

Article

PPAR γ Expression in Human Spermatozoa and Its Relationship with Seminal F₂-Isoprostanes and Resolvin D1 in the Presence of Varicocele and Urogenital Infections

Giulia Collodel ¹, Elena Moretti ^{1,*}, Caterina Marcucci ¹, Laura Liguori ¹, Daniela Marchini ², Roberta Corsaro ¹, Gabriele Centini ¹ and Cinzia Signorini ¹

¹ Department of Molecular and Developmental Medicine, University of Siena, 53100 Siena, Italy; giulia.collodel@unisi.it (G.C.); caterin.marcucci@student.unisi.it (C.M.); laura.liguori@student.unisi.it (L.L.); r.corsaro@student.unisi.it (R.C.); gabriele.centini@unisi.it (G.C.); cinzia.signorini@unisi.it (C.S.)
² Department of Life Sciences, University of Siena, 53100 Siena, Italy; daniela.marchini@unisi.it
* Correspondence: elena.moretti@unisi.it

Simple Summary: One of the most important knowledge acquisitions in the field of male infertility involves the relevant role of oxidative stress that, together with inflammation, is a common finding in many reproductive pathologies as varicocele, urogenital infections. Here, we studied the impact of these pathologies on sperm PPAR γ , a transcription factor activated by polyunsaturated fatty acids and fatty acid derivatives and a regulating agent in antioxidant response. PPAR γ causes a decrease in the production of proinflammatory cytokines, and it therefore has a significant involvement in controlling inflammation. Considering oxidative stress and inflammatory status, F₂-IsoPs, prostaglandin-like products derived from arachidonic acid, and RvD1, a resolvin acting as lipid mediator of resolution of inflammation, were measured. The main results obtained in this study indicate a reduced PPAR γ expression and high levels of F₂-IsoPs and RvD1 in cases with varicocele and urogenital infections. Therefore, the reduced expression of PPAR γ in human ejaculated spermatozoa is influenced by seminal oxidative stress and inflammation. PPAR γ could be potentially modulated by its natural or artificial ligand, affording new therapeutic opportunities for a potential treatment for male infertility, increasing the performance of spermatozoa.

Academic Editor: Hiromitsu Tanaka

Received: 29 November 2024

Revised: 23 January 2025

Accepted: 27 January 2025

Published: 28 January 2025

Citation: Collodel, G.; Moretti, E.; Marcucci, C.; Liguori, L.; Marchini, D.; Corsaro, R.; Centini, G.; Signorini, C. PPAR γ Expression in Human Spermatozoa and Its Relationship with Seminal F₂-Isoprostanes and Resolvin D1 in the Presence of Varicocele and Urogenital Infections. *Biology* **2025**, *14*, 137. <https://doi.org/10.3390/biology14020137>

Copyright: © 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) is a regulating agent in antioxidant response also involved in controlling inflammation. The impact of varicocele and urogenital infections on sperm PPAR γ expression was studied. The PPAR γ gene expression was investigated in spermatozoa of 26 normozoospermic men grouped according to their clinical conditions: normal semen parameters (N), normal semen parameters and varicocele (N + V), and normal semen parameters and urogenital infections (N + UI). Sperm PPAR γ expression was correlated with F₂-isoprostanes (F₂-IsoPs), as markers of lipid peroxidation, and Resolvin D1 (RvD1), a pro-resolving mediator in inflammation. Sperm PPAR γ expression was evaluated through comparative real-time PCR, and F₂-IsoPs and RvD1 were quantified in the seminal plasma via GC/NCI-MS/MS and immunoassay, respectively. PPAR γ expression correlates positively with sperm morphology and vitality and negatively with F₂-IsoPs and RvD1. Sperm morphology positively correlates with vitality and negatively with F₂-IsoP and RvD1 levels. Despite the normozoospermia in the three examined groups, sperm morphology and PPAR γ expression were significantly reduced in N + V and N + UI groups compared to the N group. Additionally, F₂-IsoP and RvD1 levels were elevated in N + V and N + UI patients.

These data suggest that PPAR γ expression is compromised by inflammation and lipoperoxidation, providing new insights to further explore new possibilities of targeted treatment of male infertility.

Keywords: F₂-isoprostanes; human spermatozoa; normozoospermia; PPAR γ expression; resolvin D1; urogenital infections; varicocele

1. Introduction

Spermatogenesis is a complex process in which spermatogonia develop into spermatozoa within seminal tubules. This transformation relies on various cell types and an optimal fatty acid (FA) profile composing phospholipids, both in plasma membrane and seminal plasma [1,2].

Moreover, mammalian spermatozoa, during their maturation, undergo crucial metabolic modifications. Nuclear receptors found in spermatozoa may play a role in orchestrating these metabolic changes [3]; in particular, Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) belongs to the nuclear hormone receptor superfamily, serving as a ligand-dependent transcription factor. PPAR γ regulates the gene expression networks needed for cell proliferation, differentiation, morphogenesis, and metabolic balance.

Moreover, PPAR γ serves as a critical transcriptional regulator modulating cellular glucose, lipid metabolism, and inflammatory responses [4].

Due to the dimensions of its binding cavity, the PPAR γ ligand domain can interact with a range of natural or synthetic lipophilic acids, specifically FA or their derivatives, known as eicosanoids, including prostaglandins, leukotrienes, and other arachidonic acid (ARA) metabolites [5,6].

Aquila and colleagues [7] were the first group who reported the presence of PPAR γ and the occurrence of PPAR γ function in human-ejaculated spermatozoa. Indeed, it is also well known that PPAR γ plays a critical role in ejaculated sperm motility and fertilization, since it is predominantly expressed in specific regions of these cells: in the apical region of the head, the post-acrosomal region, and mostly in the midpiece [8].

In addition, PPAR γ 's non-nuclear localization in ejaculated sperm aligns with the hypothesis of the non-genomic mechanism of action in this specific cell type, and it might be the exclusive regulatory pathway in ejaculated spermatozoa [3,7,9].

Currently, semen analysis is the cornerstone of the evaluation of male fertilizing potential. According to the latest edition of WHO guidelines [10], the 5th percentile represents the lower reference value for normal semen parameters. In some cases, even in the presence of normal semen parameters, medical history, and physical examination, the men are still infertile. This occurs in approximately 15% of infertile men, who are classified as having unexplained infertility [11,12]. Varicocele and urogenital infections represent two of the most common pathologies that can hamper male fertility, and they share a common condition based on inflammation-mediated oxidative stress (OS) [13–15]. OS that plays a relevant role in male infertility arises from an imbalance between reactive oxygen species [16,17] and antioxidant defense, leading to sperm dysfunction and damage to proteins, lipids, and DNA [18,19].

F₂-Isoprostanes (F₂-IsoPs) are isomers of prostaglandin F₂ alpha (PGF_{2 α}) belonging to the isoprostanoid class and resulting from the non-enzymatic oxidation of ARA, one of the molecular targets of OS damage. The mechanism leading to the formation of F₂-IsoPs from the non-enzymatic oxidation of ARA and the nomenclature system to classify F₂-IsoPs have been widely studied [20]. F₂-IsoPs have emerged as a potent and reliable marker of *in vivo* lipid peroxidation (LPO, the term used to indicate the reaction of lipids

with molecular oxygen and one of the most relevant processes in OS damage in biological systems), demonstrating their potential to accurately reflect OS status in various human diseases [20,21]. Among the 64 compounds that are generated by the mechanism for the formation of F₂-IsoPs [20], 8-epi-PGF_{2α} is one of the most represented and is one of the most evaluated isomers for the measurement of F₂-IsoPs.

In the management of inflammatory processes, specialized pro-resolving lipid mediators facilitate the resolution of inflammation. Specialized pro-resolving lipid mediators are formed by the enzymatic oxygenation of polyunsaturated fatty acids (PUFA) and, in addition to lipoxins, protectins, and maresins, include resolvins. As a component of specialized pro-resolving lipid mediators, Resolvin D1 (RvD1), derived from docosahexaenoic acid, exerts anti-inflammatory and pro-resolving activities in acute inflammation [22]. Signorini et al. [23] suggested a link between RvD1, OS, inflammation, and FA profile in human spermatozoa. That study provided strong evidence that RvD1 can represent a potential biomarker for identifying pathological seminal conditions.

The aim of this study was to quantify the PPAR γ relative mRNA levels in spermatozoa from patients with normal semen parameters (above the 5th percentile according to the WHO [10]), and with normal semen parameters and varicocele or positive semen culture (classified as urogenital infections). This approach allows us to explore whether the presence of these reproductive pathologies can influence PPAR γ expression. In the seminal plasma of the same cases, F₂-IsoPs and RvD1 were dosed in order to investigate the relationship between these two markers of OS and inflammation, respectively, and PPAR γ expression.

2. Materials and Methods

2.1. Patients

The semen samples were obtained from 26 selected donors (aged between 28 and 39 years) recruited at the Department of Molecular and Developmental Medicine, University of Siena.

Samples with semen parameters above the 5th percentile of the WHO guidelines for semen analysis [10] were included in this research. The participants had a normal karyotype, showed a BMI < 25 kg/m², and had normal serum hormonal levels (follicle-stimulating hormone, luteinizing hormone, testosterone, prolactin); they did not have any chronic diseases and were not receiving radiotherapy, chemotherapy, or medication. Donors did not take oral antioxidant supplements for at least 6 months before the study, or take recreational drugs or consume alcohol. All subjects with a smoking habit were excluded.

Among these 26 normozoospermic donors, 7 individuals had urogenital infections and 9 had varicocele. The specific infectious agent and, for patients with varicocele, the grade of the pathology, were recorded in each patient's medical history. The donors were grouped according to their clinical conditions:

Normal parameters with negative medical history (no problems declared during anamnesis) and normal clinical investigation in terms of absence of varicocele and urogenital infections (n° 10, N);

Normal parameters and urogenital infections (n° 7, N + UI);

Normal parameters and varicocele (n° 9, N + V).

The participants were informed about the study protocol and their privacy was ensured. They signed an informed consent form before participating in this research, agreeing that their semen samples could be used for scientific purposes. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethic Committee Siena University Hospital (ID CEAVSE 25612).

2.2. Semen Analysis and Sample Preparation

The semen samples were collected in sterile containers by masturbation after 3–5 days of sexual abstinence, and the semen was preserved from temperature fluctuations and it was ensured that it was delivered to the laboratory within 30 min of collection.

Semen analysis was performed following the WHO guidelines [10]. Normal semen parameters typically fall above the 5th percentile.

After semen analysis, the samples were centrifuged at $400\times g$ for 10 min and spermatozoa and the seminal plasma were stored separately at $-80\text{ }^{\circ}\text{C}$.

2.3. Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) Determination

The determination of PPAR γ expression was performed using PPAR γ relative mRNA levels, starting with mRNA extraction from frozen sperm cell pellets using the RNeasy[®] Plus Universal Mini Kit (QIAGEN, Hilden, Germany), following the protocol provided by the manufacturer. Briefly, each sample was lysed by adding QIAzol Lysis Reagent, and gDNA Eliminator Solution was also added. After centrifugation at $12,000\times g$ for 15 min at $4\text{ }^{\circ}\text{C}$, RNA was further purified using a RNeasy Mini spin column (QIAGEN, Hilden, Germany). Following RNA extraction, quantification was performed using NanoDrop[™] One (Thermo Scientific[™] NanoDrop[™] One Microvolume UV-Vis Spectrophotometers, Fisher Scientific, Waltham, MA, USA). To synthesize single-stranded cDNA from the extracted RNA, reverse transcription (RT) was performed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA). The reaction volume was set to 20 μL , without the use of an RNase Inhibitor. A $2\times$ RT Master Mix was prepared as indicated in the kit instructions. For each sample, 500 ng of RNA was reverse-transcribed in a final reaction volume to 20 μL . The reaction mix was centrifuged briefly to remove air bubbles and kept on ice before loading into the thermal cycler (PTC-200 Thermal Cycler, MJ Research, Waltham, MA, USA), which was programmed according to the instructions.

Comparative real-time PCR was performed using the QuantStudio[™] 5 Real-Time PCR System (ThermoFisher, Waltham, MA, USA). The analysis was carried out in a 96-well plate; each sample was amplified in triplicate for enhanced accuracy for both PPAR γ and glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), the housekeeping gene used as a control. The corresponding primers were purchased from Bio-Rad (Assay ID qHsaCED0044425—PPAR γ ; Assay ID qHsaCED0038674—GAPDH, Biorad, Hercules, CA, USA).

Reagents are prepared following the SYBR[®] Green Assay protocol (Bio-Rad, Hercules, CA, USA). The number of cycles and temperatures and the time for each cycle were set according to the manufacturer's instruction. In this study, PPAR γ expression results were normalized to the GAPDH mRNA level. The relative quantification of the comparative real-time PCR data was determined using the double delta C_T ($2^{-\Delta\Delta C_T}$) method [24].

2.4. F₂-Isoprostane (F₂-IsoP) Determination

After thawing, the levels of total F₂-IsoPs in seminal plasma were quantified using a procedure also applied in other types of biological samples [25]. Briefly, basic hydrolysis was carried out in the presence of 1N KOH by means of incubation at $45\text{ }^{\circ}\text{C}$ for 45 min. Then, 1N HCl was added to acidify and 500 pg of PGF_{2 α} -d₄ was also added to each sample. Afterward, each sample was purified by two solid phase extractions, the first using an octadecylsilane (C₁₈) cartridge, and the second using an aminopropyl (NH₂) cartridge. In the subsequent derivatization process, the F₂-IsoP carboxylic group was derivatized into pentafluorobenzyl esters, and the hydroxyl groups were converted to trimethylsilyl ethers.

The final quantification of F₂-IsoPs was performed using gas chromatography/negative ion chemical ionization tandem mass spectrometry (GC/NICI-MS/MS) analysis (Trace GC and PolarisQ Ion Trap, Thermo Finnigan, San Jose, CA, USA). F₂-IsoPs were quantified by measuring the *m/z* 299 ion, referred to as the 8-iso-PGF_{2 α} , which is known as a relevant F₂-IsoPs isomer. Results are reported as ng/mL.

2.5. Resolvin D1 (RvD1) Determination

In seminal plasma, the levels of RvD1 were quantified using a double-antibody sandwich, Enzyme-Linked Immunosorbent Assay (MyBioSource, San Diego, CA, USA). The assay utilized a pre-coated anti-RvD1 monoclonal antibody as the capture antibody and a biotin-labeled polyclonal antibody as the detecting antibody. The optical density (OD) was measured at 450 nm.

2.6. Statistical Analysis

Statistical analysis was performed with SPSS version 17.0 for Windows software package (SPSS Inc, Chicago, IL, USA). Data were reported as medians and interquartile range (IQR 25°–75° percentiles). Spearman's rho (*r*) coefficient was calculated to measure the correlation between variables for all enrolled cases. The comparisons between groups (N, N + V, N + UI) were evaluated using a Kruskal–Wallis test, followed, for significant cases only, by Dunnet's post hoc test applied for pairwise comparisons. A *p* value < 0.05 (two-tailed) was considered statistically significant.

3. Results

Semen volume, sperm concentration, progressive motility, normal morphology, and vitality were assessed in semen samples of 26 individuals with normal semen parameters (>5th percentile, [10]). F₂-IsoPs and RvD1 were dosed in seminal plasma, and PPAR γ expression was assessed in spermatozoa.

In Table 1, the medians (25° and 75° percentiles) for each variable are shown.

Table 1. Considered parameters in the studied population. Median (25° and 75° percentiles) of the considered variables in the 26 cases included in this study.

Variables	Median (25°–75° percentiles)
Semen volume (mL)	3.50 (2.70–4.50)
Sperm concentration (10 ⁶ per mL)	75.00 (44.00–17.80)
Sperm progressive motility (PR, %)	47.50 (42.00–56.60)
Normal sperm morphology (%)	11.00 (6.75–14.25)
Sperm vitality (%)	77.00 (70.00–85.25)
Seminal F ₂ -Isoprostanes (F ₂ -IsoPs, ng/mL)	48.87 (16.05–82.01)
Seminal Resolvin-D1 (RvD1, pg/mL)	40.65 (31.63–83.32)
PPAR γ (relative mRNA expression)	0.61 (0.29–1.10)

To understand the possible correlations among the studied variables, we used Spearman's rank correlation coefficient, considering the whole population of interest. In Table 2, the significant correlations are shown.

Table 2. Significant correlations are reported, * $p < 0.05$, ** $p < 0.01$.

	Normal Sperm Morphology (%)	Sperm Vitality (%)	Seminal F ₂ -Isoprostanes (F ₂ -IsoPs, ng/mL)	Seminal Resolvin-D1 (RvD1, pg/mL)
Sperm vitality (%)	$r = 0.54^{**}$ 95% C. I. = 0.19 to 0.78			
Seminal F ₂ -Isoprostanes (F ₂ -IsoPs, ng/mL)	$r = -0.40^*$ 95% C. I. = -0.69 to -0.01			
Seminal Resolvin-D1 (RvD1, pg/mL)	$r = -0.46^*$ 95% C. I. = -0.73 to -0.08	$r = -0.48^*$ 95% C. I. = -0.74 to -0.11		
PPAR γ (relative mRNA expression)	$r = 0.45^*$ 95% C. I. = 0.06 to 0.72	$r = 0.58^{**}$ 95% C. I. = 0.24 to 0.80	$r = -0.54^{**}$ 95% C. I. = -0.77 to -0.18	$r = -0.60^{**}$ 95% C. I. = -0.81 to -0.27

Regarding sperm characteristics, sperm morphology positively correlated with sperm vitality ($r = 0.54, p < 0.01$) and negatively with F₂-IsoP levels ($r = -0.40, p < 0.05$) and RvD1 ($r = -0.46, p < 0.05$). RvD1 also showed a negative correlation with sperm vitality ($r = -0.48, p < 0.05$).

PPAR γ expression had a positive correlation with sperm morphology and vitality (respectively, $r = 0.45, p < 0.05$; $r = 0.58, p < 0.01$) and it was negatively correlated with F₂-IsoP levels ($r = -0.54, p < 0.01$) and RvD1 ($r = -0.60, p < 0.01$).

The same variables were compared in the three groups (Table 3): the group with normal semen parameters (N), the group with normal parameters and urogenital infections (N + UI), and the group with normal parameters and varicocele (N + V).

Table 3. Medians (interquartile range) of sperm characteristics, seminal F₂-isoprostanes (F₂-IsoPs), seminal Resolvin-D1 (RvD1), and sperm PPAR γ expression evaluated in semen samples of 26 men, divided into three groups according to their clinical condition (normal parameters: 10 men, N; normal parameters and urogenital infections: 7 men (N + UI); normal parameters and varicocele: 9 men, N + V). Statistics are also reported: NS non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Variables	N	N + UI	N + V	Kruskal–Wallis Test	Dunnet’s Post Hoc Test
Semen volume (mL)	3.70 (2.60–4.75)	3.50 (3.25–5.75)	2.80 (2.40–3.80)	NS	/
Sperm concentration (10 ⁶ per mL)	54.50 (44.25–112.25)	86.00 (54.00–135.00)	82.00 (43.00–110.00)	NS	/
Sperm progressive motility (PR, %)	50.00 (47.25–61.00)	42.00 (40.00–54.50)	46.00 (44.00–48.00)	NS	/
Normal sperm morphology (%)	13.50 (11.25–20.00)	8.00 (6.00–14.50)	8.00 (7.00–11.00)	$p < 0.05$	N + V vs. N * N + UI vs. N *
Sperm vitality (%)	82.00 (78.50–89.00)	70.00 (65.00–73.00)	72.00 (70.00–85.00)	$p < 0.001$	N + UI vs. N ***
Seminal F ₂ -IsoPs (ng/mL)	15.14 (9.42–27.87)	45.27 (38.94–68.49)	98.49 (67.11–111.05)	$p < 0.01$	N + UI vs. N * N + V vs. N *** N + V vs. N + UI *
Seminal RvD1 (pg/mL)	31.38 (29.85–33.01)	98.49 (74.07–103.25)	45.96 (37.65–78.26)	$p < 0.001$	N + UI vs. N *** N + V vs. N **
PPAR γ (relative mRNA expression)	1.24 (0.72–1.51)	0.34 (0.25–0.71)	0.31 (0.20–0.46)	$p < 0.01$	N + UI vs. N ** N + V vs. N **

Sperm morphology was significantly reduced in the cases of N + V and N + UI with respect to the N group ($p < 0.05$); sperm vitality was also significantly decreased in N + UI with respect to the N group ($p < 0.001$). The levels of F₂-IsoPs were significantly lower in the N group with respect to those measured in the N + UI group ($p < 0.05$) and N + V ($p < 0.001$). The N + V group showed significantly higher levels of F₂-IsoPs than the N + UI group ($p < 0.05$).

Despite the condition of normozoospermia, the N + UI patients ($p < 0.01$) and N + V group ($p < 0.01$) showed higher RvD1 levels with respect to those measured in group N.

The PPAR γ expression was significantly increased in the N + UI and N + V groups compared to that detected in the N group ($p < 0.01$) (Figure 1).

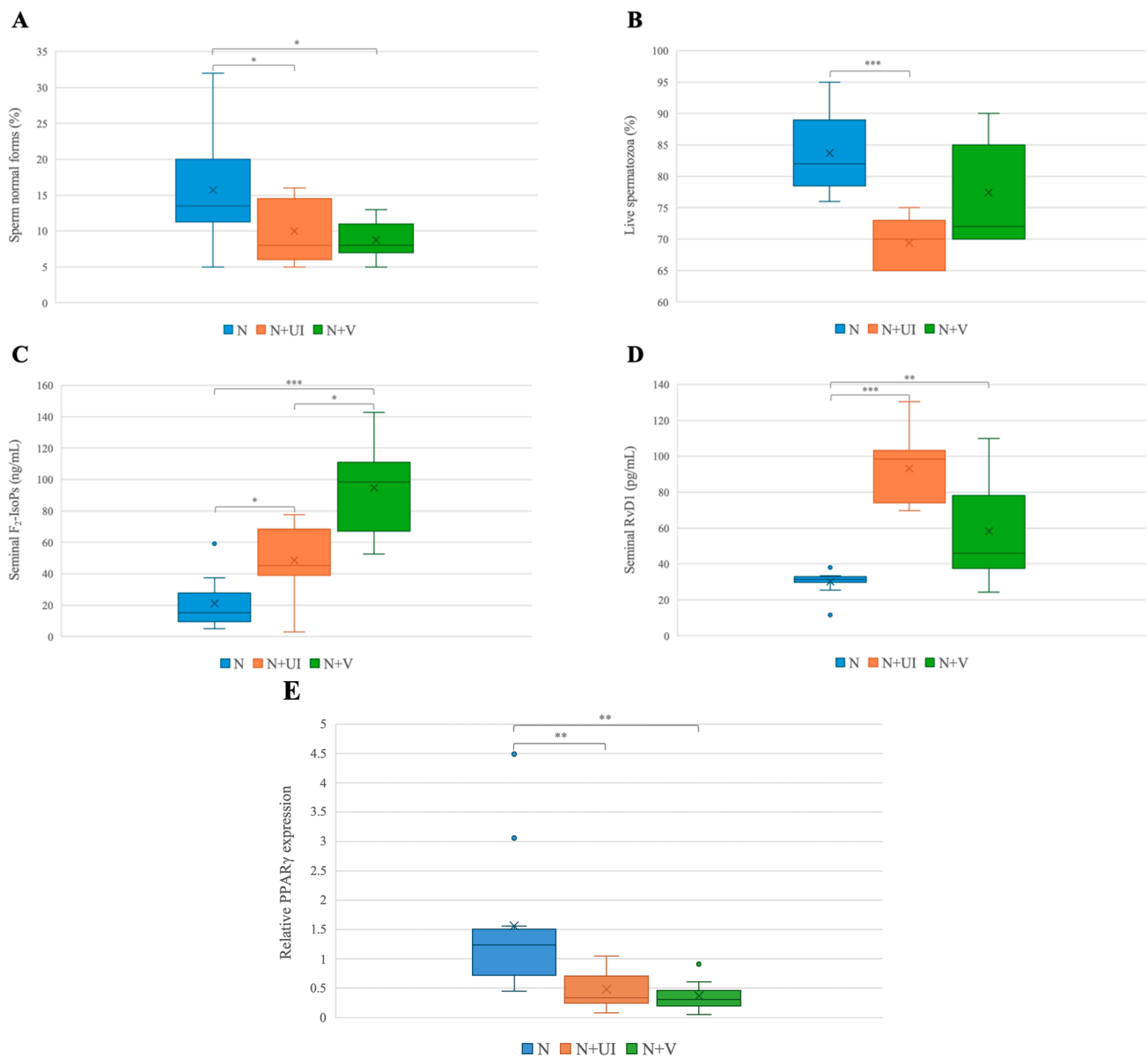


Figure 1. Box plots representing the sperm morphology % (A), the sperm vitality % (B), the seminal F₂-IsoPs pg/mL (C), the seminal RvD1 pg/mL (D), and sperm PPAR γ relative expression (E) in the three analyzed groups. N = donors with normal parameters with negative anamnesis and clinical investigation; N + UI = donors with normal parameters and urogenital infections; N + V = donors with normal parameters and varicocele. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

In this study, the expression of PPAR γ was evaluated in the spermatozoa of men with normal semen parameters (N), and in men with normal semen parameters affected by pathological reproductive conditions such as varicocele (N + V) or urogenital infections (N + UI). The use of samples with normal sperm parameters allows the study of the direct impact of the pathology in PPAR γ expression, OS, and inflammatory status.

PPAR γ belongs to the family of nuclear receptors; however, while other nuclear receptors have a single specific ligand, numerous natural PPAR γ ligands, in particular FAs or their derivatives eicosanoids, are available. Due to the close relationship between PPAR γ and lipid metabolites, in this study, we considered the seminal levels of F₂-IsoPs, oxygenated products from ARA metabolism, and RvD1, a resolvins acting as a lipid mediator of resolution of inflammation. Cumulative findings indicate that seminal F₂-IsoPs and RvD1 are promising biomarkers for the evaluation of semen quality. Fertile men have lower levels of seminal F₂-IsoPs and RvD1 with respect to those observed in infertile men with reproductive pathologies associated with inflammation [23,26]. Polvani's group [27] proposed that PPAR γ exerts an anti-inflammatory effect mediated by a non-genomic pathway. It is known that by stimulating PPAR γ through its ligands, a decrease in the production of proinflammatory cytokines is achieved and this reduction is facilitated mainly through the suppression of NF- κ B [28].

PPAR γ is expressed in human ejaculated spermatozoa [7], in the Sertoli cells and spermatocytes, indicating a critical role of this receptor in spermatogenesis [8].

The present study indicated that the expression of PPAR γ in spermatozoa was associated with seminal lipid markers of inflammation and OS. Sperm PPAR γ showed negative correlations with seminal RvD1 and F₂-IsoPs, suggesting that this transcription factor is reduced in conditions of inflammation and LPO in human semen.

Remarkably, PPARs are a group of fatty acid-regulated transcription factors that control both lipid metabolism and inflammation, and for PPAR γ , unsaturated fatty acids are listed as endogenous ligands. In particular, the main mechanisms responsible for the anti-inflammatory activity of PPAR γ is the crosstalk with the nuclear factor erythroid 2-related factor 2 (NRF2) [29]. In addition, several antioxidant genes are under transcriptional control of PPARs [30] and the cooperation between the transcription factors, Nrf2 and PPAR γ , against oxidative damage has been described [31]. Interestingly, PPAR γ has been reported to be involved in the regulation of anti-inflammatory mechanisms and OS in the brain [32]. Thus, PPAR γ is indicated as an anti-inflammatory and antioxidant gene [27].

The positive relationship between sperm PPAR γ and sperm morphology and vitality, observed in the present study, supports its protective action on human spermatozoa. The link between PPAR γ and vitality was also observed in pig spermatozoa [33]. We did not find any relationship between progressive motility and PPAR γ expression, probably due to the reduced number of cases included in the study. Conversely, other authors reported that sperm PPAR γ mRNA expression and protein levels showed a tendency to be higher in normozoospermic than asthenozoospermic men [3], indicating a positive link between PPAR γ and human sperm motility.

The comparison between the three considered categories (N; N + V; N + UI) showed a reduced PPAR γ expression in N + V and N + UI groups with respect to that detected in the N group, suggesting that reproductive pathologies, such as varicocele or urogenital infections, in cases where increased F₂-IsoP and RvD1 levels indicate the presence OS and inflammation, can influence PPAR γ expression. The increased RvD1 values indicate that the presence of OS and inflammation push for the resolution of this process in the two pathological conditions investigated.

Despite normal sperm parameters, the percentages of sperm with normal morphology and normal vitality were reduced in the presence of varicocele and urogenital infections, suggesting a protective role of PPAR γ in the regulation of sperm maturation and morphology. To support this hypothesis, studies have shown a significant decrease in PPAR α expression in the testes of a mice model of varicocele [34]. It is also reasonable to suppose that reduced PPAR γ activity might contribute to abnormal sperm morphology, since PPAR γ shares a similar modular structure with steroid receptors that are under-expressed in men affected by varicocele [35].

The morphology of spermatozoa is a parameter that indicates their overall health and function, and the presence of morphological abnormalities results in an impairment of sperm's ability to move, survive, and fertilize the oocyte. Defective morphology can make them more vulnerable to damage from external factors such as changes in pH, temperature, and the presence of ROS [36–38].

The sperm concentration was not significantly different in the three groups. However, the N + UI and N + V groups, despite the presence of pathologies, showed a sperm concentration higher than that observed in the N group, although non-significantly. In the analyzed cases, all the other parameters except for sperm concentration were decreased in the presence of pathologies, meaning that in the case of normozoospermia, the sperm concentration is the sperm parameter less affected by these pathologies, at least in the selected cases.

Our results regarding the role of PPAR γ in regulating male reproduction are also validated by a study that reported a significant downregulation of PPAR γ expression in both the testes and kidneys of hypothyroid rats. It is known that untreated hypothyroidism may result in several health problems, including infertility and, in this case, PPAR γ inhibition contributed to testicular damage, exacerbating OS and inflammation [39].

PPAR γ down-regulation concomitant with high levels of OS is well documented both in vitro and in vivo [40–42], while PPAR γ activation inhibits the inflammatory responses up-regulating antioxidant genes such as catalase and glutathione peroxidase [43].

The experimental data suggest that during OS and inflammation, ligands of PPAR γ , such as oxidized lipids and 15-deoxy-delta-prostaglandin J₂ (15d-PGJ₂), are produced. This implies that PPAR γ , if already expressed, could serve as one of the initial responders to OS by directly triggering the production of antioxidant compounds, inhibiting prooxidants while simultaneously safeguarding cells against apoptosis [27]. In the spermatozoon, the central role of PPAR γ in limiting OS and consequently ROS damage might mediate rapid responses to OS and adjustments to such environments to preserve sperm function.

The level of PPAR γ expression found in ejaculated sperm might be influenced by altered spermatogenesis. In fact, spermatozoa are highly differentiated cells, with a low transcriptional activity, containing a wide variety of both coding and non-coding RNA molecules packaged during spermatogenesis and epididymal transit [44]. However, several evidence coming from the literature cannot exclude that mature spermatozoa can accomplish some level of transcription [45,46]. It is reported that the transcripts can vary during sperm freezing [47,48], suggesting that also the modifications of the environment, for example, seminal plasma components, can have an impact on gene expression.

At this purpose, both varicocele and urogenital infections likely create an inflammatory environment within the male reproductive tract, which can increase ROS production suppressing PPAR γ activity as discussed above. PPAR γ is a regulator of lipid metabolism and may be positively influenced by n-3 PUFA dietary sources supporting sperm maturation and reducing F₂-IsoP levels [49].

This is the first study reporting the relationship between RvD1 and PPAR γ in human semen and spermatozoa. In other systems as lung or human placenta, the ability of RvD1

in attenuating inflammatory process by a mechanism that is partially dependent on PPAR γ activation was already observed [50,51]. The influence of diet on the concentration of RvD1 was recently demonstrated in a rabbit model [52].

In conclusion, reduced expression of PPAR γ in human ejaculated spermatozoa appears to be influenced by the presence of seminal OS, this could play a role in reproductive pathological conditions associated with OS as varicocele, urogenital infections, or others.

Nevertheless, it is important to highlight that PPARs are regulated by multiple molecules that can act as co-activators or co-repressors [53]. Thus, further molecules and factors might be involved in the relationship between of PPAR γ and LPO in male infertility.

We are aware that the study involves a small number of patients, which will need to be increased, however the strict selection criteria make the groups reliable.

Future perspectives can include the evaluation of experimental or clinical conditions, for example idiopathic infertility, that lead to investigating whether the variation in PPAR γ expression and the increase in LPO and RvD1 are concurrent events, or which is dependent and secondary to the other.

PPAR γ protein is highly expressed in ejaculated spermatozoa and the possibility to use natural or synthetic ligands is a relevant strategy to improve the sperm performance [44]. Given the close link between energy balance and reproduction, activation of PPAR γ may have promising metabolic implications in male reproductive functions.

Author Contributions: Conceptualization, E.M., G.C. (Giulia Collodel) and C.S.; data curation, E.M., G.C. (Giulia Collodel) and C.S.; formal analysis, G.C. (Gabriele Centini); investigation, R.C., L.L. and C.M.; methodology, C.M., L.L. and D.M.; supervision, E.M., G.C. (Giulia Collodel) and C.S.; validation, E.M., G.C. (Giulia Collodel) and C.S.; writing—original draft, C.S. and G.C. (Giulia Collodel); writing—review and editing, E.M., C.S., G.C. (Giulia Collodel) and G.C. (Gabriele Centini). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Ethic Committee Siena University Hospital (ID CEAVSE 25612; 22 November 2023).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data generated and analyzed during this study are included in this published article and are available from the corresponding author.

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

References

1. Lenzi, A.; Gandini, L.; Maresca, V.; Rago, R.; Sgrò, P.; Dondero, F.; Picardo, M. Fatty acid composition of spermatozoa and immature germ cells. *Mol. Hum. Reprod.* **2000**, *6*, 226–231. <https://doi.org/10.1093/molehr/6.3.226>.
2. Vallés, A.S.; Aveldaño, M.I.; Furland, N.E. Altered lipid homeostasis in Sertoli cells stressed by mild hyperthermia. *PLoS ONE* **2014**, *9*, e91127. <https://doi.org/10.1371/journal.pone.0091127>.
3. Mousavi, M.S.; Shahverdi, A.; Drevet, J.; Akbarinejad, V.; Esmaeili, V.; Sayahpour, F.A.; Topraggaleh, T.R.; Rahimizadeh, P.; Alizadeh, A. Peroxisome Proliferator-Activated Receptors (PPARs) levels in spermatozoa of normozoospermic and asthenozoospermic men. *Syst. Biol. Reprod. Med.* **2019**, *65*, 409–419. <https://doi.org/10.1080/19396368.2019.1677801>.
4. Moghadam, F.; Hajian, M.; Rouhollahi Varnosfaderani, S.; Jafarpour, F.; Nasr Esfahani, M.H. Effect of rosiglitazone on developmental competence of mouse embryos treated with lipopolysaccharide. *Theriogenology* **2021**, *161*, 57–64. <https://doi.org/10.1016/j.theriogenology.2020.11.022>.
5. Marion-Letellier, R.; Savoye, G.; Ghosh, S. Fatty acids, eicosanoids and PPAR gamma. *Eur. J. Pharmacol.* **2016**, *785*, 44–49. <https://doi.org/10.1016/j.ejphar.2015.11.004>.

6. Moretti, E.; Collodel, G.; Salvatici, M.C.; Belmonte, G.; Signorini, C. New insights into sperm with total globozoospermia: Increased fatty acid oxidation and centrin1 alteration. *Syst. Biol. Reprod. Med.* **2019**, *65*, 390–399. <https://doi.org/10.1080/19396368.2019.1626934>.
7. Aquila, S.; Bonofiglio, D.; Gentile, M.; Middea, E.; Gabriele, S.; Belmonte, M.; Catalano, S.; Pellegrino, M.; Andò, S. Peroxisome proliferator-activated receptor (PPAR) γ is expressed by human spermatozoa: Its potential role on the sperm physiology. *J. Cell. Physiol.* **2006**, *209*, 977–986. <https://doi.org/10.1002/jcp.20807>.
8. Liu, L.L.; Xian, H.; Cao, J.C.; Zhang, C.; Zhang, Y.H.; Chen, M.M.; Qian, Y.; Jiang, M. Peroxisome proliferator-activated receptor gamma signaling in human sperm physiology. *Asian J. Androl.* **2015**, *17*, 942–947. <https://doi.org/10.4103/1008-682X.150253>.
9. Shibata, T.; Bhat, S.A.; Cao, D.; Saito, S.; Bernstein, E.A.; Nishi, E.; Medenilla, J.D.; Wang, E.T.; Chan, J.L.; Pisarska, M.D.; et al. Testicular ACE regulates sperm metabolism and fertilization through the transcription factor PPAR γ . *J. Biol. Chem.* **2024**, *300*, 105486. <https://doi.org/10.1016/j.jbc.2023.105486>.
10. WHO. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 6th ed.; WHO: Geneva, Switzerland, 2021.
11. Baskaran, S.; Finelli, R.; Agarwal, A.; Henkel, R. Diagnostic value of routine semen analysis in clinical andrology. *Andrologia* **2021**, *53*, e13614. <https://doi.org/10.1111/and.13614>.
12. Boeri, L.; Belladelli, F.; Capogrosso, P.; Cazzaniga, W.; Candela, L.; Pozzi, E.; Valsecchi, L.; Papaleo, E.; Viganò, P.; Abbate, C.; et al. Normal sperm parameters per se do not reliably account for fertility: A case-control study in the real-life setting. *Andrologia* **2021**, *53*, e13861. <https://doi.org/10.1111/and.13861>.
13. Jensen, C.F.S.; Østergren, P.; Dupree, J.M.; Ohl, D.A.; Sønksen, J.; Fode, M. Varicocele and male infertility. *Nat. Rev. Urol.* **2017**, *14*, 523–533. <https://doi.org/10.1038/nrurol.2017.98>.
14. Signorini, C.; Moretti, E.; Collodel, G. Role of isoprostanes in human male infertility. *Syst. Biol. Reprod. Med.* **2020**, *66*, 291–299. <https://doi.org/10.1080/19396368.2020.1793032>.
15. Agarwal, A.; Baskaran, S.; Parekh, N.; Cho, C.L.; Henkel, R.; Vij, S.; Arafa, M.; Panner Selvam, M.K.; Shah, R. Male infertility. *Lancet* **2021**, *397*, 319–333. [https://doi.org/10.1016/S0140-6736\(20\)32667-2](https://doi.org/10.1016/S0140-6736(20)32667-2).
16. Aitken, R.J.; Drevet, J.R.; Moazamian, A.; Gharagozloo, P. Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidants* **2022**, *11*, 306. <https://doi.org/10.3390/Antiox11020306>.
17. Aitken, R.J.; Drevet, J.R. The Importance of Oxidative Stress in Determining the Functionality of Mammalian Spermatozoa: A Two-Edged Sword. *Antioxidants* **2020**, *9*, 111. <https://doi.org/10.3390/Antiox9020111>.
18. Bdeir, R.; Aljabali, S.M.; Banihani, S.A. Role of pyridoxine and oxidative stress in asthenozoospermia. *Heliyon* **2024**, *10*, e34799. <https://doi.org/10.1016/j.heliyon.2024.e34799>.
19. O’flaherty, C. Peroxiredoxin 6: The Protector of Male Fertility. *Antioxidants* **2018**, *7*, 173. <https://doi.org/10.3390/Antiox7120173>.
20. Milne, G.L.; Dai, Q.; Roberts, L.J., 2nd. The isoprostanes—25 years later. *Biochim. Biophys. Acta* **2015**, *1851*, 433–445. <https://doi.org/10.1016/j.bbali.2014.10.007>.
21. Collodel, G.; Moretti, E.; Noto, D.; Corsaro, R.; Signorini, C. Oxidation of Polyunsaturated Fatty Acids as a Promising Area of Research in Infertility. *Antioxidants* **2022**, *11*, 1002. <https://doi.org/10.3390/antiox11051002>.
22. Recchiuti, A. Resolvin D1 and its GPCRs in resolution circuits of inflammation. *Prostaglandins Other Lipid Mediat.* **2013**, *107*, 64–76. <https://doi.org/10.1016/j.prostaglandins.2013.02.004>.
23. Signorini, C.; Moretti, E.; Noto, D.; Micheli, L.; Ponchia, R.; Collodel, G. Fatty Acid Oxidation and Pro-Resolving Lipid Mediators Are Related to Male Infertility. *Antioxidants* **2022**, *11*, 107. <https://doi.org/10.3390/antiox11010107>.
24. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- $\Delta\Delta$ CT} Method. *Methods* **2001**, *25*, 402–408. <https://doi.org/10.1006/meth.2001.1262>.
25. Micheli, L.; Collodel, G.; Moretti, E.; Noto, D.; Menchiari, A.; Cerretani, D.; Crispino, S.; Signorini, C. Redox imbalance induced by docetaxel in the neuroblastoma SH-SY5Y cells: A study of docetaxel-induced neuronal damage. *Redox Rep.* **2021**, *26*, 18–28. <https://doi.org/10.1080/13510002.2021.1884802>.
26. Moretti, E.; Signorini, C.; Ferretti, F.; Noto, D.; Collodel, G. A Study to Validate the Relevance of Semen F₂-Isoprostanes on Human Male Infertility. *Int. J. Environ. Res. Public Health* **2022**, *19*, 1642. <https://doi.org/10.3390/ijerph19031642>.
27. Polvani, S.; Tarocchi, M.; Galli, A. PPAR γ and Oxidative Stress: Con(β) Catenating NRF2 and FOXO. *PPAR Res.* **2012**, *2012*, 641087. <https://doi.org/10.1155/2012/641087>.
28. Xia, H.; Wang, J.; Sun, S.; Wang, F.; Yang, Y.; Chen, L.; Sun, Z.; Yao, S. Resolvin D1 Alleviates Ventilator-Induced Lung Injury in Mice by Activating PPAR γ /NF- κ B Signaling Pathway. *BioMed Res. Int.* **2019**, *2019*, 6254587. <https://doi.org/10.1155/2019/6254587>.

29. Varga, T.; Czimmerer, Z.; Nagy, L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. Biophys. Acta* **2011**, *1812*, 1007–1022. <https://doi.org/10.1016/j.bbadis.2011.02.014>.
30. Muzio, G.; Barrera, G.; Pizzimenti, S. Peroxisome Proliferator-Activated Receptors (PPARs) and Oxidative Stress in Physiological Conditions and in Cancer. *Antioxidants* **2021**, *10*, 1734. <https://doi.org/10.3390/antiox10111734>.
31. Lee, C. Collaborative Power of Nrf2 and PPAR γ Activators against Metabolic and Drug-Induced Oxidative Injury. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 1378175. <https://doi.org/10.1155/2017/1378175>.
32. Villapol, S. Roles of Peroxisome Proliferator-Activated Receptor Gamma on Brain and Peripheral Inflammation. *Cell. Mol. Neurobiol.* **2018**, *38*, 121–132. <https://doi.org/10.1007/s10571-017-0554-5>.
33. Santoro, M.; Guido, C.; De Amicis, F.; Sisci, D.; Vizza, D.; Gervasi, S.; Carpino, A.; Aquila, S. Sperm metabolism in pigs: A role for peroxisome proliferator-activated receptor gamma (PPAR γ). *J. Exp. Biol.* **2013**, *216*, 1085–1092. <https://doi.org/10.1242/jeb.079327>.
34. Antonuccio, P.; Marini, H.R.; Micali, A.; Romeo, C.; Granese, R.; Retto, A.; Martino, A.; Benvenga, S.; Cuzzocrea, S.; Impellizzeri, D.; et al. The Nutraceutical N-Palmitoylethanolamide (PEA) Reveals Widespread Molecular Effects Unmasking New Therapeutic Targets in Murine Varicocele. *Nutrients* **2021**, *13*, 734. <https://doi.org/10.3390/nu13030734>.
35. Perrotta, I.; Aquila, S. Steroid receptors in human ejaculated sperm as molecular markers of the detrimental effects related to the pathophysiology of testicular varicocele. *Histol. Histopathol.* **2016**, *31*, 819–831. <https://doi.org/10.14670/HH-11-761>.
36. Aziz, N.; Agarwal, A.; Lewis-Jones, I.; Sharma, R.K.; Thomas, A.J., Jr. Novel associations between specific sperm morphological defects and leukocytospermia. *Fertil. Steril.* **2004**, *82*, 621–627. <https://doi.org/10.1016/j.fertnstert.2004.02.112>.
37. Franken, D.R. How accurate is sperm morphology as an indicator of sperm function? *Andrologia* **2015**, *47*, 720–723. <https://doi.org/10.1111/and.12324>.
38. Björndahl, L.; Kirkman Brown, J.; other Editorial Board Members of the WHO Laboratory Manual for the Examination and Processing of Human Semen. The sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen: Ensuring quality and standardization in basic examination of human ejaculates. *Fertil. Steril.* **2022**, *117*, 246–251. <https://doi.org/10.1016/j.fertnstert.2021.12.012>.
39. Osama, H.M.; Khadrawy, S.M.; El-Nahass, E.S.; Othman, S.I.; Mohamed, H.M. Eltroxin and Hesperidin mitigate testicular and renal damage in hypothyroid rats: Amelioration of oxidative stress through PPAR γ and Nrf2/HO-1 signaling pathway. *Lab. Anim. Res.* **2024**, *40*, 19. <https://doi.org/10.1186/s42826-024-00204-8>.
40. El Midaoui, A.; Wu, L.; Wang, R.; de Champlain, J. Modulation of cardiac and aortic peroxisome proliferator-activated receptor- γ expression by oxidative stress in chronically glucose-fed rats. *Am. J. Hypertens.* **2006**, *19*, 407–412. <https://doi.org/10.1016/j.amjhyper.2005.11.006>.
41. Blanquicett, C.; Kang, B.Y.; Ritzenthaler, J.D.; Jones, D.P.; Hart, C.M. Oxidative stress modulates PPAR γ in vascular endothelial cells. *Free Radic. Biol. Med.* **2010**, *48*, 1618–1625. <https://doi.org/10.1016/j.freeradbiomed.2010.03.007>.
42. Cai, R.; Yu, T.; Huang, C.; Xia, X.; Liu, X.; Gu, J.; Xue, S.; Yeh, E.T.; Cheng, J. SUMO-specific protease 1 regulates mitochondrial biogenesis through PGC-1 α . *J. Biol. Chem.* **2012**, *287*, 44464–44470. <https://doi.org/10.1074/jbc.M112.422626>.
43. Yu, Y.; Correll, P.H.; Vanden Heuvel, J.P. Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: Evidence for a PPAR γ -dependent mechanism. *Biochim. Biophys. Acta* **2002**, *1581*, 89–99. [https://doi.org/10.1016/s1388-1981\(02\)00126-9](https://doi.org/10.1016/s1388-1981(02)00126-9).
44. McSwiggan, H.M.; O'Doherty, A.M. Epigenetic reprogramming during spermatogenesis and male factor infertility. *Reproduction* **2018**, *156*, R9–R21. <https://doi.org/10.1530/REP-18-0009>.
45. Ren, X.; Chen, X.; Wang, Z.; Wang, D. Is transcription in sperm stationary or dynamic? *J. Reprod. Dev.* **2017**, *63*, 439–443. <https://doi.org/10.1262/jrd.2016-093>.
46. Santiago, J.; Silva, J.V.; Howl, J.; Santos, M.A.S.; Fardilha, M. All you need to know about sperm RNAs. *Hum. Reprod. Update* **2021**, *28*, 67–91. <https://doi.org/10.1093/humupd/dmab034>.
47. Deng, S.L.; Sun, T.C.; Yu, K.; Wang, Z.P.; Zhang, B.L.; Zhang, Y.; Wang, X.X.; Lian, Z.X.; Liu, Y.X. Melatonin reduces oxidative damage and upregulates heat shock protein 90 expression in cryopreserved human semen. *Free Radic. Biol. Med.* **2017**, *113*, 347–354. <https://doi.org/10.1016/j.freeradbiomed.2017.10.342>.
48. Santonastaso, M.; Mottola, F.; Iovine, C.; Colacurci, N.; Rocco, L. Protective Effects of Curcumin on the Outcome of Cryopreservation in Human Sperm. *Reprod. Sci.* **2021**, *28*, 2895–2905. <https://doi.org/10.1007/s43032-021-00572-9>.
49. Passaponti, S.; Signorini, C.; Collodel, G.; Moretti, E.; Mattioli, S.; Belmonte, G.; Noto, D. Expression and immunolocalisation of PPAR γ in reproductive tissues of rabbits fed n-3 PUFA-enriched diets. *J. Men's Health* **2022**, *18*, 72. <https://doi.org/10.31083/j.jomh1803072>.

50. Liao, Z.; Dong, J.; Wu, W.; Yang, T.; Wang, T.; Guo, L.; Chen, L.; Xu, D.; Wen, F. Resolvin D1 attenuates inflammation in lipopolysaccharide-induced acute lung injury through a process involving the PPAR γ /NF- κ B pathway. *Respir. Res.* **2012**, *13*, 110. <https://doi.org/10.1186/1465-9921-13-110>.
51. Li, A.; Zhang, L.; Li, J.; Fang, Z.; Li, S.; Peng, Y.; Zhang, M.; Wang, X. Effect of RvD1/FPR2 on inflammatory response in chorioamnionitis. *J. Cell. Mol. Med.* **2020**, *24*, 13397–13407. <https://doi.org/10.1111/jcmm.15963>.
52. Mattioli, S.; Moretti, E.; Castellini, C.; Signorini, C.; Corsaro, R.; Angelucci, E.; Collodel, G. Can Dietary n-3 Polyunsaturated Fatty Acids Affect Apelin and Resolvin in Testis and Sperm of Male Rabbits? *Molecules* **2023**, *28*, 6188. <https://doi.org/10.3390/molecules28176188>.
53. Santoro, M.; De Amicis, F.; Aquila, S.; Bonofiglio, D. Peroxisome proliferator-activated receptor gamma expression along the male genital system and its role in male fertility. *Hum. Reprod.* **2020**, *35*, 2072–2085. <https://doi.org/10.1093/humrep/deaa153>.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.