

Article

Sex-Dependent Regulation of Lipid Metabolism and Oxidative Stress in Cardiovascular-Relevant Tissues: A Porcine Model

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Abstract

Sex-related differences in lipid metabolism and oxidative stress are recognized as important modulators of cardiovascular physiology, but they are still insufficiently explored in large animal models. This study examined the influence of sex and gonadal status on oxidative stress, lipid metabolism, and hematological parameters in clinically healthy domestic pigs. Twenty sexually mature pigs (10 months old) were divided into four groups: boars, barrows, gilts, and ovariectomized females ($n = 5$ per group). In serum, the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-RD), total superoxide dismutase (TSOD), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH), together with concentrations of triglycerides, phospholipids, total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), low-density lipoprotein cholesterol (LDL cholesterol), non-esterified fatty acids (NEFAs), and malondialdehyde (MDA), were determined. In myocardial and liver tissues, antioxidant enzymes, lipid parameters, and MDA were analyzed. Spectrophotometric methods were used for enzyme activities and lipid parameters, while MDA was quantified by high-performance liquid chromatography. Hematological parameters were determined using an automated hematology analyzer, and serum hormone concentrations were measured by chemiluminescent immunoassays. Group-dependent differences were observed, although the relatively small sample size warrants cautious interpretation. In serum, gilts had higher phospholipid, total cholesterol, and HDL cholesterol concentrations, along with the most favorable LDL: HDL ratio, whereas boars showed the least favorable ratio. Lipid peroxidation, assessed by MDA, was lowest in gilts and highest in barrows. In myocardial tissue, gilts showed higher GSH-RD and TSOD activities and lower MDA concentrations than barrows, suggesting potentially stronger antioxidant protection. In the liver, differences were found in cholesterol, triglycerides,



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and MDA concentrations, with higher cholesterol in barrows, higher triglycerides in boars, and higher MDA in gilts and ovariectomized females. Hematological differences were also evident, with lower leukocyte and lymphocyte counts in gilts and higher platelet count and plateletcrit in barrows and ovariectomized females. Overall, sex and gonadal status were associated with distinct patterns of metabolic and oxidative responses across serum and tissues. Given the small sample size and cross-sectional design, these findings should be interpreted cautiously and as associative rather than causal.

Keywords: oxidative stress; lipid metabolism; domestic pigs; sex differences; antioxidant enzymes; cardiovascular health

1. Introduction

The healthy mammalian heart relies predominantly on fatty acid oxidation for energy production, with fatty acids providing approximately 60–70% of cardiac ATP, whereas carbohydrates, lactate, and ketone bodies contribute to a lesser extent [1–3]. Although this high oxidative capacity enables efficient energy production, it also increases susceptibility to oxidative stress due to continuous generation of reactive oxygen species (ROS) during mitochondrial respiration [2,4].

Reactive oxygen species are continuously generated during cellular metabolism, primarily within mitochondria, and in excess can damage lipids, proteins, and nucleic acids [5,6]. In highly oxidative tissues such as the myocardium, increased mitochondrial activity enhances the potential for oxidative injury. A key consequence of oxidative stress is lipid peroxidation, in which ROS attack polyunsaturated fatty acids in biological membranes, leading to membrane disruption and the formation of reactive aldehydes. Malondialdehyde (MDA), a stable end-product of this process, is widely used as a biomarker of oxidative stress in biological systems [7].

To maintain redox balance, organisms rely on a coordinated antioxidant defense system comprising enzymatic and non-enzymatic components. Key enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-RD), which act together to neutralize ROS and limit oxidative damage [8,9]. In addition, enzymes such as gamma-glutamyl transferase (GGT) contribute to glutathione metabolism and support intracellular redox homeostasis [10]. The balance between ROS production and antioxidant defense is essential for maintaining cellular integrity, particularly in metabolically active tissues [9,11]. In pigs, oxidative stress has been shown to play an important role in physiological responses to environmental and metabolic challenges, influencing enzyme activity, growth performance, and overall health [9,12].

Oxidative stress and lipid metabolism are closely interconnected. Impaired antioxidant defense can enhance lipid peroxidation, while altered lipid profiles may further increase ROS production, highlighting the need to evaluate both systems simultaneously [13,14]. Beyond the myocardium, oxidative stress has systemic implications, particularly in the liver and hematological system. The liver plays a central role in lipid metabolism, detoxification, and protein synthesis, making it both a target and regulator of oxidative processes [15,16]. Hepatic biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), primarily reflect hepatocellular integrity and liver function, whereas gamma-glutamyl transferase (GGT) is additionally involved in glutathione metabolism and contributes to intracellular redox homeostasis [10,17].

Hematological parameters provide additional insight into systemic metabolic and oxidative status. Reactive oxygen species can affect erythrocyte integrity, hemoglobin func-

tion, and leukocyte activity, while metabolic and hormonal factors influence erythropoiesis and immune responses [18,19]. Thus, hematological indices represent a functional link between oxidative stress and metabolic regulation.

Sex and gonadal hormones are important modulators of oxidative stress, lipid metabolism, hepatic function, and hematological profiles [20]. Females generally exhibit lower oxidative stress and higher antioxidant capacity, largely due to estrogen-mediated effects on mitochondrial function and antioxidant enzyme activity [20–23]. Estrogens also influence lipid metabolism by increasing high-density lipoprotein cholesterol (HDL cholesterol) levels and modulating hepatic lipoprotein synthesis [24–27]. In contrast, androgens affect fatty acid metabolism, lipoprotein dynamics, and erythropoiesis [25,28]. Consistent evidence indicates higher oxidative stress in males and greater antioxidant capacity in females across species [21,29–33]. Experimental studies in pigs further demonstrate that gonadal status significantly influences hepatic metabolism, antioxidant defense, and lipid profiles [34].

Domestic pigs (*Sus scrofa domesticus*) represent a valuable translational model for studying cardiovascular, hepatic, and metabolic physiology due to their anatomical and physiological similarity to humans [35–37]. However, despite extensive evidence on oxidative stress and lipid metabolism, comprehensive studies addressing sex- and gonadal status-dependent differences across multiple tissues in pigs remain limited.

The present study aimed to investigate sex- and gonadal status-related differences in oxidative stress and lipid metabolism in serum, myocardium, and liver, as well as hematological parameters, in intact males, castrated males, intact females, and ovariectomized females. The specific knowledge gap addressed was the limited understanding of whether sex and gonadal status are associated with coordinated but tissue-specific differences in systemic lipid metabolism, hepatic oxidative balance, and myocardial antioxidant defense. By assessing these parameters simultaneously across multiple biological compartments, this study provides an integrated characterization of sex-related metabolic and redox patterns and generates hypotheses for future mechanistic investigations.

2. Materials and Methods

All experimental observations and measurements used in the present analysis were collected in 2019.

2.1. Ethics Approval and Consent to Participate

No animals were specifically sacrificed for the purposes of this study. Blood samples were collected during routine farm procedures, and tissue samples were obtained post-mortem from clinically healthy pigs slaughtered under standard commercial conditions, without additional pain or distress. All procedures complied with Directive 2010/63/EU on the protection of animals used for scientific purposes, Regulation (EC) No 1099/2009 on the protection of animals at the time of killing, and national animal welfare legislation (Animal Protection Act, Official Gazette No. 102/2017, as amended). The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb, Croatia (24 January 2019; Class: 640-01/18-17/83; Registry No. 251-61-44-19-02). Written consent for the use of samples for research purposes was obtained from the farm and abattoir owners.

2.2. Animal Handling

The study was conducted at the swine farm of the Požega Penitentiary (Požega, Croatia), where pigs were reared under standard commercial husbandry conditions for pork production. Animals were housed in age-appropriate pens according to routine farm

management practices and had free access to water throughout the experimental period. The study included 20 clinically healthy, sexually mature domestic Swedish Landrace pigs (10 months old; 147 ± 20 kg), divided into four groups: intact males (boars), castrated males (barrows), intact females (gilts), and ovariectomized females ($n = 5$ per group). Animals originated from two closely related litters, which may reduce genetic variability among experimental groups. This breeding structure was considered when interpreting between-group variability, although it does not substitute for formal prospective statistical power calculations. At 3 weeks of age, five male pigs underwent surgical castration by bilateral orchiectomy through a scrotal approach, whereas five female pigs underwent bilateral ovariectomy using a paralumbar approach. General anaesthesia was induced with ketamine (10 mg/kg body weight) and xylazine (2 mg/kg body weight). Acepromazine was administered intramuscularly at a dose of 0.2 mg/kg body weight for preoperative sedation. In addition, animals received tetanus antitoxin (5 mL, subcutaneously) and long-acting penicillin (0.07 mL/kg body weight, intramuscularly) as perioperative prophylaxis. The corresponding intact male and female control animals underwent sham surgery following the same surgical procedure, but without removal of the gonads. Following recovery, all animals were returned to their respective group pens. After weaning, piglets were housed in $3 \text{ m} \times 2 \text{ m}$ pens ($0.42 \text{ m}^2/\text{animal}$) and fed a complete pelleted starter diet (Starter for piglets 20%, TSH d.d., Čakovec, Croatia) *ad libitum* until 4 months of age. From 4 months of age, animals were transferred to $2.5 \text{ m} \times 4.3 \text{ m}$ pens ($1.5 \text{ m}^2/\text{animal}$) and fed three times daily with a complete growing–finishing diet (BEK-1, TSH d.d., Čakovec, Croatia) up to 60 kg body weight. From 6 months until slaughter, pigs were housed in $3 \text{ m} \times 6 \text{ m}$ pens ($2.6 \text{ m}^2/\text{animal}$) and fed three times daily with a growing–finishing diet for pigs over 60 kg. During the final month, a supplemental diet (Superconcentrate 40%, TSH d.d., Čakovec, Croatia) was combined with ensiled maize grain. Pigs had *ad libitum* access to water throughout the rearing period. Feed intake was not individually recorded; however, all animals were group-housed and received the same diets under identical feeding regimes throughout the experimental period. Feed was provided either *ad libitum* or in equal portions per animal, depending on the production phase. Body weight was monitored during the rearing period, and all animals showed a uniform growth pattern, reaching the target slaughter weight without significant deviations. No health disorders or abnormal behaviour were observed during the study.

2.3. Sampling and Laboratory Analyses

Blood collection and serum preparation

At the end of the fattening period, blood samples were collected once from the cranial vena cava into Vacutainer tubes: EDTA-containing tubes for hematological analysis and tubes without anticoagulant (BD Vacutainer® SST™ II, Plymouth, UK) for serum separation.

After clotting, samples were centrifuged at $2205 \times g$ for 10 min. Serum was stored at -20°C for 24 h and subsequently at -80°C until analysis. Serum activities of glutathione peroxidase (GSH-Px; EC 1.11.1.9), glutathione reductase (GSH-RD; EC 1.6.4.2), total superoxide dismutase (TSOD; EC 1.15.1.1), gamma-glutamyl transferase (GGT; EC 2.3.2.2), and lactate dehydrogenase (LDH; EC 1.1.1.27) were determined. Serum concentrations of triacylglycerols, phospholipids, total cholesterol, HDL cholesterol, low-density lipoprotein cholesterol (LDL cholesterol), non-esterified fatty acids (NEFAs), and MDA were measured, together with estradiol, progesterone, and testosterone.

Hematological analysis

Hematological samples were stored at 4°C and analyzed using an automated hematology analyzer (Abacus Junior Vet, Diatron, Hungary). Samples were mixed prior to analysis and gently inverted (approximately 10 times) at room temperature. Analyses were per-

formed within 2 h 30 min after collection. All hematological measurements were performed in triplicate, with a maximum analytical variation of <4%, using manufacturer-provided reagents (Diatro Lyse_DIFF (Cat. No. D2041), Diatro Cleaner (Cat. No. D5011), and Diatro-Rinse (Cat. No. D4011; Diatron, Budapest, Hungary), according to the manufacturer's instructions. The hematology analyzer measured the following parameters: leukocyte count, lymphocyte count, granulocyte count, erythrocyte count, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDWc).

Tissue collection and biochemical analyses

Animals were slaughtered at 10 months of age. Samples of the left ventricular myocardium and liver were collected immediately post-mortem, placed on ice, and trimmed of visible adipose and connective tissue. Tissue samples (~1.5 g) were stored at −20 °C for 24 h and subsequently at −80 °C. For biochemical analyses, samples were homogenized in 0.14 M KCl (1:5, *w/v*) using a Teflon–glass Schüthomogen plus homogenizer (Schütt labortechnik, Göttingen, Germany) (2800 rpm; 90 s for myocardium and 60 s for liver) under cooling conditions. Homogenates were centrifuged with the centrifuge Sigma 3 K 15 (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 20,000 × *g* for 30 min at 4 °C. In myocardial and liver homogenates, enzyme activities (GSH-Px, GSH-RD, TSOD, GGT, LDH) and concentrations of triglycerides, phospholipids, cholesterol, and MDA were determined. In liver tissue, NEFA, glucose, total protein (TP), creatinine, urea nitrogen (BUN), and total bilirubin (TBI) were additionally measured.

Analytical procedures

Enzyme activities and lipid parameters, as well as all biochemical analyses except hormone measurements, were performed at the Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb (Zagreb, Croatia).

All biochemical parameters in serum and tissue samples were determined using the same analytical methods and assay kits to ensure comparability between sample types. Analyses were performed spectrophotometrically on an automated SABA 18 analyzer (AMS, Rome, Italy), according to the manufacturers' instructions. Commercial assay kits were used for glutathione peroxidase (Cat. No. RS505), glutathione reductase (Cat. No. GR2368), total superoxide dismutase (Cat. No. SD125), and non-esterified fatty acids (Cat. No. FA11530) (Randox Laboratories, Crumlin, UK); gamma-glutamyl transferase (Cat. No. TR19103), lactate dehydrogenase (Cat. No. TR20015), and total cholesterol (Cat. No. TR13303) (Thermo Scientific, Vantaa, Finland); glucose (Cat. No. TR-2000), triglycerides (Cat. No. TR-1258), HDL cholesterol (Cat. No. TR1035), LDL cholesterol (Cat. No. TR53101), BUN (Cat. No. TR-1054/KT-1054), creatinine (Cat. No. TR-0081/KL-0081), total bilirubin (Cat. No. TR-1120); and total protein (Cat. No. TR-1020) (Dijagnostika d.d., Sisak, Croatia); phospholipids (PAP 150, Cat. No. 61491) (bioMérieux, Lyon, France).

Total malondialdehyde concentrations were determined by high-performance liquid chromatography following the method of De Grotto et al. [38] using a TSP-130 system (Thermo Fisher Scientific, Waltham, MA, USA).

Serum results were expressed per liter, whereas myocardial and liver results were expressed per gram of wet tissue.

Serum concentrations of testosterone, progesterone, and estradiol were measured using the Access Testosterone (Cat. No. 33560), Access Progesterone (Cat. No. 33550), and Access Estradiol (Cat. No. 33540) assays, respectively, on the Access Immunoassay System (Beckman Coulter, Brea, CA, USA). The assays were based on paramagnetic particle

chemiluminescent immunoassays and were performed according to the manufacturer's instructions. The analytical performance and reliability of immunoassays for steroid hormone determination have been previously evaluated [39,40].

Biochemical analyses were performed using validated automated analytical methods according to the manufacturers' instructions. Measurements yielding questionable or inconsistent results were repeated to ensure analytical reliability.

2.4. Statistical Analysis

All statistical analyses were performed in R (version 4.3.1) using RStudio (version 1 September 2023). Data normality was assessed using the Shapiro–Wilk test. Variables that did not meet the assumption of normality were analyzed using non-parametric methods. Differences among groups were evaluated using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction when appropriate. Results are presented as medians with first and third quartiles (Q1–Q3). Statistically significant differences are indicated by different superscript letters in the tables. Given the small sample size ($n = 5$ per group), the study may have had limited power to detect small or moderate effects; therefore, non-significant findings should be interpreted cautiously because Type II errors cannot be excluded.

3. Results

Results for oxidative stress and lipid metabolism indicators in blood serum, liver and myocardium (left ventricle) of boars, barrows, gilts, and ovariectomized female pigs at the end of the fattening period are presented in Tables 1–4.

Table 1. Serum antioxidant enzymes, lipid profile and oxidative stress indicators, and hormones in boars, barrows, gilts, and ovariectomized females ($n = 5$ per group).

Parameter (Units)	Boars	Barrows	Gilts	Ovariectomized Females
GSH-Px (U/L)	4331 ^{ab} (4062–4805)	5207 ^b (5198–5313)	5187 ^b (4493–5265)	4478 ^{ab} (1761–5398)
GSH-RD (U/L)	118.17 ^{ab} (116.61–125.54)	144.34 ^c (130.37–195.30)	110.22 ^a (101.99–126.39)	113.34 ^{ab} (73.36–130.91)
TSOD (U/L)	80.05 (40.40–216.90)	26.65 (9.70–284.70)	43.30 (29.60–222.60)	40.07 (17.00–90.50)
GGT (U/L)	34.07 ^{ab} (23.40–42.63)	20.67 ^c (17.68–24.60)	43.11 ^a (40.33–47.96)	26.54 ^{bc} (8.74–56.32)
LDH (U/L)	719.00 ^{ab} (549–843)	752.50 ^{bc} (697–870)	899.50 ^c (801–924)	791.40 ^{bc} (680–884)
Triglycerides (mmol/L)	0.11 (0.07–0.43)	0.23 (0.08–0.31)	0.28 (0.14–0.53)	0.05 (0.03–0.11)
Phospholipids (mmol/L)	1.03 ^a (1.02–1.14)	1.23 ^b (1.14–1.36)	1.66 ^c (1.55–1.90)	1.30 ^b (1.14–1.43)
Total cholesterol (mmol/L)	2.07 ^a (2.00–2.16)	2.46 ^b (2.25–2.93)	3.05 ^c (2.89–3.29)	2.13 ^{ab} (1.75–2.68)
HDL cholesterol (mmol/L)	1.25 ^a (1.06–1.51)	1.66 ^b (1.47–1.78)	1.68 ^b (1.32–1.72)	1.35 ^{ab} (1.07–1.53)
LDL cholesterol (mmol/L)	0.17 (0.13–0.25)	0.18 (0.16–0.23)	0.16 (0.13–0.17)	0.13 (0.11–0.16)

Table 1. Cont.

Parameter (Units)	Boars	Barrows	Gilts	Ovariectomized Females
NEFA (mmol/L)	0.60 (0.37–1.18)	0.36 (0.25–0.41)	0.46 (0.36–0.50)	0.37 (0.30–0.45)
MDA (μmol/L)	4.25 ^{ab} (3.85–4.35)	4.70 ^b (4.51–4.83)	3.86 ^a (3.49–4.54)	4.07 ^{ab} (3.81–4.51)
Estradiol (pmol/L)	539 (351–771) ^a	69 (21–84) ^b	55.5 (53–75) ^b	35 (33–45) ^b
Progesterone (nmol/L)	1.09 (0.55–2.12) ^b	1.00 (0.44–2.11) ^b	12.13 (1.10–63.60) ^a	2.86 (0.73–3.45) ^b
Testosterone (nmol/L)	9.35 (6.97–15.37) ^a	1.12 (0.80–1.88) ^c	1.95 (1.28–2.52) ^b	2.81 (1.52–4.54) ^b

Glutathione peroxidase (GSH-Px); glutathione reductase (GSH-RD); total superoxide dismutase (TSOD); gamma-glutamyl transferase (GGT); lactate dehydrogenase (LDH); triglycerides; phospholipids; total cholesterol; high-density lipoprotein cholesterol (HDL cholesterol); low-density lipoprotein cholesterol (LDL cholesterol); non-esterified fatty acids (NEFA); malondialdehyde (MDA). Footnote: Values are median (Q1–Q3). Superscript letters indicate statistically significant differences between groups ($p < 0.05$) based on the Kruskal–Wallis test followed by Dunn’s post hoc test with Bonferroni adjustment, performed using R (version 4.3.1) in RStudio (1 September 2023).

Table 2. Myocardial antioxidant enzymes, lipid profile and oxidative stress indicators (left ventricle) in boars, barrows, gilts, and ovariectomized females ($n = 5$ per group), expressed per gram of wet tissue.

Parameter (Units)	Boars	Barrows	Gilts	Ovariectomized Females
GSH-Px (U/g wt)	2.60 (2.10–3.20) ^a	2.40 (2.30–3.10) ^a	3.20 (2.90–3.30) ^a	3.40 (3.10–3.80) ^a
GSH-RD (U/g wt)	0.86 (0.66–1.17) ^b	1.21 (0.99–1.45) ^{ab}	1.45 (1.38–1.56) ^a	1.49 (1.46–1.54) ^a
TSOD (U/g wt)	446.95 (412–480) ^b	450.80 (447–471) ^b	606.97 (508–665) ^a	645.20 (424–945) ^{ab}
LDH (U/g wt)	417 (363–546) ^a	417 (358–472) ^a	603 (494–781) ^a	523 (452–538) ^a
GGT (U/g wt)	0.04 (0.02–0.06) ^{ab}	0.03 (0.02–0.04) ^b	0.04 (0.02–0.07) ^{ab}	0.05 (0.04–0.10) ^a
Triglycerides (μmol/g wt)	5.26 (4.60–6.33) ^a	4.49 (3.77–4.98) ^b	4.84 (3.79–7.38) ^{ab}	4.66 (4.04–5.92) ^{ab}
Phospholipids (μmol/g)	0.79 (0.59–1.52) ^b	0.92 (0.72–1.07) ^b	1.17 (1.09–1.41) ^a	1.17 (0.94–1.29) ^{ab}
Cholesterol (μmol/g)	0.66 (0.38–0.83) ^{ab}	0.66 (0.50–0.92) ^a	0.62 (0.56–0.84) ^{ab}	0.69 (0.62–0.82) ^{ab}
MDA (nmol/g)	43.69 ^{ab} (38.53–58.07)	50.56 ^b (39.02–52.17)	33.42 ^a (32.38–38.87)	47.30 ^{ab} (35.32–64.42)

Glutathione peroxidase (GSH-Px); glutathione reductase (GSH-RD); total superoxide dismutase (TSOD); lactate dehydrogenase (LDH); gamma-glutamyl transferase (GGT); triglycerides; phospholipids; cholesterol; malondialdehyde (MDA); wet tissue weight (wt). Footnote: Values are median (Q1–Q3). Superscript letters indicate statistically significant differences between groups ($p < 0.05$) based on the Kruskal–Wallis test followed by Dunn’s post hoc test with Bonferroni adjustment, performed using R (version 4.3.1) in RStudio (1 September 2023).

Table 3. Liver antioxidant enzymes, lipid profile and oxidative stress indicators in boars, barrows, gilts, and ovariectomized females ($n = 5$ per group), expressed per gram of wet tissue.

Variable (Units of Wet Tissue (wt))	Boars	Barrows	Gilts	Ovariectomized Females
GSH-Px (U/g wt)	64.85 (50.86–69.25)	37.57 (15.30–118.69)	68.21 (66.96–83.32)	91.10 (84.80–98.96)
TSOD (U/g wt)	0.00002 (0.00001–0.00002)	0.00002 (0.00002–0.00002)	0.00002 (0.00001–0.00002)	0.00002 (0.00002–0.00002)
Cholesterol (μmol/g wt)	2.90 (2.83–3.39) ^{ab}	3.07 (3.01–3.56) ^a	2.72 (2.62–2.89) ^b	2.67 (2.59–2.73) ^b
Triglycerides (mmol/g wt)	0.0126 (0.0123–0.0143) ^a	0.0117 (0.0111–0.0123) ^{ab}	0.0085 (0.0073–0.0099) ^b	0.0113 (0.0107–0.0120) ^{ab}

Table 3. Cont.

Variable (Units of Wet Tissue (wt))	Boars	Barrows	Gilts	Ovariectomized Females
NEFA (mmol/g wt)	0.0145 (0.0078–0.0166)	0.0074 (0.0061–0.0105)	0.0049 (0.0048–0.0056)	0.0049 (0.0041–0.0061)
Glucose (mmol/g wt)	0.057 (0.035–0.071)	0.094 (0.093–0.095)	0.090 (0.088–0.096)	0.092 (0.092–0.094)
GGT (U/g wt)	3.57 (3.22–4.55)	2.42 (2.09–2.87)	2.90 (2.65–3.42)	2.77 (1.97–2.93)
LDH (U/g wt)	12.18 (10.52–13.29)	13.84 (12.73–13.84)	13.29 (12.18–15.50)	17.72 (16.61–20.49)
BUN (μ mol/g wt)	0.055 (0.046–0.066)	0.068 (0.051–0.071)	0.061 (0.045–0.070)	0.073 (0.071–0.081)
TBI (μ mol/g wt)	0.146 (0.126–0.179)	0.224 (0.176–0.244)	0.172 (0.165–0.235)	0.164 (0.132–0.183)
TP (g/g wt)	0.127 (0.118–0.148)	0.165 (0.151–0.196)	0.148 (0.136–0.161)	0.161 (0.140–0.161)
Creatinine (μ mol/g wt)	0.339 (0.258–0.366)	0.401 (0.357–0.429)	0.301 (0.292–0.330)	0.334 (0.267–0.356)
Phospholipids (mmol/g wt)	35.3 (31.9–42.9)	30.8 (24.2–41.3)	34.5 (27.6–36.6)	34.2 (32.6–52.6)
MDA (μ mol/g wt)	159.08 (104.27–227.66) ^b	244.21 (235.01–256.19) ^{ab}	312.35 (227.03–324.17) ^a	312.81 (297.14–337.65) ^a

Glutathione peroxidase (GSH-Px); total superoxide dismutase (TSOD); gamma-glutamyl transferase (GGT); lactate dehydrogenase (LDH); triglycerides; non-esterified fatty acids (NEFA); glucose; blood urea nitrogen (BUN); total bilirubin (TBI); total protein (TP); creatinine; phospholipids; malondialdehyde (MDA); wet tissue weight (wt). Footnote: Values are median (Q1–Q3). Superscript letters indicate statistically significant differences between groups ($p < 0.05$) based on the Kruskal–Wallis test followed by Dunn’s post hoc test with Bonferroni adjustment, performed using R (version 4.3.1) in RStudio (1 September 2023).

Table 4. Hematological parameters in boars, barrows, gilts, and ovariectomized females ($n = 5$ per group).

Parameter (Unit)	Boars	Barrows	Gilts	Ovariectomized Females
Leukocytes ($\times 10^9$ /L)	21.25 (20.71–22.44) ^a	16.55 (13.69–24.53) ^{ab}	16.77 (12.19–23.39) ^b	20.28 (14.63–25.93) ^{ab}
Lymphocytes ($\times 10^9$ /L)	13.32 (11.84–13.41) ^a	11.76 (9.67–12.47) ^{ab}	8.51 (6.34–16.93) ^b	11.13 (10.77–11.48) ^{ab}
Granulocytes ($\times 10^9$ /L)	9.27 (8.89–15.72) ^a	3.98 (3.91–12.53) ^b	6.33 (3.59–10.31) ^{ab}	5.13 (3.74–6.52) ^b
Erythrocytes ($\times 10^{12}$ /L)	7.55 (6.95–7.71) ^a	7.39 (7.04–7.57) ^a	6.80 (5.13–7.45) ^b	7.05 (6.25–7.85) ^{ab}
Hb (g/L)	123.50 (122.50–132.00) ^a	128.50 (119.50–134.50) ^a	112.50 (88.00–130.00) ^b	115.00 (101.00–129.00) ^{ab}
Hct (L/L)	0.399 (0.384–0.441) ^a	0.401 (0.373–0.421) ^a	0.365 (0.298–0.414) ^b	0.373 (0.328–0.418) ^{ab}
MCV (fL)	57 (51–58) ^a	55 (53–56) ^a	56 (54–58) ^a	53 (53–53) ^b
MCH (pg)	17.15 (16.20–17.80) ^a	17.40 (16.95–17.75) ^a	17.20 (16.55–17.40) ^a	16.28 (16.10–16.45) ^b
MCHC (g/L)	310 (300–319) ^a	320 (320–321) ^a	309 (296–313) ^a	308 (308–308) ^a
RDWc (%)	21.40 (19.80–21.70) ^a	20.60 (20.50–22.35) ^a	20.45 (20.40–21.30) ^a	20.85 (20.50–21.20) ^a
Platelet count ($\times 10^9$ /L)	244.00 (199.50–244.00) ^b	347.00 (304.50–367.00) ^a	183.50 (101.00–326.50) ^b	335.75 (274.00–397.50) ^a
PCT	0.002 (0.001–0.002) ^b	0.003 (0.003–0.003) ^a	0.002 (0.001–0.003) ^b	0.003 (0.002–0.003) ^a
MPV (fL)	7.40 (7.20–8.55) ^b	7.85 (7.50–7.95) ^a	7.75 (7.55–7.95) ^{ab}	7.80 (7.65–7.95) ^{ab}
PDWc (%)	40.40 (39.60–43.95) ^b	41.05 (40.25–41.30) ^a	40.65 (39.30–41.80) ^b	41.68 (41.55–41.80) ^a

Haemoglobin (Hb); haematocrit (Hct); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red cell distribution width (RDWc); plateletcrit (PCT); mean platelet volume (MPV); platelet distribution width (PDWc). Hematological parameters are grouped into Leukocyte indices, Erythrocyte indices, and Platelet indices, presented as median (Q1–Q3) with superscript letters indicating statistically significant differences (Kruskal–Wallis followed by Dunn’s test with Bonferroni correction, $p < 0.05$).

3.1. Serum Biochemical Parameters

Serum antioxidant enzyme activities, lipid parameters, oxidative stress indicators, and hormone concentrations showed group-dependent differences among boars, barrows, gilts, and ovariectomized females (Table 1). Barrows showed higher GSH-RD activity and MDA concentrations but lower GGT activity compared with gilts, while LDH activity was higher in gilts compared with boars. Serum GSH-Px activity was higher in barrows and gilts than in boars and ovariectomized females. Phospholipid and total cholesterol concentrations were highest in gilts and lowest in boars, with HDL cholesterol also lower in boars compared with gilts and barrows, whereas LDL cholesterol, NEFAs, triglycerides, and TSOD activity did not differ significantly among groups. Sex hormone concentrations varied by group: estradiol concentrations were highest in boars, progesterone concentrations were highest in gilts, whereas testosterone concentrations were highest in boars and lowest in barrows.

In gilts, total cholesterol was positively correlated with triglycerides ($r = 0.830$), phospholipids ($r = 0.966$), and NEFAs ($r = 0.968$) (Figure 1).

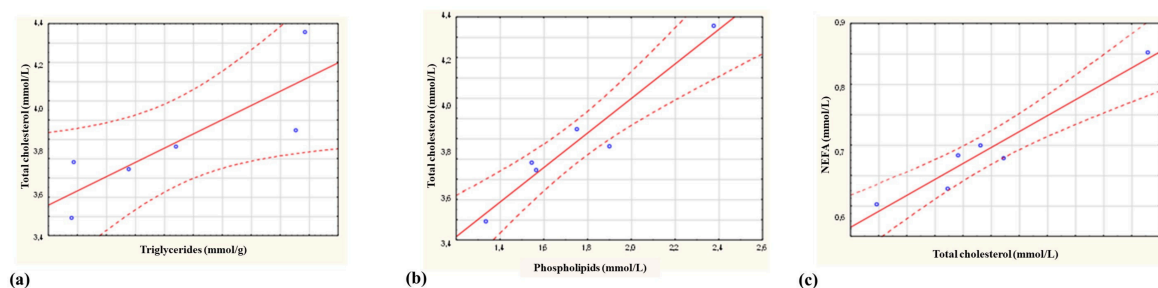


Figure 1. Correlations between lipid parameters in serum of gilts: (a) total cholesterol and triglycerides; (b) total cholesterol and phospholipids; (c) non-esterified fatty acids (NEFAs) and total cholesterol.

Calculated LDL:HDL ratios indicated a more favorable lipid profile in gilts (0.095) and ovariectomized females (0.096), intermediate values in barrows (0.108), and the least favorable profile in boars (0.136). In gilts, a negative correlation between HDL cholesterol and MDA levels was also observed ($r = -0.917$).

3.2. Myocardial Biochemical Parameters

Antioxidant enzyme activities, lipid profile parameters, and oxidative stress indicators in the left ventricular myocardium are presented in Table 2. Significant group differences were observed for GSH-RD, TSOD, GGT, triglycerides, phospholipids, and MDA ($p < 0.05$). GSH-RD activity was higher in gilts and ovariectomized females compared with boars. TSOD activity and phospholipid concentrations were higher in gilts than in boars and barrows. MDA levels were higher in barrows compared with gilts. Triglyceride concentrations were higher in boars compared with barrows. GGT activity was higher in ovariectomized females compared with barrows. No significant differences were found for GSH-Px, LDH, or cholesterol.

3.3. Liver Biochemical Parameters

Liver antioxidant enzyme activities, lipid profile parameters, and oxidative stress indicators are presented in Table 3. Significant differences among groups were detected for cholesterol, triglycerides, and MDA concentrations ($p < 0.05$), while no significant differences were observed for other parameters.

Barrows had higher cholesterol concentrations than gilts and ovariectomized females, while boars showed intermediate values and did not differ significantly from either group.

Triglyceride concentrations were higher in boars than in gilts, whereas barrows and ovariectomized females showed intermediate values and did not differ significantly from either group. MDA levels were higher in gilts and ovariectomized females compared with boars, with barrows again showing intermediate values.

3.4. Hematological Parameters

Hematological parameters in boars, barrows, gilts, and ovariectomized females are presented in Table 4. Significant differences ($p < 0.05$) were observed in hematological parameters among groups. Boars exhibited higher leukocyte and lymphocyte counts compared with gilts, while granulocyte counts were also higher in boars than in barrows and ovariectomized females. Erythrocyte indices (MCV and MCH) were lower in ovariectomized females compared with all other groups, whereas no significant differences were observed in MCHC or RDWc. Platelet-related parameters showed clear group-dependent variation. Platelet count and plateletcrit (PCT) were significantly higher in barrows and ovariectomized females compared with boars and gilts. Mean platelet volume (MPV) was higher in barrows than in boars, while platelet distribution width (PDWc) was higher in barrows and ovariectomized females compared with boars and gilts.

Group-dependent differences were observed. However, the relatively small sample size warrants cautious interpretation, particularly for non-significant findings, for which limited statistical power and potential Type II errors cannot be excluded. To provide a more comprehensive interpretation of the observed group differences beyond statistical significance alone, the principal pairwise comparisons, their approximate effect sizes, and the corresponding confidence intervals are summarized in Table 5.

Table 5. Exploratory non-parametric effect-size estimates and adjusted p -values for selected pairwise comparisons.

Variable	Comparison	Median Difference	Hodges–Lehmann Estimate	Approx. 95% CI	Cliff's δ	Bonferroni-Adjusted Dunn p -Value	Interpretation
Serum GGT	Gilts vs. Barrows	22.3490	18.7170	−81.660 to 94.657	0.2	1	*
Serum LDH	Gilts vs. Boars	66.3844	37.4546	−61.152 to 117.999	0.6	0.538116131	*
Myocardial GSH-RD	Gilts vs. Boars	0.3032	0.3032	0.207 to 0.400	0.3	1	*
Myocardial TSOD	Gilts vs. Boars	0.1287	0.1287	0.088 to 0.170	0.3	1	*
Myocardial MDA	Barrows vs. Gilts	−28.4964	−23.5726	−148.870 to 120.035	−0.2	1	*
Liver MDA	Gilts vs. Boars	83.7348	48.3757	−78.715 to 151.670	0.6	0.538116131	*
Platelet count	Barrows vs. Gilts	15.7675	15.3037	−6.678 to 26.990	0.8	0.94157046	*

* Interpretation: Descriptive non-parametric effect estimate; interpret with caution. Footnote: Effect sizes are presented as exploratory descriptive measures intended to complement, rather than replace, the primary Kruskal–Wallis and Dunn analyses. Because the primary analyses were non-parametric and the study included a small sample size, these estimates should be interpreted cautiously. Wide confidence intervals, particularly those including zero, indicate substantial uncertainty in the estimated effects. Cliff's δ is a non-parametric effect-size measure. Hodges–Lehmann estimates and 95% confidence intervals are based on pairwise differences. Dunn p -values are Bonferroni-adjusted across the six pairwise comparisons among the four groups.

4. Discussion

The present study investigated sex-related differences in lipid metabolism, oxidative stress, and hematological parameters in the serum, myocardium, and liver of sexually mature domestic pigs. The results revealed clear group-dependent differences, most consistently reflected in lipid metabolism, antioxidant enzyme activity, and lipid peroxidation markers. These observations are consistent with the possibility that sex-related endocrine

factors contribute to metabolic and redox regulation across tissues; however, given the cross-sectional and observational design, the present findings do not establish causal effects of sex hormones or gonadal status. Rather, they identify associations that may provide a basis for future mechanistic investigations.

Serum lipid metabolism and oxidative stress

In serum, gilts exhibited higher concentrations of total cholesterol, phospholipids, and HDL cholesterol compared with males, resulting in a more favorable lipid profile. The lower LDL: HDL ratio observed in gilts is further consistent with a more favorable lipid profile that may be associated with cardiovascular health. However, because cardiovascular function and clinical outcomes were not directly assessed, these findings should not be interpreted as evidence of cardiovascular protection.

Although circulating estradiol concentrations were highest in boars, the metabolic profile observed in gilts indicates that absolute hormone levels alone do not fully explain differences in lipid metabolism. Rather, tissue-specific sensitivity to sex hormones, receptor expression, and the balance between estrogenic and androgenic signaling are likely to play a more important role in regulating metabolic outcomes [20,41–43]. Elevated estradiol levels in boars are physiologically plausible and have been associated with testicular steroidogenesis and increased aromatase activity, which converts androgens to estrogens in male pigs [44,45]. However, these circulating concentrations do not necessarily translate into equivalent biological effects across tissues or sexes.

Similar sex-related differences in lipid metabolism have been reported in various mammalian species, including pigs, where estrogens influence hepatic lipid synthesis, lipoprotein metabolism, and cholesterol transport [24,26,27,34]. Estrogens are known to stimulate the expression of enzymes involved in lipid metabolism and to enhance HDL formation while reducing LDL accumulation, thereby contributing to improved lipid homeostasis and reduced cardiovascular risk [46]. The apparent discrepancy between circulating estradiol levels and metabolic outcomes in the present study likely reflects differences in endocrine regulation, including local hormone metabolism, receptor-mediated signaling, and interactions with other hormones such as testosterone [20].

The significant correlations observed between serum triglycerides, cholesterol, phospholipids, and NEFAs in gilts further support coordinated regulation of lipid metabolism in females. However, given the small sample size, these correlations should be considered exploratory and hypothesis-generating. The associations may be sensitive to individual observations and do not establish causality; confirmation in larger cohorts is warranted. Such relationships likely reflect the integrated control of lipid synthesis, mobilization, and utilization under hormonal regulation. Comparable associations between lipid parameters have been reported in metabolic and cardiovascular studies, where coordinated lipid regulation is considered an important determinant of metabolic stability [13].

Differences in oxidative stress markers were also evident. The lowest serum MDA concentrations were observed in gilts, whereas barrows showed the highest levels. MDA is a widely used indicator of lipid peroxidation and reflects the extent of oxidative damage to cellular membranes. The reduced MDA levels detected in gilts are consistent with a different redox profile compared with males; however, the present data do not establish that this difference results from more efficient redox regulation or from sex hormones specifically. Although estrogens are known to exert antioxidant effects through both direct scavenging of ROS and stimulation of endogenous antioxidant defense systems [20,22,23], sex-related differences in oxidative status cannot be attributed solely to circulating hormone levels. Instead, they likely reflect an integrated effect of hormonal signaling, mitochondrial function, and antioxidant system regulation [20]. Consequently, females frequently exhibit lower oxidative stress compared with males in both animal models and human populations [32].

These findings are also consistent with recent studies highlighting the role of oxidative stress in metabolic and physiological responses in pigs [12,46–48].

In addition, the observed negative association between HDL cholesterol and MDA further supports the protective role of HDL against oxidative damage, as HDL particles possess antioxidant and anti-inflammatory properties. Lower leukocyte and lymphocyte counts observed in gilts may reflect reduced systemic oxidative stress and a lower pro-inflammatory state. Reactive oxygen species and lipid peroxidation products are known to influence immune cell activation, proliferation, and cytokine signaling, linking redox balance with hematological responses [6,19,49]. These findings suggest that hematological parameters may, at least in part, mirror underlying oxidative status and metabolic regulation.

More importantly, the metabolic differences observed between the two groups need to be understood in the context of hormonal regulation in the physiology of reproduction. Sex hormones, such as estrogens and androgens, are not only involved in regulating the overall metabolic process but are also essential for controlling reproductive functions like gonadality, steroidogenesis, and reproductive tissues [20,25,26].

The better lipid profile and lower oxidative stress observed in gilts may be associated with the physiological conditions relevant to reproductive function; however, reproductive performance was not assessed in the present study, and causal or functional implications cannot be established. For instance, estrogens in females have been known to maintain ovarian function, folliculogenesis, and uterine physiology while simultaneously exerting their antioxidant actions to prevent oxidative stress from damaging reproductive tissues [22,23,50]. Lower lipid peroxidation in gilts could thus be one factor contributing to a favorable milieu for reproductive activities. Alternatively, the modified metabolic and redox states observed in barrows and ovariectomized females suggest that interference with gonad hormone signaling might affect not only metabolic processes throughout the body but also the physiological parameters required for proper reproduction. It is known that oxidative stress can contribute to gamete development impairment, decreased fertility, and abnormalities within reproductive tissues in different organisms [41,50,51]. From the above information, it can be concluded that the process of lipid metabolism is tightly connected with the endocrine regulation responsible for reproduction, and the reproductive condition affects not only overall metabolism but also reproductive physiology via hormonal mechanisms [20,25].

Myocardial antioxidant defense and lipid metabolism

Differences in myocardial antioxidant enzyme activities and lipid peroxidation markers were observed among the four experimental groups, indicating that both biological sex and gonadal status contributed to myocardial redox homeostasis. In both preclinical and clinical studies, females typically exhibit enhanced cardiac resilience compared to males, including improved endothelial function, reduced myocardial remodeling, and attenuated oxidative stress [37,52,53].

Gilts showed higher activities of GSH-RD and TSOD, together with lower MDA concentrations than barrows, suggesting more effective antioxidant protection in the female myocardium. Ovariectomized females exhibited antioxidant enzyme activities comparable to those of gilts, whereas MDA concentrations did not differ significantly from the other groups. The glutathione system, including GSH-Px and GSH-RD, represents a key cellular defense mechanism against oxidative damage by reducing hydrogen peroxide and lipid hydroperoxides [8].

The higher antioxidant capacity observed in gilts is consistent with more efficient redox regulation. Although estrogen-mediated effects on mitochondrial function and oxidative metabolism may contribute to this pattern, circulating hormone levels alone do not fully explain the observed differences, and the present study cannot determine the underlying

mechanisms. Experimental studies have shown that estrogens can improve mitochondrial efficiency, increase ATP production, and reduce ROS generation [21,22]. However, sex differences in myocardial redox balance also involve mitochondrial function, redox signaling pathways, and gene expression, independent of absolute hormone concentrations [20,54]. Because the myocardium contains a high density of mitochondria and relies heavily on oxidative metabolism for energy production, these mechanisms are particularly important for maintaining cardiac function and limiting oxidative damage [55,56].

Differences were also observed in myocardial lipid parameters. Triglyceride concentrations were higher in boars, whereas phospholipids were elevated in gilts. Under physiological conditions, the heart relies predominantly on fatty acid oxidation for ATP production, with intracellular triglycerides serving as a temporary energy reserve [2]. However, excessive myocardial lipid accumulation may impair cardiac function through lipotoxic mechanisms [57]. The higher triglyceride levels observed in males may therefore reflect differences in lipid utilization and metabolism; however, the specific contribution of androgen-related mechanisms cannot be determined from the present observational data. From a reproductive endocrinology perspective, these myocardial differences may reflect broader systemic effects of sex hormone signaling, as estrogens and androgens influence not only reproductive organs but also cardiovascular tissues through shared endocrine pathways.

Hepatic metabolism and oxidative stress regulation

The liver plays a central role in systemic lipid metabolism and redox homeostasis, and the present results indicate that sex-related differences in hepatic tissue are primarily driven by lipid metabolism rather than antioxidant enzyme activity. In contrast to other tissues, most hepatic antioxidant enzymes did not differ significantly among groups, suggesting a relatively stable enzymatic antioxidant capacity. Significant differences were observed in lipid-related parameters. Barrows exhibited higher hepatic cholesterol concentrations compared with gilts and ovariectomized females, while boars showed the highest triglyceride levels, indicating distinct patterns of lipid metabolism associated with sex and gonadal status. These findings are consistent with previous evidence that sex-related endocrine factors can influence hepatic lipid regulation, including fatty acid metabolism and lipoprotein synthesis; however, the present study does not establish a direct causal role for sex hormones [25,34,49].

Notably, gilts and ovariectomized females exhibited higher hepatic MDA concentrations compared with boars, indicating increased lipid peroxidation in the liver. This finding contrasts with the lower oxidative stress observed in serum and myocardial tissue of gilts and suggests a tissue-specific regulation of redox balance. The liver, as a central metabolic organ with high lipid turnover, is particularly susceptible to oxidative processes associated with fatty acid metabolism and detoxification pathways [15,16].

The apparent discrepancy between systemic and hepatic oxidative status likely reflects the distinct physiological role of the liver compared with circulating blood and myocardial tissue. As the primary organ responsible for lipid metabolism, biotransformation, and detoxification, the liver is continuously exposed to high metabolic activity and the generation of reactive oxygen species [15,16]. Consequently, increased hepatic lipid peroxidation does not necessarily indicate a generalized increase in systemic oxidative stress but may instead reflect tissue-specific differences in oxidative regulation. Moreover, because the present study was observational and based on biochemical endpoints, the underlying mechanisms responsible for this divergence cannot be determined and should be investigated in future studies.

Overall, these results indicate that hepatic responses to sex and gonadal status differ from those observed in serum and myocardium, with lipid metabolism playing a dominant

role and oxidative stress showing a distinct, tissue-specific pattern. These findings emphasize that oxidative status should not be interpreted as a uniform systemic phenomenon, as different tissues may exhibit distinct redox responses depending on their physiological function and metabolic demands.

Given the central role of hepatic in steroid hormone metabolism, including the synthesis, activation, and clearance of sex hormones, the observed hepatic differences may be associated with endocrine regulation related to reproductive status. This interpretation remains hypothetical because the cross-sectional design does not permit causal inference. The liver is a key organ in maintaining hormonal balance, and disruptions in hepatic lipid metabolism and oxidative stress may influence circulating hormone levels and, consequently, reproductive function.

Hematological differences

Sex-related variations were also evident in hematological parameters. Gilts exhibited lower leukocyte and lymphocyte counts compared with boars, whereas platelet counts and PCT values were higher in barrows and ovariectomized females. Sex hormones are known to influence immune function and hematopoiesis, and previous studies have demonstrated that both androgens and estrogens can modulate leukocyte production, erythropoiesis, and platelet activity [6,19,58,59].

The observed hematological differences occurred alongside changes in oxidative stress and lipid metabolism. However, because platelet activation and other direct inflammatory or coagulation markers were not assessed, these relationships should be considered associative rather than mechanistically established. Higher platelet counts and PCT values in barrows and ovariectomized females may be associated with oxidative and lipid-related processes, although no direct markers of platelet activation were assessed in this study, as oxidized lipids and lipid peroxidation products are known to promote platelet activation and procoagulant states.

Boars displayed higher erythrocyte counts, Hb concentrations, and Hct values compared with females. These findings are consistent with the well-established stimulatory effect of testosterone on erythropoiesis, which occurs through increased erythropoietin production and enhanced bone marrow activity [60].

In addition, lower MCV and MCH values observed in ovariectomized females suggest altered erythropoiesis associated with the absence of ovarian hormones. Estrogens are known to influence erythrocyte maturation, membrane stability, and iron metabolism, and their absence may contribute to changes in red blood cell indices. Overall, these findings suggest that hematological parameters may be associated with integrated metabolic and endocrine regulation, although the present study cannot determine the underlying mechanisms.

Implications for cardiovascular and translational research

The present study demonstrates that sex and gonadal status significantly influence lipid metabolism, oxidative stress regulation, and hematological parameters in pigs, highlighting a complex and tissue-specific pattern of metabolic regulation. The more favorable lipid profile and lower lipid peroxidation observed in gilts support a relatively protective systemic phenotype, whereas barrows exhibited a less favorable oxidative profile, indicating the impact of gonadal hormone removal on redox balance.

Although estrogens are known to exert protective effects in cardiovascular physiology, the present findings indicate that circulating hormone concentrations alone do not fully account for the observed differences. Instead, the results support the concept that sex-related variability arises from an integrated interaction between hormonal signaling, tissue-specific sensitivity, and metabolic regulation [20].

Similar sex-related differences have been described in humans, where premenopausal women generally exhibit a lower incidence of cardiovascular disease compared with men [22,61]. Experimental and comparative studies further suggest that females often display more efficient redox regulation and reduced oxidative damage; however, these differences are not uniform across tissues and conditions [62–64].

Importantly, the present study also revealed tissue-specific differences, with gilts showing reduced oxidative stress in serum and myocardium, but higher lipid peroxidation in the liver. This divergence highlights the importance of evaluating multiple organs when assessing systemic oxidative and metabolic status, as single-tissue interpretations may be misleading.

Domestic pigs represent a highly relevant translational model due to their anatomical and physiological similarity to humans, including comparable cardiac structure, coronary circulation, and metabolic regulation [35–37]. Collectively, these findings support the use of pigs as a model for investigating sex-specific mechanisms underlying cardiometabolic regulation and oxidative stress.

Finally, hematological changes observed in this study paralleled alterations in lipid metabolism and oxidative status, indicating that blood cell indices may reflect systemic metabolic and redox conditions. This integrative relationship further emphasizes the interconnected nature of lipid metabolism, oxidative balance, and hematological regulation.

Taken together, the present findings suggest that sex-related differences in metabolic and oxidative parameters extend beyond general physiological variation and may have direct implications for reproductive biology. The interaction between lipid metabolism, oxidative stress, and endocrine signaling is particularly relevant in the context of reproductive tissues, where redox balance plays a crucial role in processes such as folliculogenesis, oocyte quality, implantation, and spermatogenesis. Therefore, the observed patterns in gilts, characterized by a more favorable lipid profile and lower oxidative stress, may indicate conditions that support reproductive efficiency, whereas altered profiles in castrated or ovariectomized animals may reflect endocrine disruption with potential consequences for reproductive physiology.

Recent studies further support the importance of biological sex as a determinant of metabolic and oxidative responses in porcine models. Sex-related differences in serum cholesterol and triglyceride concentrations in Meishan pigs have been linked to differences in hepatic lipid biosynthesis and androgen-associated regulation, providing further evidence that sex may influence systemic lipid metabolism through hepatic pathways [34]. Similarly, biological sex has been shown to affect oxidative stress responses in porcine skeletal muscle, indicating that redox regulation may differ between males and females in a tissue- and physiological context-dependent manner [65]. Sex-specific metabolic differences have also been identified in porcine circulating immune cells, including differences in metabolic flux through the pentose phosphate pathway [66]. In addition, recent studies have reported sex-related differences in porcine mitochondrial function and have demonstrated sex-specific effects on cardiac function and metabolism in swine models of cardiometabolic disease [67,68]. Collectively, these findings are consistent with the present observation that sex and gonadal status are associated with distinct metabolic and oxidative patterns across serum, liver, and myocardium. However, these studies also highlight the tissue- and context-dependent nature of sex-related biological responses and support cautious interpretation of the present findings without assuming a single uniform hormonal mechanism.

Integrated interpretation of serum and tissue findings

The simultaneous assessment of serum, hepatic, and myocardial parameters provides an opportunity to consider the findings within an integrated physiological framework. The

more favorable circulating lipid profile and lower serum and myocardial MDA concentrations observed in gilts occurred alongside increased myocardial antioxidant enzyme activities, whereas the liver showed a different pattern, characterized by higher MDA concentrations in gilts and ovariectomized females. This tissue-specific divergence suggests that systemic lipid metabolism and oxidative balance may not be regulated uniformly across organs. Sex-related endocrine factors may contribute to these differences through interactions with lipid handling, antioxidant defense, and tissue-specific hormone responsiveness. Nevertheless, these mechanisms remain hypothetical in the present study and cannot be established from the cross-sectional design. The findings therefore support a model of coordinated but tissue-specific metabolic and redox regulation rather than a single uniform effect of sex or gonadal status.

Implications for livestock production, veterinary medicine, precision nutrition, and biomedical research

The observed sex- and gonadal status-related differences may have relevance beyond the experimental setting. In livestock production, understanding sex-associated variation in lipid metabolism and oxidative balance may contribute to improved characterization of metabolic phenotypes and animal health. In veterinary medicine, such information may assist in interpreting biochemical and hematological parameters in relation to sex and reproductive status. The findings may also provide a rationale for future precision-nutrition studies investigating whether nutritional strategies should account for sex-specific metabolic characteristics. From a biomedical perspective, pigs remain relevant models for cardiovascular and metabolic research because of their physiological similarities to humans. Nevertheless, these implications are prospective and should not be interpreted as direct production, clinical, or therapeutic recommendations based solely on the present findings. Validation in larger and mechanistically focused studies is required.

Study Limitations

A major limitation of the present study is the relatively small sample size. The experimental design was predetermined as part of a collaborative study, and the same cohort of pigs was used for all analyses. The animals originated from two closely related litters, which may have reduced genetic variability within the study population; however, this does not substitute for an a priori power analysis or eliminate uncertainty associated with limited statistical power. Consequently, the study may have had insufficient power to detect small or moderate effects, and Type II errors cannot be excluded. Therefore, non-significant findings should not be interpreted as definitive evidence that no biological differences exist.

In addition, the study was based on biochemical and hematological parameters without detailed molecular analyses of signaling pathways, receptor expression, or gene regulation. Therefore, the underlying mechanisms linking sex and gonadal status with oxidative stress and lipid metabolism could not be fully elucidated. Furthermore, the cross-sectional design does not allow assessment of dynamic changes over time or causal relationships between sex and gonadal status and metabolic outcomes. Future studies incorporating larger cohorts, a priori power calculations, longitudinal designs, effect-size estimates with confidence intervals, and molecular approaches will be important for confirming the observed patterns and clarifying the mechanisms underlying sex-specific metabolic and redox regulation in pigs.

5. Conclusions

The present findings indicate that sex and gonadal status are associated with distinct patterns of metabolic and oxidative regulation across serum and tissues. Gilts exhibited

a more favorable systemic lipid profile and lower oxidative stress markers in serum and myocardium, whereas the liver showed a different pattern characterized by increased lipid peroxidation in gilts and ovariectomized females. These findings emphasize the tissue-specific nature of sex-related metabolic and redox regulation. Given the small sample size and cross-sectional design, the results should be interpreted as associative and hypothesis-generating rather than causal. Further studies with larger cohorts, formal power calculations, and mechanistic approaches are warranted to determine the biological pathways underlying these observations.

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Institutional Review Board Statement: No animals were specifically sacrificed for the purposes of this study. Blood samples were collected during routine farm procedures, and tissue samples were obtained post-mortem from clinically healthy pigs slaughtered under standard commercial conditions, without additional pain or distress. All procedures complied with Directive 2010/63/EU on the protection of animals used for scientific purposes, Regulation (EC) No 1099/2009 on the protection of animals at the time of killing, and national animal welfare legislation (Animal Protection Act, Official Gazette No. 102/2017, as amended). The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb, Croatia (24 January 2019; Class: 640-01/18-17/83; Registry No. 251-61-44-19-02). Written consent for the use of samples for research purposes was obtained from the farm and abattoir owners.

Informed Consent Statement: Not applicable. Since the animals were institutionally owned by the Correctional Institution and Prison in Požega, no separate informed consent form for privately owned animals was applicable.

Data Availability Statement: The original data presented in the study are openly available in the Zenodo repository at <https://doi.org/10.5281/zenodo.19545023> (accessed on 2 July 2026).

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