• Reduced number of spermatozoa compared to WT • Increased % of spermatocytes at leptotene and zygotene stages compared to WT • Suggests arrest of spermatogenesis at zygotene stage; later stages rarely observed • Increased germ cell apoptosis	• Decreased fertilization rates (3% vs 94% in WT) under standard breeding conditions	Yes	Yes	Xie et al., 2020	E2f5 is a transcriptional repressor during cell-cycle progression
Marine medaka (*Oryzias melastigma*)	0.1 mg/L of DEHP for 6 months from larval stage	• Contained mostly spermatocytes (Sp) and spermatids (Sd) with few spermatozoa especially in this treatment	• Significant decrease in fecundity compared to control (21.78 vs 29.89 eggs/f/d) • Significant decrease in	Yes	Yes	Ye et al., 2014	DEHP - phthalate MEHP - active metabolite of DEHP; fertilization success defined as proportion of fertilized eggs

			fertilization success (84.12 vs 94.21%)				
	0.5 mg/L of DEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fecundity compared to control (20.44 vs 29.89 eggs/f/d) • Significant decrease in fertilization success (81.61 vs 94.21%)	Yes	Yes		
	0.1 mg/L of MEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fertilization success vs control (87.46% vs 94.21%)	Yes	Yes		
	0.5 mg/L of MEHP for 6 months from larval stage	• Contained mostly Sp and Sd with few spermatozoa	• Significant decrease in fertilization success vs control (82.16% vs 94.21%)	Yes	Yes		

Uncertainties and Inconsistencies

- When exposed to 10 and 100 ng/L of EE2 for 62 days leading to spawning, rainbow trout exhibited an increase in sperm density, concentration, and spermatocrit and decrease in GSI but overall there were no significant changes to spermatogenesis. Despite this, there was a decrease in viability of embryos (Schultz et al., 2003).
- Two-generation zebrafish study with 1 nM bisphenol A (BPA) showed a significant decrease in sperm density along with decreased sperm quality, however, no significant different in egg fertilization (Chen et al., 2015).
- There are multiple other factors involved in producing viable offspring, including but not limited to oocyte maturation and ovulation, development including successful organogenesis, and adequate nutrition.

Quantitative Understanding of the Linkage

Response-response relationship

Empirical response-response data is very limited; thus, the response-response relationship has not yet been evaluated.

Time-scale

- The duration of spermatogenesis in humans is reported to be 74 days (Griswold, M.D, 2016). Consequently, effects on spermatogenesis may not manifest as observable impacts on fertility until perhaps 74 days after impacts on spermatogenesis began. This may vary depending on the stage(s) of spermatogenesis that are impacted by the stressor.
- The duration of the meiotic and spermiogenic phases in zebrafish is reported to be 6 days which means there could be a delay of at least 6 days before signs of impaired fertility and downstream effects may be detected (Leal et al., 2009).

Known Feedforward/Feedback loops influencing this KER

Feedforward/feedback loops haven't been evaluated yet. However, given that that oocyte fertilization and production of viable offspring are external to the male it seems unlikely there would feedback that impacts spermatogenesis.

References

Boulanger, G., Cibois, M., Viet, J., Fostier, A., Deschamps, S., Pastezeur, S., Massart, C., Gschloessl, B., Gautier-Courteille, C., & Paillard, L. (2015). Hypogonadism Associated with Cyp19a1 (Aromatase) Posttranscriptional Upregulation in Celf1 Knockout Mice. Molecular and cellular biology, 35(18), 3244–3253. https://doi.org/10.1128/MCB.00074-15

Chen, J., Jiang, D., Tan, D., Fan, Z., Wei, Y., Li, M., & Wang, D. (2017). Heterozygous mutation of eEF1A1b resulted in spermatogenesis arrest and infertility in male tilapia, Oreochromis niloticus. *Scientific reports*, *7*, 43733. https://doi.org/10.1038/srep43733

Chen, J., Xiao, Y., Gai, Z., Li, R., Zhu, Z., Bai, C., Tanguay, R. L., Xu, X., Huang, C., & Dong, Q. (2015). Reproductive toxicity of low level bisphenol A exposures in a two-generation zebrafish assay: Evidence of male-specific effects. Aquatic toxicology (Amsterdam, Netherlands), 169, 204–214. https://doi.org/10.1016/j.aquatox.2015.10.020

Chen, J., Xiao, Y., Gai, Z., Li, R., Zhu, Z., Bai, C., Tanguay, R. L., Xu, X., Huang, C., & Dong, Q. (2015). Reproductive toxicity of low level bisphenol A exposures in a two-generation zebrafish assay: Evidence of male-specific effects. *Aquatic toxicology (Amsterdam, Netherlands)*, *169*, 204–214. https://doi.org/10.1016/j.aquatox.2015.10.020

Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., and Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (*Danio rerio*). Reprod Biol. 13(3):195-202.

Dai, X., Shu, Y., Lou, Q., Tian, Q., Zhai, G., Song, J., Lu, S., Yu, H., He, J., & Yin, Z. (2017). Tdrd12 Is Essential for Germ Cell Development and Maintenance in Zebrafish. *International journal of molecular sciences*, *18*(6), 1127. https://doi.org/10.3390/ijms18061127

Griswold M. D. (2016). Spermatogenesis: The Commitment to Meiosis. Physiological reviews, 96(1), 1–17. https://doi.org/10.1152/physrev.00013.2015

Hill, R.L Jr and Janz, D.M. (2003). Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio breeding success. Aquat Toxicol. 63(4):417-429.

Jobling, S., Coey, S., Whitmore, J.G., Kime, D.E., Van Look, K.J.W., McAllister, B.G., Beresford, N., Henshaw, A.C., Brighty, G., Tyler, C.R., and Sumpter, J.P. (2002). Wild intersex roach (*Rutilus rutilus*) have reduced fertility. Biol Reprod. 67(2):515–524.

Kang, I.J., Yokota, H., Oshima, Y., Tsuruda, Y., Yamaguchi, T., Maeda, M., Imada, N., Tadokoro, H., and Honjo, T. (2002). Effect of 17β-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*). Chemosphere 47(1): 71-80,

Leal, M. C., Cardoso, E. R., Nóbrega, R. H., Batlouni, S. R., Bogerd, J., França, L. R., & Schulz, R. W. (2009). Histological and stereological evaluation of zebrafish (Danio rerio) spermatogenesis with an emphasis on spermatogonial generations. Biology of reproduction, 81(1), 177–187. https://doi.org/10.1095/biolreprod.109.076299

Leal, M. C., Feitsma, H., Cuppen, E., França, L. R., & Schulz, R. W. (2008). Completion of meiosis in male zebrafish (Danio rerio) despite lack of DNA mismatch repair gene mlh1. *Cell and tissue research*, *332*(1), 133–139. https://doi.org/10.1007/s00441-007-0550-z

Ma, Yan-Bo, Jia, Pan-Pan, Junaid, Muhammad, Yang, Li, Lu, Chun-Jiao, & Pei, De-Sheng. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution (1987), 237, 1050-1061.

Ma, Yan-Bo, Jia, Pan-Pan, Junaid, Muhammad, Yang, Li, Lu, Chun-Jiao, & Pei, De-Sheng. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. *Environmental Pollution* (1987), 237, 1050-1061.

Nash, J.P, Kime, D.E., Van der Ven, Leo T.M., Wester, P.W., Brion, F., Maack, G., Stahlschmidt-Allner, P., and Tyler, C.R., (2004). Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ Health Perspect 112(17):1725-1733.

Oakes, J. A., Li, N., Wistow, B., Griffin, A., Barnard, L., Storbeck, K. H., Cunliffe, V. T., & Krone, N. P. (2019). Ferredoxin 1b Deficiency Leads to Testis Disorganization, Impaired Spermatogenesis, and Feminization in Zebrafish. *Endocrinology*, *160*(10), 2401–2416. https://doi.org/10.1210/en.2019-00068

Saito, K., Siegfried, K. R., Nüsslein-Volhard, C., & Sakai, N. (2011). Isolation and cytogenetic characterization of zebrafish meiotic prophase I mutants. *Developmental dynamics : an official publication of the American Association of Anatomists*, *240*(7), 1779–1792. https://doi.org/10.1002/dvdy.22661

Saju, J. M., Hossain, M. S., Liew, W. C., Pradhan, A., Thevasagayam, N. M., Tan, L., Anand, A., Olsson, P. E., & Orbán, L. (2018). Heat Shock Factor 5 Is Essential for Spermatogenesis in Zebrafish. *Cell reports*, *25*(12), 3252–3261.e4. https://doi.org/10.1016/j.celrep.2018.11.090

Schultz, I. R., Skillman, A., Nicolas, J. M., Cyr, D. G., & Nagler, J. J. (2003). Short-term exposure to 17 alpha-ethynylestradiol decreases the fertility of sexually maturing male rainbow trout (Oncorhynchus mykiss). Environmental toxicology and chemistry, 22(6), 1272–1280.

Schulz, R. W., de França, L. R., Lareyre, J. J., Le Gac, F., Chiarini-Garcia, H., Nobrega, R. H., & Miura, T. (2010). Spermatogenesis in fish. *General and comparative endocrinology*, *165*(3), 390–411. https://doi.org/10.1016/j.ygcen.2009.02.013

Seki, M., Yokota, H., Matsubara, H., Tsuruda, Y., Maeda, M., Tadokoro, H. and Kobayashi, K. (2002). Effect of ethinylestradiol on the reproduction and induction of vitellogenin and testis-ova in medaka (Oryzias latipes). Environ. Toxicol. Chem. 21(8):1692-1698.

Tang, H., Chen, Y., Wang, L., Yin, Y., Li, G., Guo, Y., Liu, Y., Lin, H., Cheng, C., & Liu, X. (2018). Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor. Biology of reproduction, 98(2), 227–238. https://doi.org/10.1093/biolre/iox165

Tang, H., Chen, Y., Wang, L., Yin, Y., Li, G., Guo, Y., Liu, Y., Lin, H., Cheng, C., & Liu, X. (2018). Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor. *Biology of reproduction*, *98*(2), 227–238. https://doi.org/10.1093/biolre/iox165

Uhrin, P., Dewerchin, M., Hilpert, M., Chrenek, P., Schöfer, C., Zechmeister-Machhart, M., Krönke, G., Vales, A., Carmeliet, P., Binder, B. R., & Geiger, M. (2000). Disruption of the protein C inhibitor gene results in impaired spermatogenesis and male infertility. *The Journal of clinical investigation*, *106*(12), 1531–1539. https://doi.org/10.1172/JCI10768

Uren-Webster, Tamsyn M, Lewis, Ceri, Filby, Amy L, Paull, Gregory C, & Santos, Eduarda M. (2010). Mechanisms of toxicity of di(2-ethylhexyl) phthalate

on the reproductive health of male zebrafish. Aquatic Toxicology, 99(3), 360-369.

Uren-Webster, Tamsyn M, Lewis, Ceri, Filby, Amy L, Paull, Gregory C, & Santos, Eduarda M. (2010). Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. *Aquatic Toxicology*, *99*(3), 360-369.

Wang, H., Zhao, R., Guo, C., Jiang, S., Yang, J., Xu, Y., Liu, Y., Fan, L., Xiong, W., Ma, J., Peng, S., Zeng, Z., Zhou, Y., Li, X., Li, Z., Li, X., Schmitt, D. C., Tan, M., Li, G., & Zhou, M. (2016). Knockout of BRD7 results in impaired spermatogenesis and male infertility. *Scientific reports*, *6*, 21776. https://doi.org/10.1038/srep21776

Wu, H., Sun, L., Wen, Y., Liu, Y., Yu, J., Mao, F., Wang, Y., Tong, C., Guo, X., Hu, Z., Sha, J., Liu, M., & Xia, L. (2016). Major spliceosome defects cause male infertility and are associated with nonobstructive azoospermia in humans. Proceedings of the National Academy of Sciences of the United States of America, 113(15), 4134–4139. https://doi.org/10.1073/pnas.1513682113

Xia, H., Zhong, C., Wu, X., Chen, J., Tao, B., Xia, X., Shi, M., Zhu, Z., Trudeau, V. L., & Hu, W. (2018). *Mettl3* Mutation Disrupts Gamete Maturation and Reduces Fertility in Zebrafish. *Genetics*, *208*(2), 729–743. https://doi.org/10.1534/genetics.117.300574

Xie, H., Kang, Y., Wang, S., Zheng, P., Chen, Z., Roy, S., & Zhao, C. (2020). E2f5 is a versatile transcriptional activator required for spermatogenesis and multiciliated cell differentiation in zebrafish. *PLoS genetics*, *16*(3), e1008655. https://doi.org/10.1371/journal.pgen.1008655

Ye, Ting, Kang, Mei, Huang, Qiansheng, Fang, Chao, Chen, Yajie, Shen, Heqing, & Dong, Sijun. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). *Aquatic Toxicology, 146*, 115-126

Relationship: 2938: Decreased, Viable Offspring leads to Decrease, Population growth rate

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARalpha Agonism Leading to Decreased Viable Offspring via Decreased 11-Ketotestosterone	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
teleost fish	teleost fish		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Key Event Relationship Description

Population growth rate which measures the per capita rate of population increase over a time interval is proportional to the instantaneous birth rate (number of births per individual per unit of time and the instantaneous death rate (number of deaths per individual per unit of time) (Caswell 2001, Miller and Ankley 2004, Gotelli 2008, Vandermeer and Goldberg 2013, Murray and Sandercock 2020). Decreases in viable offspring could therefore lead to decreased population growth rate, recognizing that other factors (e.g., immigration/emigration, intraspecific and interspecific competition, predation, disease) influence population growth. Population models could be employed to aide in understanding how changes to population growth rate result from various levels of decline in recruitment of young of year fish.

Evidence Supporting this KER

There is no empirical data suitable for evaluating the dose-response, temporal, or incidence concordance between a reduction in the number of viable offspring and decrease in population growth rate. However, population modeling/simulation approaches could be applied in investigating this KER.

Biological Plausibility

A decrease in population growth rate whereby the per capita rate of population change is negative over time can result from either a decline in the instantaneous birth rate and/or an increasein the instantaneous death rate (Caswell 2001, Miller and Ankley 2004, Gotelli 2008, Vandermeer and Goldberg 2013, Murray and Sandercock 2020). While the number of eggs produced by female fish would not be directly impacted, impaired spermatogenesis in male fish that results in decreased oocyte fertilization and/or a reduction in viable offspring would reduce the population

growth rate over time as fewer eggs on average would survive to become young of year fish. Thus, the reproductive potential of female fish adjusted for the inability of fertilized eggs to progress and hatch into viable offspring would be expected to result in a decline in recruitment and contribution of offspring to the next generation (a decline in net reproductive rate) (Caswell 2001, Gotelli 2008, Vandermeer and Goldberg 2013).

Empirical Evidence

There is very limited empirical data for this KER; thus, evidence is based on biological plausibility and population models.

Uncertainties and Inconsistencies

There is limited empirical data for this KER. Population models are often parameterized based on information from a single species. Studies at the population level rely upon observation and estimation of a number of species-specific variables that influence population growth rate (e.g. age or stage specific estimates of survival and fecundity), each of which has an associated uncertainty. There are also uncertainties in extending the population model (extrapolation of model predictions) to be applicable to other species.

Quantitative Understanding of the Linkage

Response-response relationship

Decreased oocyte fertilization and/or a reduction in viable offspring would result in reduced survival of eggs to become young of year fish. This in turn would result in a lower population growth rate over time.

Time-scale

The time-scale at which decrease in viable offspring would impact population levels is dependent on a species life cycle, with the potential for impacts in the short term (i.e. days or weeks) for short-lived species and much longer (years) for long-lived species.

References

Caswell H. 2001. Matrix Population Models. Sinauer Associates, Inc., Sunderland, MA, USA.

Galic N, Hommen U, Baveco JM, van den Brink PJ (2010) Potential application of population models in the European ecological risk assessment of chemicals. II. Review of models and their potential to address environmental protection aims. Integr Environ Assess Manag 6:338–360.

Gotelli NJ. 2008. A Primer of Ecology. Sinauer Associates, Inc., Sunderland, MA, USA.

Miller DH, Ankley GT. 2004. Modeling impacts on populations: Fathead minnow (Pimephales promelas) exposure to the endocrine disruptor 17b-trenbolone as a case study. Ecotox Environ Saf 59:1–9.

Mittelbach GG, McGill BJ (2019) Community ecology. Oxford University Press, Oxford.

Murray DL, Sandercock BK. 2020. Population ecology in practice. Wiley-Blackwell, Oxford UK, 448 pp.

Vandermeer JH, Goldberg DE. 2013. Population ecology: first principles. Princeton University Press, Princeton, NJ USA.