A Review of the Effect of Estrogen on Immune Efficacy in Zebrafish (*Danio rerio*) with Comparisons to Human and Murine Homologs

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ABSTRACT
A review was conducted on current research surrounding the effect of estrogen, and the estrogen receptor, on immune development. Estrogen can regulate many processes and genes throughout immune development, from modulating complement activation and regulating genes crucial for hematopoiesis, to elevating toll-like receptor gene expression. Estrogen has also been shown to have a pronounced effect on regulating certain cancers through inducing macrophage infiltration. It has also been demonstrated to play an important role in the regulation of microRNAs that are important for proper immune development. A greater understanding of this hormone's effect gained through the zebrafish model can lead to the development of better practices to improve both human and ecological health. Contemporary reviews typically examine the effect of estrogen-like compounds (oftentimes referred to as estrogenic endocrine disrupting compounds) on a sequestered part of immune system development. A distinct lack of cohesion exists in combining contemporary and past reports of the effects of estrogen on various aspects of immune system development in zebrafish. This review serves to fill that gap in knowledge, and to provide a gateway for other researchers interested in this topic.

KEYWORDS
Zebrafish; Immune development; Zebrafish immunology; Estrogen; Estrogen receptor; Autoimmunity; Altered signaling; Hematopoiesis

INTRODUCTION

Estrogen is an important hormone for development in vertebrate species, including zebrafish (*Danio rerio*), an increasingly popular animal model for its ease of care, transparency during development, and the similarity of many of its systems to humans. While estrogen activity is primarily associated with development of sexual organs and reproduction within female members of a species, it has an effect in a variety of systems, both during initial development and throughout the life of the organism. As with other hormones, estrogen operates within tightly controlled levels, with significant variation in these levels resulting in a variety of disorders. For example, estrogen deficiency can result in loss of bone density,[1] metabolic disorders resulting in increased weight gain and LDL cholesterol,[2] and fertility problems in adults.[3] An overabundance of estrogen can be just as damaging, leading to a variety of conditions including breast tumor development,[4] with the increased expression of this hormone in females being correlated with higher rates of autoimmunity.[5]

There have been two primary estrogen receptor subtypes identified in zebrafish, the Esrα and Esrβ (also known as the ERα and ERβ, respectively).[6] Within the Esrβ subtype, there are two additional receptors, and these are the Esr2a, and the Esr2b receptors.[7] There also exists the somewhat enigmatic GPER, or G protein-coupled estrogen receptor, which is a recently discovered membrane bound receptor that may regulate innate immunity.[8, 9] These receptors have been found in a variety of tissues within the zebrafish, including the liver and kidney.[10] Prior research has determined that the human ERβ is homologous to zebrafish ERβa, in both function and structure.[11] The human ERα has also been confirmed to be orthologous to the zebrafish's ERα.[12]

This study will explain where these estrogen receptors and estrogen signaling might have the strongest effect in the zebrafish model pertaining to immune development, based on contemporary research. Due to the increased prevalence of estrogenic chemicals that can signal through the estrogen receptor, such as bisphenols, it is essential that the research community investigates the mechanism of estrogen- and estrogenic-induced immune function manipulation, in aquatic and terrestrial wildlife as well as human populations.[13, 14] Different chemicals can bind to these estrogen receptors other than the hormone after which it is named. For example, BPA and some of its analogs can interfere with estrogen signaling.[15] These chemicals, which belong to a class of

substances called estrogen disrupting chemicals (EDCs), can also include dichlorodiphenyltrichloroethane (DDT), dioxin, phthalate esters, atrazine, etc.[16]
Female zebrafish can typically respond to estrogens through the expression of biomarkers such as vitellogenin, which is a yolk precursor protein.[17] This expression is atypical in males but can occur in hepatocytes after exposure to both natural and synthetic estrogens.[17, 18] Estrogens can also lead to reduced gonadal maturation and even reverse sex differentiation in males.[19]
Published research from a variety of peer-reviewed sources was used to construct this review. Topics chosen for coverage in this review ranged from estrogen's effect on complement activation, toll-like receptor signaling, thymic growth, notch signaling, and cytokines, to innate cells and micro RNAs.

Complement
Complement activation is a crucial part of humoral innate immunity. It is a series of three unique pathways of plasma proteins that form a membrane attack complex (MAC) once activated by pathogens; in turn, it opsonizes the pathogens to neutralize them, or aids in the recruitment of specialized phagocytic cells such as macrophages.[20] Therefore, reducing its effectiveness in some capacity from excess estrogen exposure can cause serious issues for immune development, with the results being potentially fatal. Recent research has demonstrated that zebrafish larval exposure to elevated 17 β-estradiol (or E2) levels can cause a toxic accumulation of factor H, which is a regulatory protein of the alternative pathway of the complement system that is responsible for ensuring the MAC does not assemble on self-cells.[21, 22]

Estradiol is an important estrogenic hormone that plays a key role in the proper development of multiple functional systems throughout the body, and serves as an agonist of the two classical subclasses of estrogen receptors.[23, 24] When challenged by *C. albicans* with and without E2 exposure, it was determined that zebrafish exposed to 1μM of E2 yielded a 63% decrease in the survival rate post-infection.[21] While C3b, an important complement factor, was able to bind to the fungal cell surfaces, the heightened factor H recruitment, effectively prevented phagocytosis by inhibiting the membrane attack complex from forming.[21] The usual function of factor H is to regulate complement activation through accelerating the degradation of C3b on either the surface of targeted cells or in the fluid-phase.[25] As C3b is an integral component of both the alternative and classical pathways, this allows factor H to prevent two of the three pathways that are available (although it is generally associated with the alternative pathway).[21, 26] Factor H is essential in regulating a pro-inflammatory response in the body; in fact, its very absence or downregulation has been linked to diseases such as hemolytic uremic syndrome or glomerulonephritis.[27, 28] In the case of estrogen-induced overexpression, however, it can prevent the formation of the MAC and halt two of the three complement pathways altogether; this in turn can lead to higher infection susceptibility and elevated fatality rates in zebrafish.[21] Future research could be performed on other regulatory components of the complement system that work in conjunction with Factor H, such as complement receptor type 1 (CR1) or decay accelerating factor (DAF), to further establish if estrogen can cause other aberrant effects during development of the zebrafish innate immunity.

Toll-like Receptors
Estrogen is also involved in the expression and regulation of the transmembrane toll-like receptors (TLRs) in zebrafish. TLRs are one of multiple subsets of PRRs, or pattern recognition receptors, which are used for the early detection of foreign substances through its recognition of distinctly non-self molecules, such as double stranded RNA (dsRNA), flagellin, or even lipopolysaccharide (LPS).[20] TLR's are found on a variety of cell types, from keratinocytes and innate immune cells such as dendritic cells, to adaptive immune cells like B lymphocytes; this early TLR-mediated detection is vital for downstream cytokine signaling to occur, which in turn recruit neutrophils, macrophages, and dendritic cells in the early immune response.[29-31]

Male zebrafish embryos treated with high levels of E2 were associated with an elevated expression of genes coding for Esr and TLRs, which resulted in additional nuclear factor kappa B (NF-ϰB) expression.[32] When TLRs detect the presence of a pathogen associated molecular pattern (PAMP), such as the bacterial cell wall component LPS, this triggers a complex cascade within cells that eventually results in the release of NF-ϰB.[33, 34] NF-ϰB is a regulatory transcription factor that, once activated, ends a cascade that causes the nuclear transcription of a variety of pro-inflammatory cytokines and adhesion molecules.[35] See **Figure 1**.

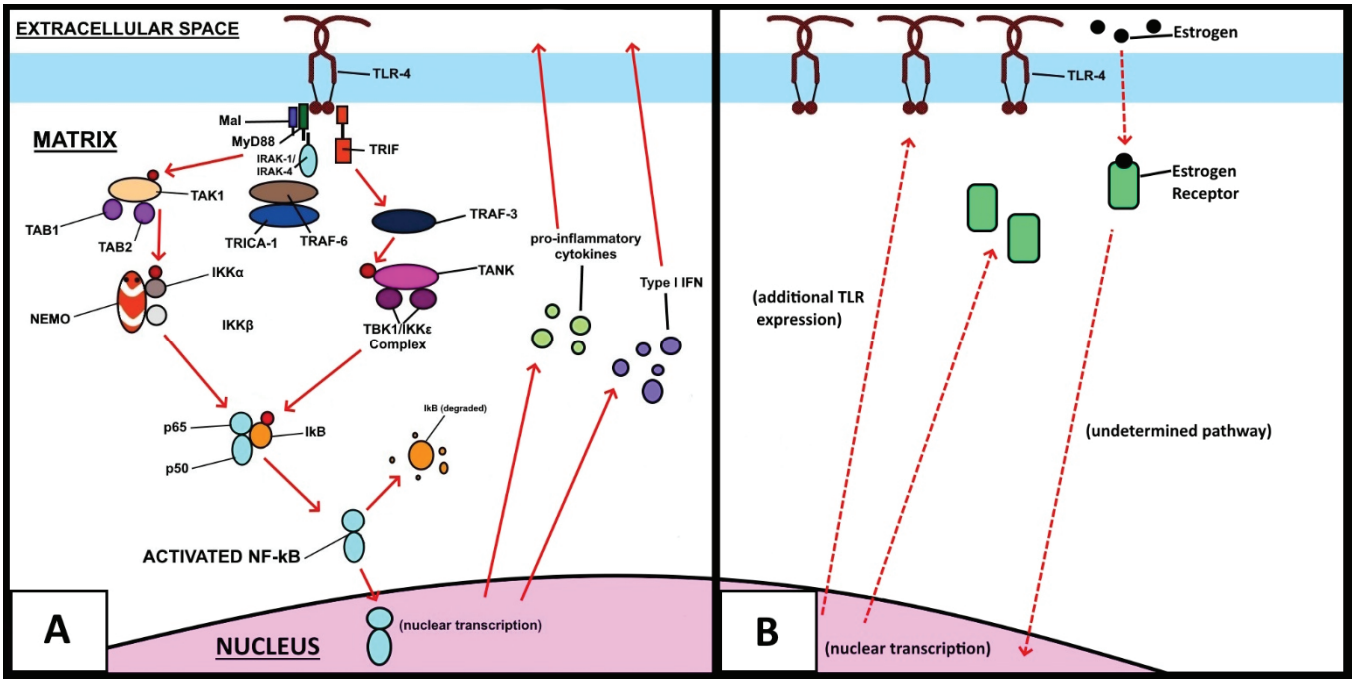

Figure 1. (A) TLR signaling. The figure above details both the canonical NF-ϰB pathway through MyD88, and the non-canonical pathway through TRIF. The canonical pathway results in the release of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6, while the non-canonical pathway sees a release of Type I interferons into the microenvironment. **(B)** An established pathway for E2's modulatory effect on zebrafish toll-like receptor and estrogen receptor expression has not been entirely elucidated. Elevated estrogen levels in zebrafish were correlated with increased expression of both estrogen receptors and TLRs. *Original Illustration by Michael S. Chembars.*

Sun et al. postulates that heightened TLR expression results in overall greater NFϰB expression within zebrafish hepatic and gonadal cells, due to the prevalence of estrogen receptors across multiple tissue types.[32] However, the increased NFϰB expression did not correlate with elevated inflammatory activity, but rather the opposite. In murine macrophages, it has been shown that E2 binding to its receptor EsrA can prevent p65 (also known as REIA) translocation through activation of the PI3K pathway.[36, 37] The essential transcription factor p65 regulates NF-ϰB through its production of inhibitory factor IϰB.[38] In another study, Valentine et al. determined that E2 can signal through both EsrA in addition to EsrB to suppress p65 in murine HeLa and COS-1 cells,[39] which further supports E2's ability to suppress NF-ϰB through the canonical pathway. Thus, in cell types that express the estrogen receptors, including important immune cells such as macrophages, E2 can function as a non-conventional anti-inflammatory drug and prevent the release of pro-inflammatory cytokines including TNF and IL-6 through inhibiting NF-ϰB.[40-42] In human models, E2-mediated depression is associated with post-menopausal osteoporosis during higher levels of secretion.[35, 37, 42]

While E2 could potentially serve as a useful anti-inflammatory drug to treat several diseases in humans involving hyperimmune responses, its very inhibition of NF-ϰB and ultimately cytokine secretion could also cause adverse immunosuppressive effects as well. Previous studies with loss-of-function mutations that hinder or halt the TLR pathway have also been shown to cause immunodeficiencies, which in turn lead to recurrent bacterial infections, arthritis, and even cancer.[20, 43] The use of non-steroidal anti-inflammatory drugs (NSAIDs) to decrease estradiol levels in postmenopausal women is performed as an experimental technique for preventing breast cancer.[44, 45] However, estradiol's specific anti-inflammatory mechanisms and this correlation with carcinogenesis is not well understood, and requires further research.[45] Additionally, research is available to suggest that zebrafish estrogen receptors may respond differently to environmental estrogens than human estrogen receptors. [46]

Thymus

The thymus is an important secondary immune organ, where T cells undergo much of their development, including V(d)J recombination and negative and positive selection.[47, 48] There is also some evidence that estrogen exposure can cause aberrations in adaptive immune development as well. In teleost fish, research has identified that Esr2 is especially important in the ontogenesis of the thymus, and as such is the most probable of the ERs to bind to estrogen here.[49] A 'critical window' may exist during immune development, in relation to estrogen exposure, which may determine whether the hormone causes atrophy or hypertrophy of the thymus.[50, 51] As a result, estrogen may have varying effects on this immune organ. There is not a lot of available data on the effects of natural estrogens such as E2 on thymic development in zebrafish, and thus further research is

needed to confirm the effect of natural estrogen exposure on thymic development in *D. rerio*. There is, however, research to support that synthetic estrogens such as EE2, or 17α-Ethnylestradiol, can cause thymic retardation in zebrafish embryos.[52]

Notch & Hematopoiesis

Hematopoiesis is a highly regulated process that is responsible for the differentiation of self-renewing, multi-potent hematopoietic stem cells (HSCs) into lymphoid progenitor cells and myeloid progenitor cells.[53] The former is responsible for giving rise to T and B lymphocytes and other cells of adaptive immunity, while the latter is generally associated with the development of cells of the innate response, such as monocytes and granulocytes.[54] Before this differentiation occurs though, estrogen is shown to play an important role in zebrafish HSCs in the first 24-36 hours of development through Vegf/Notch signaling, through which it acts as a morphogen and signaling molecule.[53, 55] Carrol et al. additionally suggested that excess E2 exposure in this early phase led to a decrease in notch-family genes,[55] such as Notch3 and Notch1. Notch is a receptor that commits T-cell precursors towards the T-lymphocyte lineage by activating the GATA-3 transcription factor during the first double negative stage. Froehlicher et al. treated zebrafish embryos between 72 and 96 hours post fertilization (hpf) with a high concentration of an engineered ERβ2 morpholino (a molecule designed to modify gene expression).[56] By knocking out ERβ2 RNA translation with the morpholino, the exposure during embryonic development caused an upregulation of the Notch3 gene.[56] In contrast, other studies which exposed mice to E2 demonstrated a correlation in which notch-family genes such as Notch1, and the Notch ligand Jagged1 were upregulated significantly, following higher concentrations of estradiol.[57-59] The above differences between Carrol et al. versus other researchers who reported increased expression of notch-family genes could be due to one of several major experimental factors. Carrol et al. used a concentration of 8-10 μM estradiol chemical treatments in their study on embryos between 12 and 36 hpf.[55] In contrast, a treatment of 1 nM estradiol, in addition to using murine cell lines rather than zebrafish embryos, led Soares et al. to different results and conclusions.[57] Thus, while these differing results may be useful for studies concerning estrogen's effect on the environment, further research must be done to isolate which model organism (zebrafish or murine) is best for determining the effect of estrogen on hematopoiesis in *Homo sapiens*. Different avenues of research have also been proposed in zebrafish models to further solidify the understanding of estrogen's effect on Notch, including hippocampal formation and additional cell proliferation mechanisms.[60]

Overexpression of notch has been linked to highly increased canonical and non-canonical signaling through both the RBPJK signal binding protein and mTORC2 pathways, respectively.[61, 62] Additionally, Jagged1 overexpression may cause both positive and negative effects. In dystrophin deficient zebrafish with elevated levels of Jagged1, overexpression can rescue the mutant phenotypes in a particular model, opening up new avenues of research into treating Duchenne muscular dystrophy.[63] On the flipside, however, it is also linked to increased angiogenesis and cancer.[64] Particularly, when mTORC2 pathways were increased sharply, this resulted in decreased Foxp3 expression and activity. Foxp3 is a master gene regulator whose expression is sufficient to induce T lymphocyte differentiation; therefore, a decrease in Foxp3 will also result in a decrease in T lymphocyte expression. Mutations that decrease the expression or eliminate Notch altogether can also lead to decreased cardiac health and tissue regeneration in murine models.[65]

Innate Cells

Innate immunity is often described as the first line of defense in the body's defense mechanism, and can include cell types such as eosinophils, basophils, neutrophils, and monocytes, the latter of which can differentiate into dendritic cells and macrophages.[66] Currently, it is thought that E2 primarily affects dendritic cell precursors and macrophages through Esrα, although this is still under investigation.[67]

Xu et al. reports that E2 exposure to whole male and female zebrafish embryos can also increase neutrophil counts by a factor of over 25%.[68] This, along with elevated mpo and IL-8 gene expression, are indicative of elevated immune response stimulation. These results seem to be supported by several other studies as well. Namely, Rodriguez et al. used E2 in a zebrafish model to promote increased neutrophil expression and recruitment,[69] along with TGFβ1, which resulted in metastasis of cancer cells. Because neutrophils and dendritic cells can derive from the same myeloid progenitor cell, one would also expect to see increased dendritic cell differentiation in response to E2 exposure. This is further demonstrated by Siracusa et al.'s murine study, which indeed found elevated MHC II+ dendritic cells after being exposed to E2 *in vivo*.[70]

Both neutrophils and dendritic cells are vital for innate immunity, as they are the most common immune cells which respond to infection and provide the greatest amount of antigen presentation, respectively, within the zebrafish immune arsenal. Neutrophils can phagocytose antigens, cause granule release, and release cytokines and chemokines that cause increased transmigration of additional white blood cells to the site of infection.[20] Dendritic cells, on the other hand, are the primary antigen presenting cells through their use of major histocompatibility complex (MHC) molecules and serve as the 'bridge' between the innate and adaptive branches of immunity.[20]

There is some research available indicating that E2 can also affect macrophage migration to cancerous tissue via chemoattractants. E2 can increase circulating CCL2 and CCL5 chemokines, which are known tumorigenic compounds.[71, 72] The higher chemokine levels led to cancer metastasis due to the increased influx of macrophages.[71] These results were similar to a zebrafish study by Rodriguez et al.,[69] which found that E2 can increase metastasis by inducing higher neutrophil recruitment and by promoting TGFβ1 expression. Estrogen also has regulatory effects on nitric oxide (NO) by increasing its release from macrophages.[73] Macrophages, which are well known for their production of inducible nitric oxide synthase (iNOS), utilize reactive oxygen and nitrogen species to kill captured organisms during phagocytosis.[74]

Cytokines

Endocrine disrupting chemicals (EDCs) such as E2 have also been reported to have marked effects on expression of multiple cytokines within *Danio rerio*, such as TNFα, Type II interferons (IFN-γ), IL-1β, as well as the chemokines IL-8, CXCL-Clc, and CC-chemokine.[75] Jin et al. found that TNFα, IL-1β, and IFN-γ expression levels were elevated in zebrafish embryos after exposure to higher concentrations of E2.[75] In a different study, Xu et al. reported increased transcription of TNFα after exposure to E2,[68] although their expression levels were lower when compared to those in Jin et al.'s experiment;[75] Jin et al. exposed larvae to 12.5 μg/L of E2, whereas Xu et al. treated larvae with only 5 μg/L of E2, which could demonstrate a dose-dependent effect of E2 on TNFα. TNFα is the flagship cytokine of the TNF superfamily that is mainly released by macrophages, mast cells, and dendritic cells; its expression is regulated through the NFκB pathway.[76, 78] Upon release, TNFα can cause a host of effects including apoptosis through proteins such as c-Jun, along with playing a major role in inducing inflammation, and activating MAP kinases.[78-80] In the event of sepsis, activated macrophages might be apt to release large quantities of TNFα systemically, which can lead to septic shock due to a sharp drop in blood pressure.[20] Excess amounts of TNFα have also been linked to arthritis and other inflammatory tissue diseases, such as vasculitis or ankylosing spondylitis.[80, 81] See **Figure 2.**

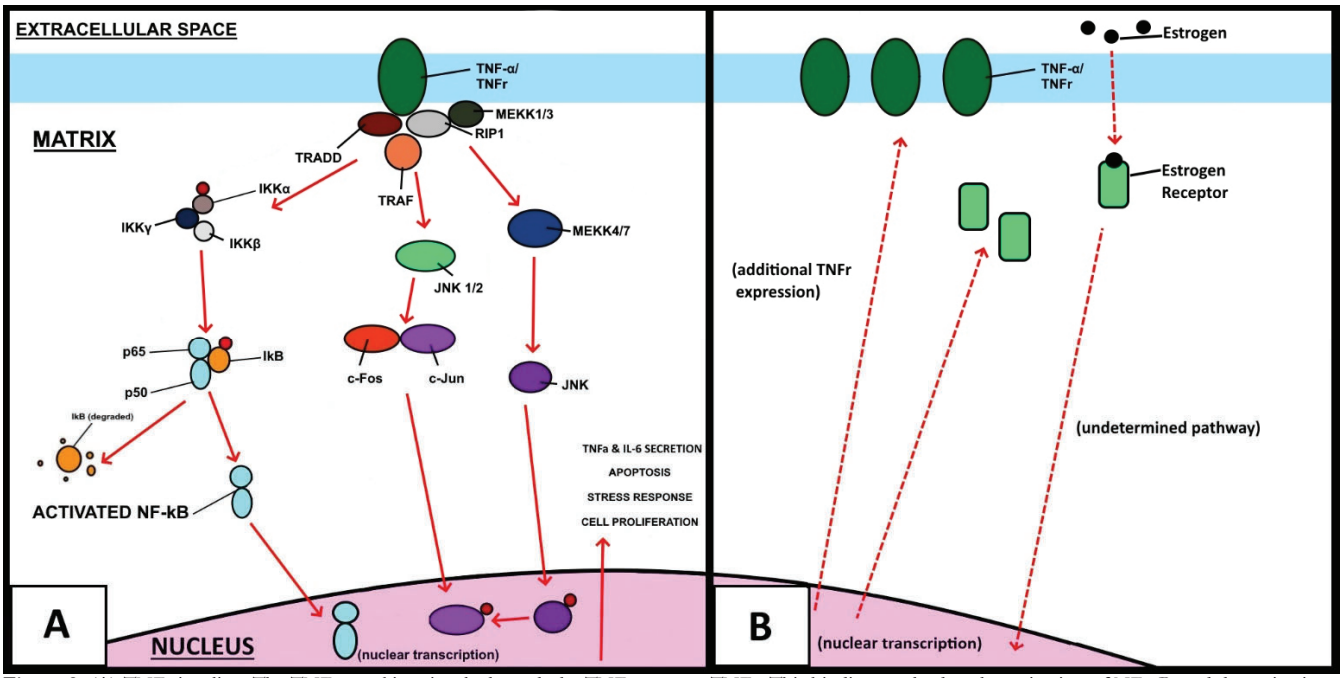

Figure 2. (A) TNF signaling. The TNFα cytokine signals through the TNF receptor, TNFr. This binding can lead to the activation of NF-κB, and the activation of cJun through the MEKK and JNK pathways. This can then lead to the release of chemicals that results in apoptosis, additional stress response, cell proliferation, and the release of additional pro-inflammatory cytokines. TNFα binding can cause a host of responses, but we chose to focus on those that are known to be affected by estrogen exposure. **(B)** An established pathway for E2's modulatory effect on TNF signaling has not been entirely elucidated. However, elevated estrogen levels in zebrafish were correlated with increased expression of both estrogen receptors and TNFr. *Original Illustration by Michael S. Chembars.*

IFN-γ is a type II interferon that is instrumental in modulating cell-mediated immunity, where it serves in multiple capacities including the amplification of antigen presentation, increasing reactive oxygen species (ROS) production for increased antiviral activity, and the induction of an anti-proliferative state to combat cancer.[82] In the innate branch of immunity, ROS and IFN production is stimulated by macrophages, which secrete cytokines to attract natural killer cells to the area of inflammation and further IFN-γ production.[83, 84] In a study by Xu et al., E2 exposure promoted higher expression of IFN-γ in both male and female zebrafish,[85] which promoted the inflammatory response and led to increased cardiac tissue regeneration. Xu et al. also determined

that E2 caused Esr2a and Esr1 expression levels to increase in zebrafish,[85] while Esr2b expression was suppressed. Additional research also confirmed E2 binding to both Esr1 and GPER, which led to increased IFN-γ expression in zebrafish.[70, 86-88]

E2 is also a known effector of the nitric oxide (NO) pathway.[89] Jin et al. reinforced this by determining that E2 exposure can lead to an increase in iNOS gene expression,[75] leading to its upregulation. Additional iNOS is expressed due to increased cytokine activity, and can cause the release of nitric oxide through catalysis.[75, 90, 91] Innate immune cells such as macrophages, neutrophils, or NK cells can utilize NO released to increase host resistance, lessen the severity of an infection, and serve as a potent antimicrobial and antiviral substance.[92, 93] Overabundant levels of iNOS in the body for a prolonged duration of time can lead to a plethora of autoimmune-induced diseases; these can include but are not limited to gastroenteritis, septic shock, or atherosclerosis.[94] A different study by Karpuzoglu-Sahin et al. suggested similar overexpression of iNOS,[87] this time due to its interaction with none other than the cytokine IFN-γ.[95] This interferon's expression can be enhanced by E2 upstream as previously defined, which causes a cascade effect down the line with iNOS at the end of it, that eventually leads to an uncontrolled amount of NO released to the body.[74]

Current research also demonstrates that E2 exhibits a developmental stage-dependent effect on numerous genes, meaning the age at which the embryos are exposed to E2 in hours or days post fertilization (hpf or dpf, respectively) is crucial. One gene that regulates ubiquitination in zebrafish, *dcaf13* (human homolog: DCAF13), experienced a significant increase in expression at 1 dpf in zebrafish embryos following exposure to 1 μM E2.[86] Ubiquitin is a protein which serves as the central player in both the processes of ubiquitination and its counterpart, deubiquitination; it is part of an immune retraction process that is essential for the regulation of both innate and adaptive responses.[96] Ubiquitin accomplishes this through seven lysine residues, one of which can cause protein degradation through the 26s proteasome, while another named K63 can create linkages which are responsible for initiating cytokine signaling.[97, 98] One of the 26s proteasome's functions is to degrade IκB, which subsequently frees NF-κB to enter the nucleus.[99] Li et al. also expounded on ubiquitination's effect on the CbI family of proteins,[97] which play a key role in the regulation of both T and B lymphocytes. Based on these reports, it is evident that E2 can affect other physiological processes through modulating ubiquitination, in addition to affecting zebrafish immune development.

The use and regulation of cytokines is crucial for a prompt and effective response from both the adaptive and innate repertoires of zebrafish immunity. Estrogen has demonstrated its ability to affect the expression and regulation of several cytokines through promoting gene transcription events. Further research should be conducted on other well-known cytokines and their related pathways in order to elucidate estrogen's specific method of action.

*Micro-RNA*Several studies have shown that E2 can influence microRNA regulation. MicroRNAs (miRNA) are small segments of RNA nucleotides that can silence gene expression post-transcriptionally by increasing the rate of degradation in mRNA.[100, 101] In immunology, miRNAs have been identified to control multiple effects in immune cells, which affect all stages of development.[102] Some miRNAs, such as miR-126 in zebrafish, have also been associated with regulating HSC differentiation, enabling them to control the myeloid and lymphoid lineages.[103] By comparison, in mice, miR-181 can increase B lymphocyte expression through lineage regulation.[104] A shift in lineage differentiation such as that caused by miR-181 may lead to the mutant organism exhibiting enhanced antibody production, at the expense of a heightened susceptibility to infection due to fewer myeloid innate cells that can professionally phagocytose.

E2 levels can either cause the upregulation of certain miRNA genes such as miR-17-92, or the downregulation of miRNA genes such as let7.[105, 106] Several members of the miR-17-92 family were found to be upregulated in female zebrafish following exposure to E2.[105] miR-17-92 is responsible for regulating numerous cell types in the immune response, ranging from B lymphocyte development, T lymphocyte activation, and expression of dendritic cells.[107] In B lymphocytes, miR-17-92 expression is higher during the early stages of development, and decreases as the B lymphocytes reach the pre-B to immature B lymphocyte stages.[108] Overexpression or failure of miR-17-92 levels to decrease in T lymphocytes have been linked to several autoimmune diseases in both murine and human models, such as systemic lupus and multiple sclerosis.[109, 110] One of the mechanisms through which it causes autoimmunity is through targeting several pro-apoptotic lipid phosphatases and proteins such as PTEN and Bim, respectively.[107, 111] This in turn promotes Th1 delayed-type hypersensitivity, and simultaneously retarding regulatory T cell production.[107] Current knowledge on E2's role in zebrafish miR-17-92 expression is not extensively documented, and may present itself as an avenue of future research in lieu of less economical murine or human models.

A miRNA credited as a master regulator of inflammation, miR-155,[112] may play a role in promoting human breast cancer when exposure to E2 is involved.[113] miR-155 is also present in zebrafish, though E2's effect in this organism is not well documented.[114] Zebrafish models testing E2's effect on miR-155 could benefit current knowledge on human breast cancer, Gaucher's disease, and kidney disease.[112, 114]

Cohen et al. discovered that E2 can also repress miRNA of the let-7 family in zebrafish;[106] these are known tumor suppressants which regulate genes such as k-Ras. k-Ras is an oncogene which can signal through the PI3K and MAPK pathways, which eventually result in the execution of important cellular functions including cell proliferation; there are numerous studies that link k-Ras mutations in some form or fashion to approximately 25% of human cancers.[115] k-Ras can also cause secretion of pro-inflammatory cytokines such as IL-6, a cytokine noted for its tumorigenic activities.[115, 116] k-Ras can also mediate IL-1 and NF-κB expression, both of which are crucial to the inflammatory response.[115, 117] A study on k-Ras transgenic zebrafish has also suggested that estrogen plays some role in inhibiting cell proliferation in hepatocellular carcinoma (HCC).[118, 119] This discovery may help provide an answer as to why human males are more susceptible to HCC than females, as females traditionally express higher estrogen levels.[119]

miRNA's suppression of k-RAS and other oncogenic genes continues to be studied in zebrafish, murine, and human models in order to fully determine the role of microRNA in regulating cancer.[120-122] As such, this is still an emerging field of study, and a promising one with many different avenues to pursue.

CONCLUSION

It is evident that estrogens, such as the naturally derived 17 β-estradiol, act as potent immunomodulatory agents in zebrafish. Many of these effects can alter critical pathways, such as the case of the complement cascade and the toxic buildup of Factor H, which reduces its effectiveness by preventing the formation of the membrane attack complex. Estrogen can affect pathways involving the expression of the protein NF-κB, which has effects ranging from development and maturation of immune cells to activation in response to infection. Estrogen can also upregulate cytokines such as TNFα, IL-1β, and IFN-γ, which affect important functions such as apoptosis, mediating the inflammatory response, antigen presentation, and much more. Immunomodulatory effects can also be seen on microRNAs from estrogen exposure, which is a vital regulator in both myeloid and lymphoid lineage development.

EDCs are not exclusively natural estrogens such as E2. Other chemicals, such as bisphenols, phthalates, etc., can also signal through the estrogen receptor similarly to E2.[123, 124] Because of an EDC's agonistic behavior, future research documenting their mechanisms of action to induce zebrafish immune disruption has been identified as a locale in research that should receive more attention, especially as it pertains to ecological toxicology.[125]

These findings highlight the necessity for researchers to further investigate the effects of estrogenic compounds both in zebrafish and in other aquatic species, as these chemicals are becoming more prevalent in aquatic environments.[126, 127] From these environments, trace amounts of contaminants can radiate to terrestrial mammals including humans, primarily, though not exclusively, through the food supply.[128-130] Furthermore, estrogen's effect on many aspects of the immune system, especially the adaptive branch of immunity, is less understood. *Danio rerio* has proven itself as a staple model organism for immunological studies due to its cost efficiency, high embryo yield, and similarity to the human immune response. Through this model, it is suggested that additional research be conducted and documented concerning estrogen's effect on environmental and human health (as well as synthetic estrogens and estrogenic compounds such as bisphenol-A and its derivatives).[131]

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PRESS SUMMARY

This study reviews current knowledge of the effect of estrogen on immune system efficacy in zebrafish. Significant research has been conducted on this topic, and primarily concludes that estrogen exposure can cause aberrant immunological development and function that decreases the overall health of the organism.