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GnRH Agonist Triptorelin Induces Hepatic Fibrosis via Sex-Specific Oxidative and Inflammatory Responses in Adolescent Rats

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Abstract

Recently, GnRHa puberty blockers have been under scrutiny, with newly identified risks of heart and brain damage and recurrent concerns about fertility, cancer risk, and bone health. This study explores the effects of GnRHa on liver morphology, function, and injury response. Peripubertal male and female Sprague-Dawley rats received daily GnRHa triptorelin (100 μg) subcutaneously. Liver oxidative stress, inflammation, and fibrosis were evaluated via malondialdehyde and 8-OHdG (oxidative damage), immunohistochemistry for CK19 (cholangiocytes) and CD45 (leukocytes), and collagen staining as well as ⟨SMA (liver fibrogenesis) and TIMP1 (extracellular matrix breakdown) expression, respectively. Following GnRHa treatment, only male rats exhibited increased ductular reaction and oxidative stress. In contrast, GnRHa-treated female rats showed increased leukocyte infiltration. In both sexes, GnRHa-treated rats showed increased fibrosis, with significantly increased collagen deposition and ⟨SMA expression. Interestingly, GnRHa-treated female rats exhibited increased TIMP1 expression, whereas male rats showed decreased TIMP1 expression. Overall, GnRHa puberty blocking leads to significantly increased liver injury in both sexes. Specifically, biological females are at increased risk of hepatic inflammation, while biological males are at increased risk of oxidative stress. Human clinical trials are crucial for further exploring these findings.

Keywords: GnRH agonist; adolescent; liver fibrosis; inflammation; sex-specific response; oxidative stress; puberty blocker

1. Introduction

With an estimated population of 1 million in the United States (US), transgender and gender-diverse (TGD) individuals face unique healthcare disparities exacerbated by a lack of TGD-focused medical research [1,2]. Healthcare needs of the LGBTQ+ (lesbian, gay, bisexual, transgender, queer, and more) community differ from those of heteronormative populations, and the limited research focusing on this population places their long-term health at risk [1,3]. Estimates of TGD adolescents in the US are anywhere between 1 and 6 million children, all of whom have unique healthcare needs [4,5]. For these individuals, Gonadotropin-releasing hormone agonist (GnRHa) puberty blockers can aid in treating peripubertal gender dysphoria, alleviating psychosocial stress caused by the incongruity between sex assigned at birth and gender identity. While the psychosocial benefits of GnRHa treatment are well founded, with recent confirmation of GnRHa puberty blocking treating

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gender dysphoria, the systemic physiological effects of this treatment remain partly a mystery – particularly in organs with high endocrine sensitivity, such as the liver.

Originally synthesized 60 years ago, GnRHa drugs are FDA-approved for treating central precocious puberty (CPP), a condition where puberty starts at a detrimentally young age [6]. In adults, GnRHa is used to treat sex-specific disorders and cancers, including endometriosis, IVF fertility, and prostate cancer [7,8]. Over the past 30 years, these agents have been used as an off-label treatment for adolescent gender dysphoria [9]. Despite their widespread use, the long-term side effects of GnRHa treatment during adolescence are not fully known. In recent FDA petitions, heart and brain damage have been added to the list of potential side effects from GnRHa use [10,11].

GnRHa induces this 'puberty blocked' phenotype by interrupting the production and secretion of sex-specific hormones such as estrogen and testosterone at the start of puberty. Disruptions in these hormone levels are often linked to an increased risk of hepatic damage in both males and females, with sex-specific injury patterns Moreover, the liver displays distinct sexual dimorphic variations, including hepatocyte size, vascular architecture, and the abundance of specific cell types within the liver [12]. Overall, female livers exhibit a greater hepatoprotective capacity and display quicker recovery rates than male livers [12,13]. Given the liver's critical role, the disruption in sex-hormone levels induced by GnRHa puberty blocking may cause liver injuries.

Hepatic injury is characterized by oxidative stress, inflammation, and liver fibrosis. Without removing the cause of injury, this can change injury-response cell populations and increase fibrogenesis due to prolonged and amplified inflammatory responses. Liver injury frequently is caused by increased cellular metabolism generating damaging reactive oxygen species (ROS). Thus increased oxidative stress is often the first sign of liver injury [14,15]. Increased levels of ROS further induces mitochondrial and DNA oxidative damage, and if not accommodated for, leads to fibrosis and hepatocellular apoptosis [14–16]. DNA and mitochondrial oxidative damage can be measured through 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA), respectively.

Beyond oxidative stress, molecular mechanisms of hepatic inflammation are coordinated through proinflammatory signals, including tissue inhibitor of metalloproteinases (TIMP)1 and transforming growth factor beta (TGFβ)1 [17,18]. Liver injury stimuli, such as TIMP1 and TGFβ1, often promote the infiltration of leukocytes (marked CD45+), including Kupffer cells, dendritic cells, neutrophils, and lymphocytes [18,19]. Similarly, other hepatic injury-response cells also recruited by TGFβ1 include hepatic stellate cells (HSC) and cholangiocytes (CK19+) [20,21]. Intrahepatic cholangiocytes can activate and incite further injury responses, including differentiating into hepatocytes and inciting biliary fibrogenesis through myofibroblast stimulation [22,23]. This process is termed ductular reaction (DR) and is characterized by an increased presence of CK19+ cells, as cholangiocytes become a reservoir for hepatocyte replacement during prolonged injury [23,24].

Hepatic inflammatory responses, including DR, are often accompanied by fibrosis, which is characterized by increased deposition of collagen fibers and expression of profibrotic biomarkers [25]. Alpha smooth muscle actin (⟨SMA) promotes this fibrotic response, and the simultaneous increased expression of TIMP1 limits the breakdown of collagen fibers [21,25]. When the injury stimulus is removed during the final stage of liver injury response, TIMP1 levels decrease, signaling tissue remodeling and replacing excess collagen fibers with healthy hepatocytes [25].

Within this context, the effect of GnRHa in the liver is largely unknown. This study, therefore, investigates whether GnRHa puberty blocking results in oxidative stress, inflammation, and fibrosis, and whether these effects differ between biological sexes.

2. Materials and Methods

2.1. Animal Trial

An adolescent rodent model was used to mimic the peripubertal timeframe of GnRHa puberty blocking. Rats enter puberty at three to four weeks of age and reach their adult form by eight weeks of age [26]. The animal trial adhered to Institutional Animal Care and Use Committee-approved protocols under the Animal Welfare Act guidelines (protocol: 2301CD-DH-R-26). Male and female

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Sprague-Dawley rats at three weeks-of-age were sourced from Envigo (Indianapolis, Indiana). Following a week of acclimation to standard housing conditions, including a 12:12 light/dark cycle and ad libitum access to water and standard rat chow, the trial started with 4-week-old rats, aligning with this species' pubertal onset [26]. Animals were randomly assigned to the control group (saline) or the treatment group (GnRHa), with five to six animals of both sexes in each group. Based on a previous study by Roth et al., GnRHa groups received a daily subcutaneous injection of 100 ʋg triptorelin (Creative Peptides (Shirley, NY USA) Cat #110967) – denoted as GnRHa, while control animals received a daily saline injection [27]. Animal body weights were monitored weekly. After 4 weeks of daily GnRHa administration (or control), animals were humanely sacrificed using a 50 mg/kg dose of sodium pentobarbital.

2.2. Tissue Processing

Following the end of the animal trial, liver tissues were collected and processed in two ways: 1) mounting in optimal cutting temperature (OCT) media, snap freezing in liquid nitrogen, and storing at -80ºC; 2) fixing in neutral buffered formalin for 24 hours, followed by incubating in 70% ethanol for 24 hours, and then the tissues are sent to iHisto company (Salem, MA) for mounting in paraffin blocks.

2.3. Oxidative Damage

Low levels of ROS, the source of oxidative damage, are expected and accommodated within healthy cells [14]. During cellular stress, ROS levels increase above the level of endogenous antioxidants, and the resulting mitochondrial, lipid, and DNA oxidative damage causes cellular dysfunction [15]. Thus, the expression of 8-OHdG (dilution 1:50; Santa Cruz Biotechnology Cat# sc-66036, RRID:AB_832272), a sign of DNA oxidative damage was evaluated via immunofluorescence (IF) performed using 8 µm thick OCT sectioned liver slides according to the following simplified steps: 1) fix slides in 10% neutral buffered formalin for 10 minutes; 2) rinse in cold phosphate-buffered saline (PBS); 3) incubate in permeabilization solution (0.1% Triton X-100 (intracellular target) or 1% Tween 20 (extracellular) in PBS); 4) rinse in PBS; 5) incubate in 1% goat serum blocking buffer for 20 minutes; 6) incubate in primary antibody overnight in 4ºC; 7) rinse slides in PBS; 8) incubate in permeabilization solution for 10 minutes; 9) incubate in secondary antibody for 60 minutes; 10) Rinse in PBS; 11) Mount in fluorescent DAPI media and seal sides with nail polish. Slides were stored in the dark at -20ºC until imaged using a Zeiss 700 confocal microscope. Further, the level of mitochondrial oxidative stress in liver tissues was measured through a thiobarbituric acid-reactive substance (TBARS) MDA assay using homogenized snap-frozen liver tissues (catalog #10009055, Caymen Chemical, Michigan, USA).

2.4. Inflammatory Response

To measure the injury response of leukocyte infiltration caused by peripubertal GnRHa administration was measured via IHC specific for CD45+ cells (dilution 1:50; Santa Cruz Biotechnology Cat# sc-1178, RRID:AB_627074) according to the following simplified protocol: 1) deparaffinize; 2) incubate in 6% H202 and methanol to block exogenous peroxides for 5 minutes; 3) rinse in water; 4) pressure cook in citrate buffer for 30 minutes; 5) rinse in water; 6) wash in tris-buffered saline with 0.1% Tween 20 (TBST) for 5 minutes; 7) incubate in horse serum blocking buffer for 30 minutes; 8) incubate in primary antibody overnight in 4ºC fridge; 9) wash slides in phosphate-buffered saline with 0.1% Tween 20 (PBST); 10) incubate in secondary antibody for 30 minutes; 11) wash in PBS; 12) incubate in VECTASTAIN ABC reagent for 30 minutes; 13) wash in PBS; 14) incubate in DAB peroxidase substrate solution for 2 to 5 minutes; 15) counterstain in hematoxylin for 2 minutes; 16) rinse in water; 17) dehydrate and mount. Furthermore, the cholangiocyte injury response to peripubertal GnRHa administration was assessed by staining CK19+ cells via IHC (dilution 1:50; Abcam Cat# ab220193, RRID: AB_2814863) using the previously described IHC protocol.

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2.5. Evaluation of Fibrotic Changes

Picrosirius red (PSR) staining highlights the presence of collagen I and III fibrosis. PSR staining was performed on 5 µm thick paraffin liver sections following these steps: 1) deparaffinize slides using xylene and 100-70% ethanol; 2) stain in hematoxylin for 1 min; 3) rinse in water; 4) stain with Picrosirius red for 1 hour; 5) rinse in acidified water (5% HCL); and 6) dehydrate and mount. Besides detecting collagen fibers, fibrosis was also measured by the expression of profibrotic ⟨SMA (dilution 1:50; Thermo Fisher Scientific Cat# 14-9760-82, RRID:AB_2572996) and TIMP1 (dilution 1:50; Santa Cruz Biotechnology Cat# sc-21734, RRID:AB_628359) via IF performed according to the above-described protocol.

2.6. Tissue Morphology

Hematoxylin and eosin (H&E) staining elucidates tissue morphology changes, parenchymal apoptosis/necrosis, and inflammatory cell loci. Using 5 µm thick sections, H&E staining was performed following these simplified steps: 1) deparaffinize slides using xylene and 100-70% ethanol, 2) stain slides in hematoxylin for 3 minutes, 3) rinse in water and moderate in 95% ethanol, 4) stain in eosin for 1 minute, and 5) dehydrate and mount. Histotechnologist evaluation of H&E slides was performed by Patrick Wilson. H&E slides were given numerical ratings based on a standardized scoring system for metabolic-dysfunction-associated fatty liver disease, assessing lobular inflammation and cell structure changes induced by peripubertal GnRHa administration [28].

2.6. Quantification and Analysis of Data

The above-detailed cellular and molecular signs of hepatic injury were imaged across multiple lobes of the liver. These images were then quantified and analyzed using ImageJ and R software, respectively. All experiments were performed using 3 or 4 animals per group. Shapiro-Wilk tests for normality were complete, and for the results that adhered to a normal distribution, a two-way ANOVA and post hoc Tukey tests were used. Non-normal distributions found by the Shapiro-Wilk tests for normality were treated as nonparametric. This data was then evaluated through Kruskal-Wallis tests, followed by Dunn's test for multiple comparisons, to identify significant differences between control and treatment groups, with an alpha level of 0.05.

3. Results

3.1. GnRHa Administration Leads to Sex-Specific Increased Oxidative Stress and Inflammatory Responses

Drug-induced hepatic injury often includes oxidative stress, leukocyte infiltration, and cholangiocyte proliferation [29]. Further, sex steroids normally regulate hepatic redox balance; thus, we first evaluated oxidative stress signs as early indicators of hepatic injury (Figure 1). TBARS assay for MDA revealed a trend towards an increase (p= 0.052) in lipid oxidative stress in GnRHa males but not in GnRHa females (Figure 1a). This same trend was noted in the IF staining for 8-OHdG for DNA oxidative damage (Figure 1b,c).

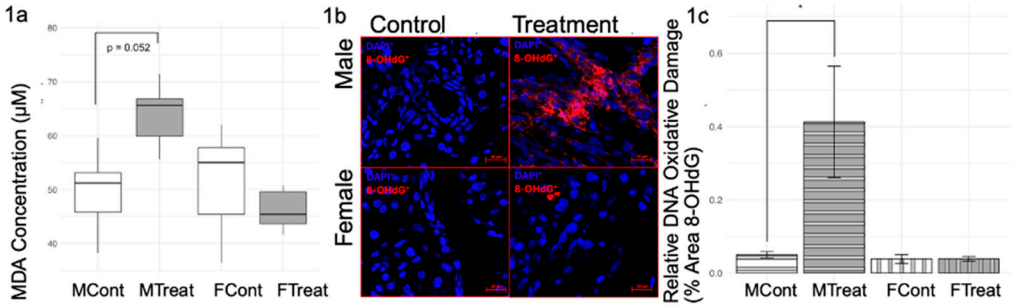

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Figure 1. GnRHa administration induces oxidative stress in male rats. 1a TBARS Assay specific for MDA concentration in whole tissue lysate, compared across treatment. 1b Histological staining of male and female Sprague Dawley rat liver tissue sections for DNA oxidative stress biomarker 8-OHdG expression (bright red) with nucleus co-staining with DAPI via immunofluorescence (IF). 1c Relative level of DNA oxidative stress via quantification of 8-OHdG-positive IF staining. Percent of positive-stained area compared across sex and treatment. * $p<0.05$.

Beyond oxidative damage, further inflammatory responses exhibit sex-specific trends that are induced during GnRHa administration (Figure 2). IHC staining for CK19 revealed increased bile duct mass in GnRHa-treated males but not in GnRHa-treated females (Figure 2a,c). Conversely, IHC staining for CD45 highlighted a significant increase in leukocyte presence in GnRHa-treated females but not in GnRHa-treated males, as shown in Figure 2b,d. Overall, GnRHa-treated males appeared to show a more advanced injury response (indicated by higher DNA oxidative stress levels and higher bile duct mass) than GnRHa-treated females (indicated by the lack of MDA oxidative stress or bile duct mass change). These results put together suggest that while GnRHa-treated males exhibit greater oxidative stress and biliary remodeling, females display a robust immune response, indicating distinct pathways of hepatic injury between biological sexes.

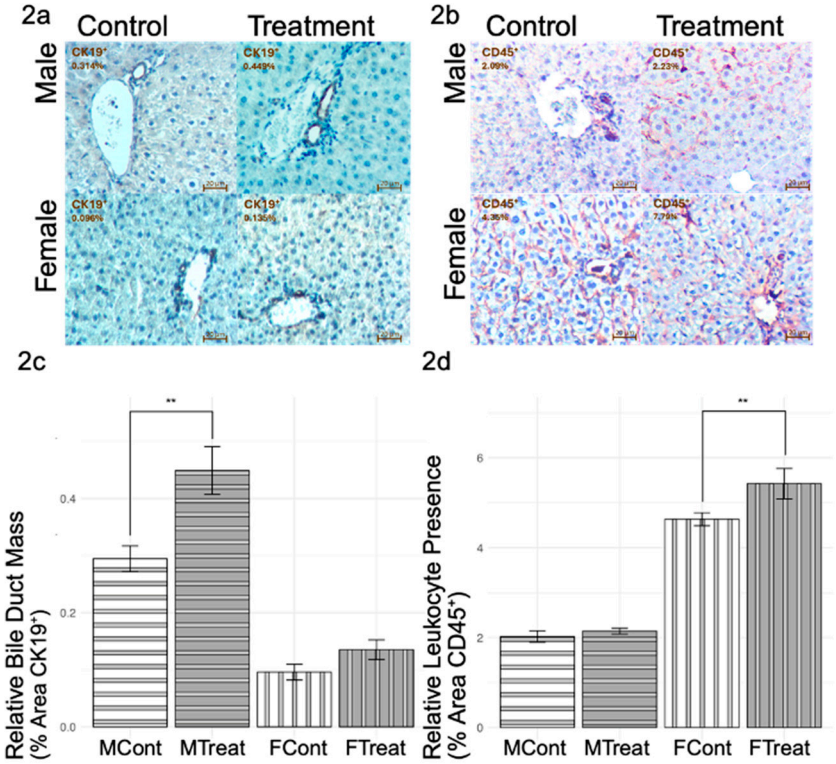

Figure 2. GnRHa administration induces inflammatory responses in a sex-specific manner. 2a-2b Histological staining of male and female Sprague Dawley rat liver tissue sections, 20x magnification. 2a Evaluation of cholangiocyte biomarker CK19 via IHC. 2b Evaluation of leukocyte biomarker CD45 via IHC. 2c-2d Percent of positive-stained area compared across sex. 2c Relative bile duct mass estimated via quantification and analysis of CK19+ IHC staining. 2d Relative leukocyte presence estimated via quantification and analysis of CD45+ IHC staining. * $p<0.05$, ** $p<0.01$.

3.2. GnRHa Administration Leads to Increased Fibrosis

Oxidative stress and inflammatory signaling pathways in hepatic injury often include profibrotic signals and increased collagen deposition. Therefore, fibrosis investigations included evaluating the level of collagen fibers and the expression levels of profibrotic biomarkers ⟨SMA and TIMP1. PSR Staining for collagen fibers (types I and III) revealed significantly increased fibrosis in

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both sexes, with males showing a greater fibrotic response than females (Figure 3a,d). Similarly, the expression of profibrotic biomarker ⟨SMA showed a significant increase in both sexes (Figure 3b,e). Notably, profibrotic TIMP1 levels in GnRHa males were significantly lowered, while GnRHa-treated females showed increased TIMP1 (Figure 3c,f). Overall, increased fibrosis was induced by GnRHa in both sexes, though TIMP1 levels suggest a difference in mechanistic pathways that lead to the same fibrotic end.

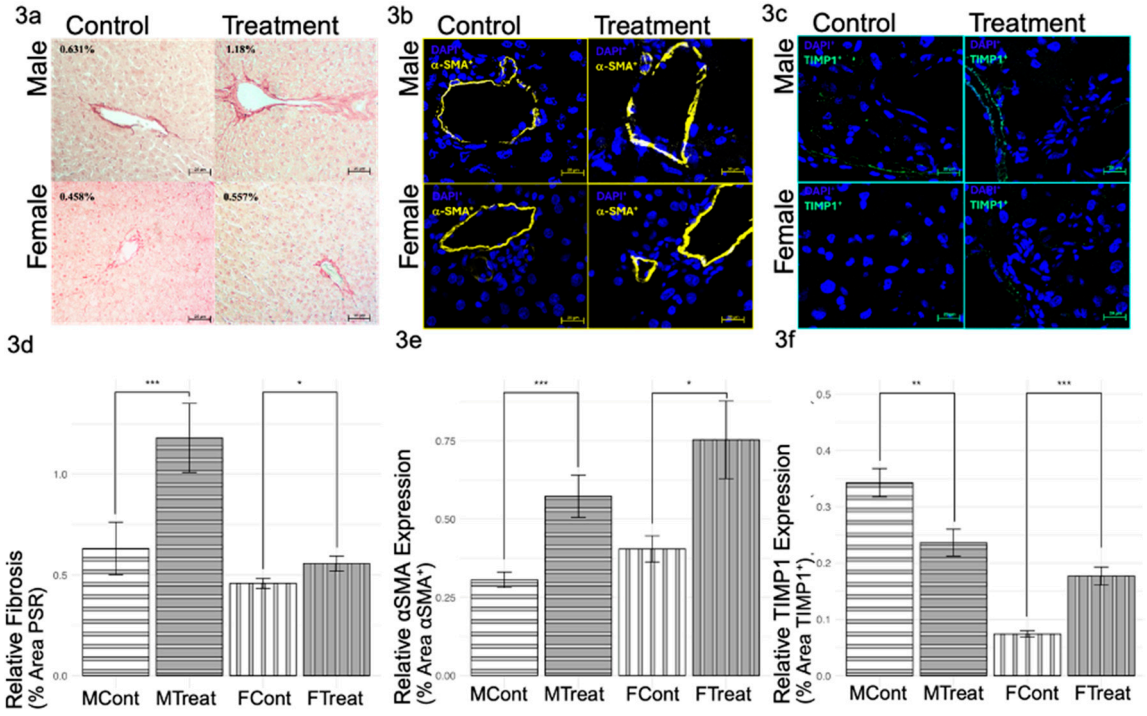

Figure 3. GnRHa Administration Induces Fibrosis. 3a-3c Histological staining of male and female Sprague Dawley rat liver tissue sections, 20x magnification. 3a Evaluation of fibrosis via PSR (dark red) liver tissue staining with average percent positive staining in top left corner for each group. 3b Evaluation of profibrotic αSMA expression (bright yellow) with nucleus co-staining with DAPI via IF. 3c Evaluation of profibrotic TIMP1 expression (bright teal) with nucleus co-staining via DAPI via IF. 3d-3f percent of positively stained area compared across sex and treatment. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. 3d Relative level of fibrosis per group via quantification and analysis of collagen-positive PSR tissue staining. 3e Relative level of αSMA per group via quantification and analysis of αSMA+ IF staining. 3f Relative level of TIMP1 per group via quantification and analysis of TIMP1+ IF staining.

3.3. Gross & Tissue Morphology Unaltered by GnRHa Puberty Blocking

Weekly body weights for male and female samples were not significantly different between the control and treatment groups (Supplemental Figures S1a,b). Further, histological examination of tissue morphology via H&E staining showed no significant GnRHa-induced changes (Supplemental Figure S1). Both treatment and control groups showed minimal but healthy levels of parenchymal cell death, with no signs of pathological steatosis. The absence of gross or histological abnormalities suggests that any GnRHa-induced hepatic alterations occur at the molecular and cellular level.

4. Discussion

This study shows that GnRHa administration induces sex-specific fibrotic liver injury responses, with male rats showing increased DNA and lipid oxidative stress in liver tissue and females not reflecting this pattern. This increased oxidative stress aligns with higher collagen deposition in male, as endogenous ROS accumulation is a strong driver of fibrogenesis. 8-OHdG and MDA are signs of ROS-induced damage, a common theme of hepatic injury and linked with the expression of

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profibrotic biomarkers, including ⟨SMA [15,30]. A recent study found that CCl4 hepatic injury increases in both ⟨SMA and MDA levels, further highlighting the link between oxidative damage and active fibrosis [31]. GnRHa-treated male rats reflected these hepatic injury trends of increased 8-OHdG and lipid oxidative stress in conjunction with increased ⟨SMA. Interestingly, female rats did not replicate this pattern, highlighting the incredible sexual dimorphism of the liver and the sex-hormone-linked molecular mechanisms present during the peripubertal period [12].

Estrogen and testosterone are the main sex-specific hormones driving puberty. The lack of testosterone in GnRHa males likely contributes to the increased oxidative stress shown in this study. Previous studies have shown that testosterone deprivation is linked to increased liver fibrosis in males. In castrated male rats, Boukari et al. found that MDA levels rise in the brain, prostate, and seminal vesicles and return to normal when supplemented with testosterone at low levels, suggesting that testosterone acts as a potent antioxidant [32]. Jin et al. conclude that adolescent males reflect the same hepatic sex-specific response. With peripubertal GnRHa treatment, male rats appeared to have an oxidative stress-driven fibrotic injury response. Recent research has shown that endothelial cells respond to GnRHa hormonal withdrawal with reduced capacity to accommodate endogenous ROS [32]. In females, increased estrogen expression has been linked to biliary injury, often accompanied by significantly increased oxidative stress, as explored by Zu et al. [33]. Interestingly, the lack of this oxidative response in GnRHa female rats is inconsistent with the link between estrogen deficiency and hepatic oxidative damage found in prior research. This suggests female resistance to oxidative stress may be estrogen independent.

Liver injury responses can include the activation of inflammatory cells, including Kupffer cells, HSCs, cholangiocytes, and other infiltrating leukocytes. While cholangiocyte injury response shares many functions with that of hepatocytes, including coordinating further injury responses through TGFβ1 signaling, cholangiocytes have not been found to utilize TIMP1 signaling to promote fibrogenesis [22,34]. Thus, it is unsurprising that male rats showed increased cholangiocytes in the absence of increased TIMP1 expression upon GnRHa administration. Defamie et al. also recently found that TIMP1-deficient progenitor cells preferentially differentiate into cholangiocytes over hepatocytes [35]. The positive relationship between oxidative damage and the cholangiocyte population in both sexes suggests that oxidative stress is a key contributor of DR during hepatic injury response.

Unlike male rats, puberty blocked females showed increased immune cell infiltration, not oxidative damage or DR. The increased presence of CD45+ immune cells with simultaneous increased TIMP1 expression supports a more leukocyte-mediated injury response pattern in females. Sharing a common progenitor cell, CD45+ inflammatory cells that respond to hepatic injury include liver-resident Kupffer macrophages, as well as circulating T-cells, B-cells, granulocytes, and macrophages [36]. These leukocytes have been the highlight of many studies, documenting the complex and cooperative way inflammatory responses are coordinated during liver injury. Similarly, the evidence that estrogen has a protective impact on the liver and can modulate inflammatory responses is well documented [12]. Thus, disruptions in estrogen levels may instigate altered injury response mechanisms in females. Increased leukocyte presence in female rats during peripubertal GnRHa administration in conjunction with increased expression of TIMP1 and ⟨SMA indicates active inflammation and the recruitment of circulating inflammatory responsive cells.

Beyond oxidative stress and changes in injury response cell populations, this study shows that GnRHa administration induces liver fibrosis in both sexes. Increased collagen deposition was accompanied by increased expression of ⟨SMA in both sexes, while TIMP1 expression was decreased in males but increased in females, highlighting TIMP1-independent fibrosis. TIMP1 levels increase during fibrogenesis to promote collagen extracellular matrix (ECM) production in early-stage injury and decrease in late-stage injury to favor MMP degradation of excess collagen ECM formed in earlier stages [25]. The sex-specific differential expression of TIMP1 does not fully explain the fibrotic response seen in this study; indeed, as Thiele et al. recently demonstrated, TIMP1 is not essential for liver fibrogenesis [37]. During GnRHa administration, male rats appeared to have a more advanced

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fibrotic response than females. This suggests that the DR and oxidative stress seen in males are critical for the progression of fibrosis. Interestingly, the sex-specific expression of TIMP1 is consistent with the differential levels of leucocyte infiltration. Studies showed that TIMP1 promotes the expression of monocyte chemoattractant protein (MCP)-1 to recruit CD45+ macrophages to the liver [17]. Our data suggest that the increased leukocytes in females following GnRHa administration may be attributed to a higher level of TIMP1 expression. Both male and female rats showed increased ⟨SMA expression, further highlighting active fibrogenesis during GnRHa administration, suggesting that at least some injury responses are similar between the sexes.

5. Conclusions

Overall, our study highlights the sex-specific nature of GnRHa-induced hepatic injury responses. While both GnRHa-treated males and females showed increased fibrosis, the male rats indicated a later injury stage with a more locally controlled damage response, evidenced by higher collagen levels, higher ⟨SMA levels, lower TIMP1 levels, and DR (CK19+). Females show a delayed injury response, potentially mediated by leukocytes, evidenced by lower collagen levels, higher αSMA levels, higher TIMP1 levels, and higher CD45+ levels.

Our research underscores the importance of considering sex-specific responses in the development and administration of treatments, suggesting that further studies are needed to fully understand the implications of GnRHa therapy on liver health and to optimize treatment protocols for all patients, including sex-specific co-treatments to supplement liver health. Additionally, dosage trials are needed to evaluate if altered GnRHa-induced hepatic damage is dose-dependent. GnRHa has a long history of use in managing central precocious puberty and other conditions, with minimal and manageable side effects [38,39]. While recent studies have raised concerns about potential risks, including increased heart [10] and brain damage [11], the therapeutic benefits of GnRHa for treating serious psychological disorders should continue to be evaluated within the context of individual patient needs and risks.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Supplemental Figure S1: GnRHa administration does not induce significant changes in body weight or obvious tissue morphology changes.

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Institutional Review Board Statement: All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee and the protocol approved under the Animal Welfare Act guidelines (protocol: 2301CD-DH-R-26, May 4, 2023).

Data Availability Statement: Data for this project is available upon request (corresponding author: Yuyan Han – yuyan.han@unco.edu). This is an ongoing project, so the full data set is not published for public access at this time.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

8-OHdG	8-hydroxy-2'-deoxyguanosine

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aSMA	alpha smooth muscle actin
CPP	central precocious puberty
DR	ductular reaction
GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone agonist
H&E	hematoxylin and eosin
HSC	hepatic stellate cells
IF	immunofluorescence
IHC	immunohistochemistry
LGBTQ+	lesbian, gay, bisexual, transgender, queer, and more
MCP-1	monocyte chemoattractant protein 1
MDA	malondialdehyde
OCT	optimal cutting temperature
PBS	phosphate-buffered saline
PBST	PBS with 0.1% Tween 20
PSR	Picro-Sirius Red
ROS	reactive oxygen species
TBARS	thiobarbituric acid-reactive substance
TBST	tris-buffered saline with 0.1% Tween 20
TGD	transgender and gender-diverse
TGFβ1	transforming growth factor beta 1
TIMP1	tissue inhibitor of metalloproteinases
US	United States

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