Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of Intracerebroventricular Metrnl Administration on the Hypothalamic-Pituitary-Testicular Axis in Male Rats

Burak Bircan
Osmaniye Korkut Ata University
Güldeniz Şekerci
İnönü University
Yavuz Erden
Bartın University
Çiğdem Tekin
İnönü University
Azibe Yıldız
İnönü University
Suat Tekin
suat.tekin@inonu.edu.tr
İnönü University

Research Article

Keywords: Metrnl, hypothalamus-pituitary-testis axis, GnRH, LH, FSH, testosterone, sperm parameters, intracerebroventricular infusion

Posted Date: April 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9196848/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Molecular Biology Reports on May 12th, 2026. See the published version at https://doi.org/10.1007/s11033-026-11938-0.

Abstract

Metrnl (Meteorin-like) is a newly discovered adipokine, and the presence of this peptide has been demonstrated in important components of the hypothalamic-pituitary-testis (HPT) axis, including brain, pituitary, and testicular tissues. This study reports the effects of Metrnl on the HPT axis in male rats. A total of forty male Wistar-Albino rats were used and equally divided into four groups (n = 10). 10 nM and 100 nM Metrnl were administered to the treatment groups by intracerebroventricular (icv) infusion for 14 days. After the treatments, GnRH gene expression in the hypothalamus, and LH, FSH, and testosterone hormone levels in serum samples were analyzed. Testicular tissue was evaluated by histological and immunohistochemical methods. Our findings showed that Metrnl administration decreased GnRH mRNA and protein expression ($p < 0.05$). Serum LH, FSH, and testosterone levels were significantly decreased in the treatment groups compared to the control group ($p < 0.05$). Testicular histology revealed that Metrnl increased seminiferous tubule diameter and decreased germinal epithelial thickness ($p < 0.05$). Immunohistochemical analyses revealed significant decreases in HSD3B1 and vimentin reactivity in the Metrnl-treated groups, with these decreases being more pronounced at the 100 nM dose. Furthermore, sperm concentration and motility were significantly lower in the Metrnl-treated groups (10 and 100 nM) compared to the control group ($p < 0.05$). Our study suggests that Metrnl may have anti-reproductive effects by reducing the levels of hormones involved in the HPT axis and negatively affecting sperm quality.

1. Introduction

The physiological functioning of the reproductive system is of vital importance for species continuity and individual fertility. The functions of this system are complexly regulated under the influence of numerous central and peripheral factors, particularly dependent on the integrated and healthy functioning of the hypothalamo-hypophyseal-gonadal (HHG) axis [1]. Gonadotropin-releasing hormone (GnRH), released in a pulsatile manner by the hypothalamus, plays a central role in regulating gonadal functions by triggering the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. These gonadotropins control; in females, folliculogenesis, ovulation, and steroid hormone production; and in males, spermatogenesis and testosterone production [2, 3]. The hypothalamic-pituitary-gonadal (HPG) axis functions in an integrated manner. In males, this structure is specifically referred to as the hypothalamus-pituitary-testis (HPT) axis and is modulated by various central and peripheral signals. Functional disorders that may occur in the HPT axis can lead to hormonal imbalances at the testicular level and gametogenic dysfunction, resulting in serious clinical conditions such as delayed puberty and infertility. In this context, a detailed understanding of the physiology of the HPT axis and the identification of new molecular factors contributing to its functioning have the potential to contribute both to basic scientific knowledge and to clinical applications. Although the specific receptor for Metrnl has not been fully characterized in all tissues, studies suggest that Metrnl may exert its effects through the KIT receptor signaling pathway or by blocking insulin resistance via PPARγ signaling. Identifying the specific target receptors of Metrnl in the HPT axis components is crucial for understanding its regulatory mechanism [4–7].

Reproductive health is a multifaceted biological process that requires the coordinated functioning of vascular, neurological, and endocrine systems [8, 9]. The anatomical proximity of the energy balance and reproductive control centers in the hypothalamus is one of the fundamental factors explaining the effect of nutritional status on reproductive functions. Studies examining the effects of obesity on the reproductive system in various experimental models have shown that many peptides can have bidirectional effects on both energy metabolism and reproductive behaviors [10]. Metabolism of energy and the endocrine functions of adipose tissue exert significant effects on fertility, and the interaction between these two systems is shaped at the hypothalamic level. Adipose tissue, beyond being merely an energy depot, plays a role in various processes such as angiogenesis, vascular hemostasis, lipid metabolism, and reproduction by secreting numerous bioactive molecules, including chemokines, cytokines, hormones, and growth factors [10–12].

Metrnl was first reported in 2009. This peptide is called Meteorin-like because it is approximately 40% similar to the Neurotrophic factor Meteorin [4, 13]. Metrnl plays important roles in energy metabolism by browning white adipose tissue and increasing insulin sensitivity. Moreover, Metrnl has been implicated in many processes, including obesity, diabetes, heart disease and improved beta-cell function [5–7]. Metrnl is expressed and secreted in many central and peripheral tissues (e.g., brain, fat, muscle, liver, spleen, skin, heart, and activated macrophages) [4]. The demonstration of Metrnl expression in key components of the HPT axis, such as brain and testis [7, 14], points to the potential role of this molecule in male reproductive physiology. Furthermore, it has been reported that Metrnl is also expressed in male reproductive system organs such as the epididymis, prostate, and seminal vesicles; and in female reproductive organs such as ovary, endometrium, fallopian tubes, and placenta [15]. Current knowledge suggests that various adipokines and myokines (e.g., leptin, adiponectin, irisin, asprosin) may influence the HHG axis and thus play roles in male fertility [16, 17]. Given the distribution of Metrnl in regions involved in reproductive physiology, we hypothesized that this peptide may have similar effects and play roles in male reproductive physiology. Our study addresses the potential relationship between Metrnl and the HHG axis, and our results provide the first evidence regarding the potential role of Metrnl in male reproductive physiology.

2. Material and method

2.1. Animals and Experimental Design

Forty male Wistar-Albino rats (12–14 weeks old, 300–350 g), approved by the İnönü University Local Ethical Committee (No: 2021/25 – 1), were used. Animals were housed individually under standard conditions (20–22°C, 12 h light/dark cycle) with ad libitum food and water and acclimatized for 3 days before experimentation. Rats were randomly assigned to four groups (n = 10): Control, Sham, low-dose (10 nM) Metrnl, and high-dose (100 nM) Metrnl. The doses of Metrnl (10 nM and 100 nM) were selected based on our previous study, which demonstrated significant metabolic and hormonal effects of central Metrnl administration at these concentrations [18].

The Control group received neither surgery nor treatment. Other groups were anesthetized with ketamine/xylazine and fixed in a stereotaxic frame (Harvard Apparatus, USA) for intracerebroventricular (icv) infusion. Coordinates for the right lateral ventricle (0.8 mm lateral, 0.8 mm posterior to bregma, 4.8 mm ventral) were determined using the rat brain atlas [19, 20].

A brain infusion kit was implanted in the right lateral ventricle of the sham and Metrnl treatment groups, followed by a 7-day recovery period. On day 8, rats were anesthetized again, and osmotic minipumps were connected to the brain infusion kit cannula, and these pumps were implanted under the skin of the neck. Artificial cerebrospinal fluid was administered to the sham group [21], and Metrnl prepared in this solvent was administered to the treatment groups for 14 days (5 µL/h, 120 µL/day). On day 24, all animals were decapitated, and hypothalamus, blood, testicular, and epididymal tissues were collected.

2.2. Hormonal Parameters

Blood samples were centrifuged at 3500 rpm for 10 minutes to obtain serum (Hettich, Germany). Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were determined using rat-specific ELISA kits (LH: Elabscience E-EL-R0026; FSH: Elabscience E-EL-R1106; Testosterone: Cayman 582701). Analyses were performed in accordance with the protocols of the respective kits.

2.3. RT-PCR Analysis

Total RNA was isolated from hypothalamic tissue samples using the EZ-10 Spin Column Total RNA Miniprep Kit (BioBasic, Canada) according to the manufacturer's protocol. Subsequently, cDNA was then generated using the obtained RNA samples. cDNA templates were amplified by quantitative Real-Time PCR using the BioRad CFX Connect Detection System (USA). Each 10 µL reaction contained 5 µL SYBR Green Master Mix, 2.5 µL nuclease-free water, 0.5 µL of each primer, and 2 µL cDNA (Table 1). β-actin served as the housekeeping gene. Thermal cycling consisted of 95°C for 10 min, followed by 45 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Change gene expression was calculated by the β-actin ratio.

Table 1
Primer sequences and genbank accession numbers used in the study

Gene	Sequence (5' → 3')	GenBank Accession No.
Beta-actin	F CGTTGACATCCGTAAAGAC	NM_031144
	R TAGGAGCCAGGGCAGTA	
GnRH	F GCCGCTGTTGTTCTGTTGACTG	NM_012767.2
	R CCTCCTCCTTGCCCATCTCTTG	

2.4. Western Blot Analysis

Hypothalamic tissues were homogenized with a bead-based homogenizer (Qiagen Tissuelyser LT, Germany) in RIPA buffer containing protease inhibitors [22]. After sonication and centrifugation, the

supernatant was collected, and protein concentrations were determined by BCA assay (Thermo Scientific, USA). Equal amounts of protein (30 µg) were separated on 15% SDS-PAGE gels and transferred onto nitrocellulose membranes (0.22 µm) using a semi-dry transfer system. Membranes were blocked with 5% non-fat milk in TBS-T and incubated overnight at 4°C with a rat GnRH primary antibody (1:1000, sc-32292, Santa Cruz). After washing, membranes were incubated for 1 h with an HRP-conjugated secondary antibody (1:3000, sc-516102, Santa Cruz). β-actin (sc-517582, Santa Cruz) was used as a loading control. Protein bands were visualized with ECL substrate (BioRad, USA) and imaged using a Fusion FX system (Vilber, Germany). Band intensities were quantified with ImageJ and normalized to β-actin [23].

2.5. Sperm Count and Motility

Epididymal tissue pieces were minced in 1 mL of isotonic solution (0.9% NaCl) and incubated at room temperature. The epididymal sperm cells in the resulting supernatant were examined microscopically (Leica light microscope) at 20x magnification to assess sperm count and motility.

2.6. Histological Analysis

Following collection, testicular tissues were fixed in 10% formalin. After fixation, tissues underwent routine processing and were embedded in paraffin. Paraffin blocks were sectioned at 5 µm thickness, and sections were stained with Hematoxylin and Eosin (H&E). Seminiferous tubule diameter and germinal epithelium thickness were measured using the Leica Q Win image analysis system at 20x magnification [24]. Testicular histological evaluation included measurements of seminiferous tubule diameter, germinal epithelium thickness.

2.7. Immunohistochemical Analysis

HSD3B1 and vimentin were used as biomarkers for Leydig and Sertoli cells, respectively. After blocking non-specific binding with protein block, primary antibodies were applied for 60 min at room temperature. Sections were then incubated with biotinylated secondary antibodies and streptavidin–peroxidase for 10 min, counterstained with hematoxylin, mounted, and visualized with AEC chromogen. Vimentin- and HSD3B1-positive cells appeared red. Staining intensity was analyzed utilizing the Leica Q Win Image Analysis system to ensure objective densitometric evaluation, minimizing manual interpretation bias. At least 300 Sertoli and Leydig cells were analyzed, and staining intensity was semi-quantitatively scored on a 4-point scale (0: none, 1: mild, 2: moderate, 3: strong). Histopathological and immunohistochemical evaluations were conducted with a Leica DFC280 light microscope and Leica Q Win Image Analysis system (Leica Microsystems Imaging Solutions Ltd., Germany, UK).

2.8. Statistical Analysis

Data were analyzed using IBM SPSS Statistics 22.0 (Windows). Quantitative variables were expressed as mean ± standard deviation (SD). Normality was tested with the Shapiro–Wilk test. Intergroup comparisons were performed using the Kruskal–Wallis H test, followed by Bonferroni-adjusted Mann–Whitney U tests for post hoc analysis. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Serum LH, FSH, and Testosterone Levels

The effects of i.c.v. Metrnl treatment on serum LH, FSH, and testosterone are shown in Fig. 1. In both Metrnl-treated groups (10 nM and 100 nM), serum LH levels decreased in a dose-dependent manner compared to the control and sham groups ($p < 0.05$). Similarly, serum FSH levels in the Metrnl groups showed a significant decrease compared to the control and sham groups ($p < 0.05$). In addition, testosterone levels also decreased in both groups following Metrnl treatment ($p < 0.05$). These results indicate that Metrnl can suppress elements of the HHT axis after central infusion.

3.2. GnRH mRNA and protein levels

Group-wise alterations in GnRH mRNA levels are shown in Fig. 2 – 1. The sham group did not differ significantly from control group. In contrast, i.c.v. Metrnl treatment (10 nM and 100 nM) significantly reduced GnRH mRNA expression compared with control and sham groups ($p < 0.05$). Expression levels were comparable between the two Metrnl-treated groups.

The effect of decreased mRNA levels on protein expression was demonstrated by Western blot analyses. Similar to mRNA levels, GnRH protein levels were lower in the Metrnl-treated groups compared to the control and sham groups (Fig. 2–2; $p < 0.05$). No significant differences were found between the control and sham groups. Our results demonstrated that Metrnl administration affected hypothalamic GnRH gene expression at both the mRNA and protein levels.

3.3. Effect of Metrnl Administration on Sperm Parameters

Epididymides were minced in 1 mL of isotonic solution (NaCl, 0.9%) and incubated at room temperature. The epididymal sperm cells within the resulting supernatant were examined under a light microscope (Leica) using a 20x objective to determine sperm count and motility.

The changes in testis and epididymal weights, as well as sperm motility and morphological images, are presented in Table 2. According to these data, no significant changes in testis and epididymal weights were observed following icv Metrnl infusion. However, a statistically significant decrease was found in both sperm density and motility in the Metrnl-treated groups (10 and 100 nM) when compared to the control and sham groups ($p < 0.05$).

Table 2
Effects of icv Metrnl administration on sperm parameters, testicular histomorphometry, and organ weights in male rats

Parameters	Control	Sham	Metrnl 10 nM	Metrnl 100 nM
Sperm Parameters				
Sperm density (million)	74.16 ± 5.19[a]	77.51 ± 5.39[a]	50.12 ± 5.81[b]	42.11 ± 3.93[c]
Sperm motility (%)	81.21 ± 4.17[a]	79.64 ± 5.12[a]	54.80 ± 5.13[b]	52.76 ± 6.14[b]
Testicular Histomorphometry				
Germinal Epithelium Thickness	58.1 ± 9.7[a]	57.5 ± 9.3[a]	49.4 ± 10.7[b]	51.1 ± 10.1[b]
Seminiferous Tubule Diameter	263.8 ± 39.6[a]	265.5 ± 40.8[a]	263.8 ± 45.2[a]	283.5 ± 47.6[b]
Organ Weights (g)				
Testis weight (g)				
Right	1.77 ± 0.05	1.83 ± 0.07	1.73 ± 0.08	1.77 ± 0.05
Left	1.79 ± 0.04	1.79 ± 0.06	1.68 ± 0.07	1.82 ± 0.06
Epididymis weight (g)				
Right	0.67 ± 0.04	0.66 ± 0.06	0.70 ± 0.04	0.64 ± 0.07
Left	0.65 ± 0.07	0.69 ± 0.04	0.66 ± 0.05	0.68 ± 0.06

(Values are expressed as mean \ SD. Different superscript letters (a, b, c) in the same row indicate a statistically significant difference between groups ($p < 0.05$), while the same letters indicate no statistically significant difference ($p > 0.05$). Data were evaluated using the Kruskal-Wallis H test, followed by the Mann-Whitney U test with Bonferroni correction.)

3.4. Effect of Metrnl Administration on Testis Histology

Seminiferous tubule diameter and germinal epithelium thickness were measured at 20x magnification in each preparation using the Leica Q Win image analysis system (Fig. 3A-D). Histological changes were assessed by counting 10 tubules per section in H&E-stained sections.

Testicular sections from the control group exhibited a normal histological appearance, characterized by Sertoli cells arranged In a concentric layer surrounding differentiating germ cells in the germinal epithelium. Mean germinal epithelium thickness and seminiferous tubule diameter were found to be similar in the control and sham groups (Fig. 3A-B). While gross tissue architecture remained intact, significant micromorphometric changes were observed. Specifically, Metrnl administration resulted in a

significant reduction in germinal epithelium thickness in both treatment groups compared to controls ($p < 0.05$). Notably, the mean germinal epithelium thickness was significantly reduced in the Metrnl 10 and Metrnl 100 groups compared to the control and sham groups ($p < 0.05$). Additionally, an increase in seminiferous tubule diameter was observed in the Metrnl 100 group, and this increase was found to be statistically significant when compared to the control and Metrnl 100 groups ($p < 0.05$). The mean germinal epithelium thickness and seminiferous tubule diameter results for each group are presented in Table 2.

3.5. Effect of Metrnl Application on Leydig and Sertoli Cells Histology

HSD3B1 immunoreactivity was prominent in the Leydig cell cytoplasm of rats across all groups (Fig. 4, Table 3). The highest immunoreactivity was observed in the Control group. In other groups (Sham, 10 nM Metrnl, and 100 nM Metrnl), HSD3B1 immunoreactivity was found to be significantly lower compared to the Control group ($p < 0.05$). On the other hand, while immunoreactivity was similar in the Sham and 10 nM Metrnl groups, it was statistically lower in the 100 nM Metrnl group compared to the Sham and 10 nM Metrnl groups ($p < 0.05$).

Table 3
HSD3B1 and Vimentin immunoreactivity intensity for each group (median (min-max))

Groups	Vimentin	HSD3B1
Control	3 (2–3)	2 (1–3)
Sham	3 (2–3)	2 (0–3)[a]
10 nM Metrnl	2 (2–3)[a]	2 (0–3)[a]
100 nM Metrnl	2 (1–3)[a]	1 (0–3)[a, b]

[a] Statistically lower compared to the Control group ($p < 0.05$). [b] Statistically lower compared to the Sham and 10 nM Metrnl groups ($p < 0.05$).

Immunoreactivity against Vimentin protein was detected in the testes across all groups (Fig. 5). It was prominent in the cytoplasm of Sertoli cells in rats from all groups, especially in the perinuclear cytoplasmic region; the apical region of these cells also showed immunoreactivity, though with less intensity. Vimentin immunoreactivity was similar in the Control and Sham groups. However, a decrease in vimentin immunoreactivity was observed in the 10 nM Metrnl and 100 nM Metrnl groups, and in these groups, vimentin immunoreactivity was determined to be statistically lower compared to the Control and Sham groups ($p < 0.05$). Although vimentin immunoreactivity was lower in the 100 nM Metrnl group compared to the 10 nM Metrnl group, no statistically significant difference was found between these groups (Fig. 5, Table 3).

4. Discussion

The regulation of reproductive functions is a complex neuroendocrine process involving the reciprocal integration of signals from both the central nervous system and the environment. Circulating steroid hormones and neuropeptides related to metabolic status send peripheral signals to the brainstem, which are then transmitted to the hypothalamus and brain for relay to the reproductive organs and peripheral tissues. The hypothalamus plays a key role in modulating the activation of the HPT axis and in the short- and long-term regulation of reproductive behaviour by integrating these signals. This centre is the site where numerous peptides and neurotransmitters, including GnRH, work together to regulate reproductive physiology [25]. The presence of Metrnl in the central area, in the male reproductive system (testicular tissue, epididymis, prostate, seminal vesicle) and in the female reproductive system (ovary, vagina, cervix, endometrium, fallopian tube and placenta) suggests that the peptide may affect certain processes in reproduction and the HPG axis [14, 26–28]. A limited number of studies have focused on this relationship between Metrnl and reproduction. Research has reported that serum Metrnl levels are reduced in individuals with PCOS [29, 30]. Rong et al. reported that serum Metrnl levels were elevated in infertile men, that sperm concentration was reduced by approximately 50 per cent in the infertile group despite similar semen volumes between groups, and that this indicated significant spermatological abnormalities occurring concurrently with the increase in Metrnl [31]. In infertile individuals, the decrease in the proportion of sperm with normal morphology, the significant reduction in progressive motility, and the increase in DNA fragmentation suggest that elevated Metrnl levels may be associated with impaired spermatogenesis and sperm function [31]. Yalçın et al. concluded that aluminium exposure in male rats caused an increase in Metrnl levels in testicular tissue and that this increase could be an attempt by the cells to adapt to changing physiological conditions [32].

In our study found that Metrnl administration significantly reduced GnRH mRNA and tissue protein levels in the hypothalamic tissue. Furthermore, our study determined that central Metrnl administration significantly reduced serum LH, FSH, and testosterone levels in rats. This decrease in hormones secreted by the pituitary (LH and FSH) and testis (testosterone) tissues, which are other components of the HPT axis, associated with Metrnl administration is thought to be due to the long-term suppressive effect of Metrnl on GnRH in particular. In our previous study, we demonstrated that central Metrnl infusion activates the hypothalamus-pituitary-thyroid (HPT) axis and increases uncoupling protein (UCP) levels, promoting energy expenditure and thermogenesis [18]. Interestingly, the current study reveals that Metrnl suppresses the HPG axis. This dichotomy may be explained by the physiological trade-off between energy metabolism and reproduction. It is well established that conditions promoting high energy expenditure (such as increased thermogenesis driven by thyroid hormones) can suppress reproductive functions to conserve energy. Therefore, Metrnl may act as a metabolic switch, enhancing energy dissipation while temporarily suppressing reproductive investment [18]. The pulsatile release of GnRH induces the synthesis and secretion of LH and FSH [33]. LH induces testosterone secretion from Leydig cells [34], while FSH increases the proliferation of Sertoli cells, their metabolic support, and the development of germ cells [35]. When examining the findings of our study, it was observed that GnRH, LH, FSH and testosterone levels decreased with Metrnl infusion, while sperm density and motility decreased without any difference in testicular and epididymal weight. When the histological

examinations of the testes, which constitute the final part of the HPT axis, were evaluated, it was determined that Metrnl infusion caused a decrease in germinal epithelial thickness. It was also determined that high-dose Metrnl infusion caused an increase in seminiferous tubule diameter. In the HSD3B1 immunoreactivity measurement performed to evaluate the steroidogenesis capacity of Leydig cells [36], it was observed that high-dose infusion of Metrnl significantly reduced HSD3B1 levels in these cells and consequently decreased testosterone synthesis capacity. In the Vimentin immunoreactivity measurement used to evaluate Sertoli cell morphology and integrity [37], it was determined that Metrnl significantly reduced Vimentin levels in the cells. The reduction in HSD3B1 levels is consistent with the decreased serum LH levels observed, as LH is the primary regulator of steroidogenic enzymes, including HSD3B1, in Leydig cells [34]. Similarly, the maintenance of vimentin filaments in Sertoli cells is strictly dependent on FSH stimulation. The observed decrease in vimentin immunoreactivity aligns with the suppression of serum FSH levels, indicating compromised Sertoli cell cytoskeletal integrity [37, 38]. Our findings suggest that central Metrnl infusion reduces GnRH mRNA and protein levels in the hypothalamus, thereby decreasing LH and FSH secretion from the pituitary gland. This, in turn, reduces HSD3B1 immunoreactivity and testosterone synthesis capacity in Leydig cells, while decreased Vimentin levels in Sertoli cells lead to impaired cell morphology and germ cell support. Thereby suggesting alterations in seminiferous tubule structure and reduced sperm concentration and motility.

This study provides important information about the potential role of Metrnl in male reproductive physiology and emphasises the need for further research into the effects of Metrnl.

Limitations of the Study

There are several limitations in this study that should be acknowledged.

1. Dose and Administration: While the icv doses were selected based on previous efficacy [18], we did not measure Metrnl levels in the cerebrospinal fluid or plasma. Therefore, we cannot rule out the possibility of peripheral leakage or fully confirm physiological concentrations.
2. Controls: A positive control group (e.g., GnRH antagonist) was not included, which could have further validated the suppression model.
3. Sperm Analysis: Sperm parameters were evaluated using standard light microscopy. While this provides a basic assessment of count and motility, more advanced techniques such as Computer-Assisted Sperm Analysis (CASA) would provide more detailed kinetic data.
4. Receptor Mechanisms: The precise receptor mechanism (e.g., KIT signaling) in the hypothalamus remains to be elucidated in future molecular studies.

Conclusion In conclusion, despite these limitations, our findings provide the first evidence that central Metrnl administration suppresses the male reproductive axis at hypothalamic, pituitary, and gonadal levels. This suppression stands in contrast to its stimulatory effect on the thyroid axis, suggesting a complex role for Metrnl in coordinating energy balance and reproduction.

Declarations

Funding

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK; Project No. 123Z074).

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure Statement

The authors declare no conflict of interest.

Ethics Statement: This study was approved by the İnönü University Local Ethical Committee (Protocol No: 2021/25-1) and performed in accordance with the ethical standards of the institution.

Author Contributions

- The study was designed by B.B., and S.T.
- B.B., S.T., and G.Ş. performed the experimental model, biochemical and sperm analyses.
- Y.E. performed the molecular analyses, and A.Y. performed the histopathological and immunohistochemical analyses.
- Ç.T. performed the statistical analyses.
- All authors contributed to the final version of the manuscript.

References

1. Knobil E, Plant T, Wildt L, Belchetz P, Marshall G (1980) Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. Science 207:1371–1373
2. Burger L, Haisenleder D, Dalkin A, Marshall J (2004) Regulation of gonadotropin subunit gene transcription. J Mol Endocrinol 33:559–584
3. Sahin Z, Aktas O, Kalkan OF, Cuce G, Alver A, Sahin E, Erdem S, Saglam N, Solak Gormus ZI, Kutlu S (2024) Investigation of effect peripheral kisspeptin treatment on hypothalamo-pituitary-gonadal axis and hypothalamo-pituitary-adrenal axis in male rats. Biol Futur 75:477–486. 10.1007/s42977-024-00241-3
4. Zheng SL, Li ZY, Song J, Liu JM, Miao CY (2016) Metrnl: a secreted protein with new emerging functions. Acta Pharmacol Sin 37:571–579. 10.1038/aps.2016.9
5. Ruperez C, Ferrer-Curriu G, Cervera-Barea A, Florit L, Guitart-Mampel M, Garrabou G, Zamora M, Crispi F, Fernandez-Sola J, Lupon J, Bayes-Genis A, Villarroya F, Planavila A (2021) Meteorin-like/Meteorin-beta protects heart against cardiac dysfunction. J Exp Med 218. 10.1084/jem.20201206

6. Hu W, Wang R, Sun B (2021) Meteorin-Like Ameliorates beta Cell Function by Inhibiting beta Cell Apoptosis of and Promoting beta Cell Proliferation via Activating the WNT/beta-Catenin Pathway. Front Pharmacol 12:627147. 10.3389/fphar.2021.627147
7. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157:1279–1291. 10.1016/j.cell.2014.03.065
8. Yilmaz N, Yildiz A (2024) Intracerebroventricular PROK2 infusion could increase the secretion of male reproductive hormones by stimulating the HPG axis. Mol Biol Rep 51:656. 10.1007/s11033-024-09604-4
9. Ulker N, Yardimci A, Coban E, Ozcan M, Canpolat S (2021) Chronic irisin exposure decreases sexual incentive motivation in female rats. Physiol Behav 232:113341
10. Shokrollahi B, Shang J-H, Saadati N, Ahmad HI, Yang C-Y (2021) Reproductive roles of novel adipokines apelin, visfatin, and irisin in farm animals. Theriogenology 172:178–186
11. Tekin S, Erden Y, Sandal S, Etem Onalan E, Ozyalin F, Ozen H, Yilmaz B (2017) Effects of apelin on reproductive functions: relationship with feeding behavior and energy metabolism. Arch Physiol Biochem 123:9–15. 10.1080/13813455.2016.1211709
12. Mathew H, Castracane VD, Mantzoros C (2018) Adipose tissue and reproductive health. Metabolism 86:18–32. 10.1016/j.metabol.2017.11.006
13. Jorgensen JR, Thompson L, Fjord-Larsen L, Krabbe C, Torp M, Kalkkinen N, Hansen C, Wahlberg L (2009) Characterization of Meteorin–an evolutionary conserved neurotrophic factor. J Mol Neurosci 39:104–116. 10.1007/s12031-009-9189-4
14. Li ZY, Zheng SL, Wang P, Xu TY, Guan YF, Zhang YJ, Miao CY (2014) Subfatin is a novel adipokine and unlike Meteorin in adipose and brain expression. CNS Neurosci Ther 20:344–354
15. Proteinatlas (2021) The Human Protein Atlas
16. Keskin T, Erden Y, Tekin S (2021) Intracerebroventricular asprosin administration strongly stimulates hypothalamic-pituitary-testicular axis in rats. Mol Cell Endocrinol 538:111451. 10.1016/j.mce.2021.111451
17. Tekin S, Beytur A, Erden Y, Beytur A, Cigremis Y, Vardi N, Turkoz Y, Tekedereli I, Sandal S (2018) Effects of intracerebroventricular administration of irisin on the hypothalamus-pituitary-gonadal axis in male rats. J Cell Physiol. 10.1002/jcp.27541
18. Şekerci G, Erden Y, Tekin S (2022) Effects of meteorin-like hormone on endocrine function of hypothalamo-hypophysial system and peripheral uncoupling proteins in rats. Mol Biol Rep 49:5919–5925
19. Paxinos G, Watson C (2006) The rat brain in stereotaxic coordinates: hard cover edition. Elsevier
20. Yilmaz U, Tekin S, Demir M, Cigremis Y, Sandal S (2018) Effects of central FGF21 infusion on the hypothalamus–pituitary–thyroid axis and energy metabolism in rats. J Physiological Sci 68:781–788

21. Tekin S, Erden Y, Ozyalin F, Onalan EE, Cigremis Y, Colak C, Tekedereli I, Sandal S (2018) Central irisin administration suppresses thyroid hormone production but increases energy consumption in rats. Neurosci Lett 674:136–141. 10.1016/j.neulet.2018.03.046
22. Erden Y (2020) Capsanthin stimulates the mitochondrial apoptosis-mediated cell death, following DNA damage in MCF-7 cells. Nutr Cancer 73:662–670
23. Erden Y (2021) Sour black mulberry (Morus nigra L.) causes cell death by decreasing mutant p53 expression in HT-29 human colon cancer cells. Food Bioscience 42:101113
24. Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences
25. Lara HE, Dorfman MD, Mayerhofer A (2025) Nervous system and reproduction: a highly integrative partnership. Frontiers Media SA, p 1621411
26. Kaya S, Yalçın T, Kuloğlu T (2023) Resveratrol may reduce oxidative stress and apoptosis in doxorubicin-induced cardiotoxicity by regulating meteorin-like and TRPM2 levels. Comp Clin Pathol 32:393–404
27. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157:1279–1291
28. Proteinatlas (2025) Metrnl
29. Deniz R, Yavuzkir S, Ugur K, Ustebay DU, Baykus Y, Ustebay S, Aydin S (2021) Subfatin and asprosin, two new metabolic players of polycystic ovary syndrome. J Obstet Gynaecol 41:279–284
30. Fouani FZ, Fadaei R, Moradi N, Zandieh Z, Ansaripour S, Yekaninejad MS, Vatannejad A, Mahmoudi M (2020) Circulating levels of Meteorin-like protein in polycystic ovary syndrome: A case-control study. PLoS ONE 15:e0231943
31. Rong C, Weng L, Li M, Zhou L, Li Y (2023) Serum interleukin-38 and-41 levels as candidate biomarkers in male infertility. Immunol Lett 255:47–53
32. Yalçın T, Kaya S, Kuloğlu T, Yiğin A (2023) N-Acetylcysteine May Regulate Altered Meteorin-Like Levels in Testicular Tissue due to Aluminum Exposure. Biol Trace Elem Res 201:5335–5345. 10.1007/s12011-023-03656-6
33. Clarkson J, Herbison AE (2006) Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty. Mol Cell Endocrinol 254:32–38
34. Oduwole OO, Huhtaniemi IT, Misrahi M (2021) The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. Int J Mol Sci 22:12735
35. Wang J-M, Li Z-F, Yang W-X, Tan F-Q (2022) Follicle-stimulating hormone signaling in Sertoli cells: a licence to the early stages of spermatogenesis. Reproductive Biology Endocrinol 20:97
36. Yokoyama C, Chigi Y, Baba T, Ohshitanai A, Harada Y, Takahashi F, Morohashi K-i (2019) Three populations of adult Leydig cells in mouse testes revealed by a novel mouse HSD3B1-specific rat monoclonal antibody. Biochem Biophys Res Commun 511:916–920

37. Saito H, Yokota S, Kitajima S (2023) Immunohistochemical analysis of the vimentin filaments in Sertoli cells is a powerful tool for the prediction of spermatogenic dysfunction. Acta Histochem 125:152046
38. Wang J, Jia Z, Dang H, Zou J (2022) Meteorin-like/Meteorin-β upregulates proinflammatory cytokines via NF-κB pathway in grass carp Ctenopharyngodon idella. Dev Comp Immunol 127:104289

Figures

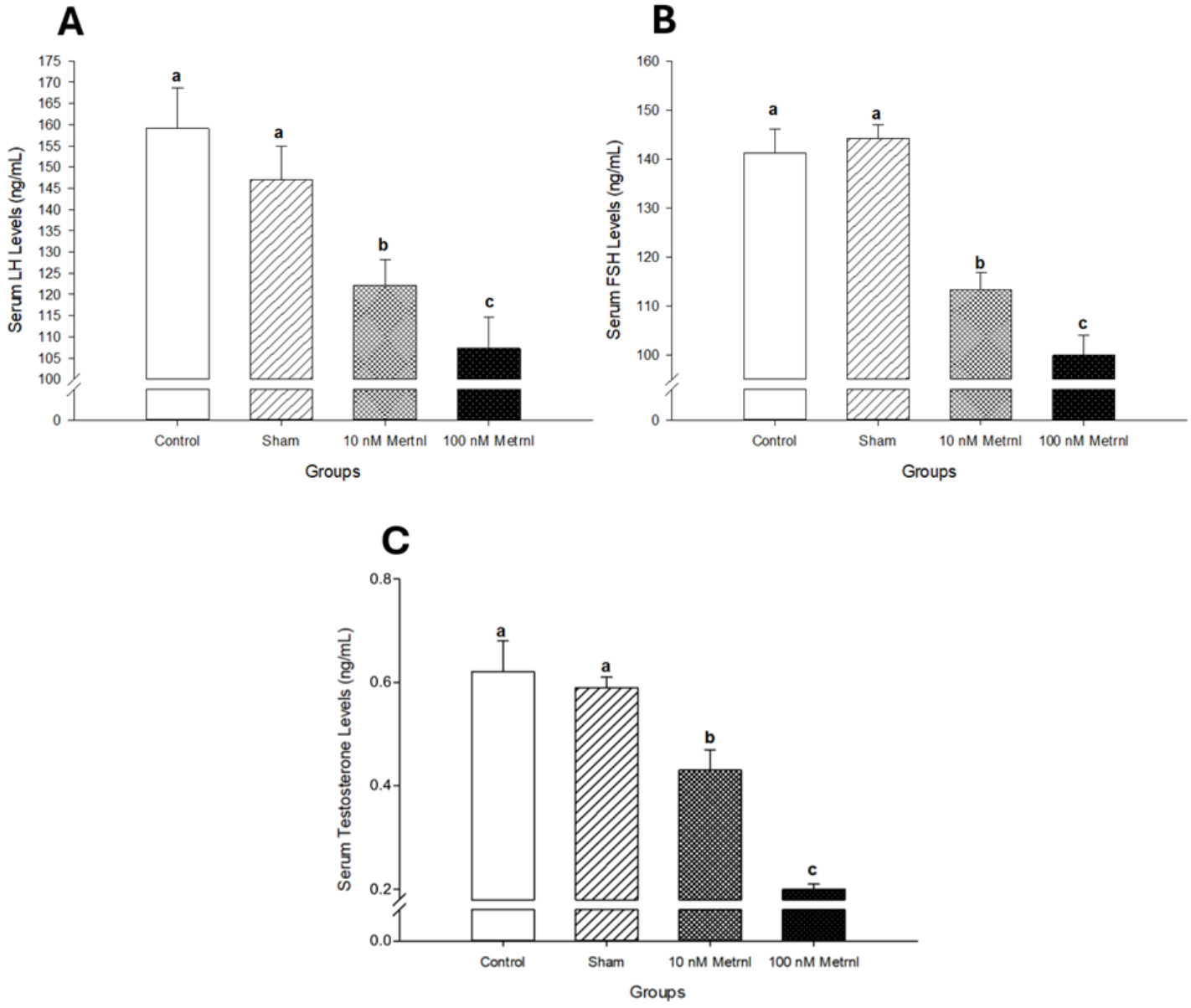


Figure 1

Effects of intracerebroventricular (icv) Metrnl administration on serum reproductive hormone levels. (A) Serum luteinizing hormone (LH) levels, (B) serum follicle-stimulating hormone (FSH) levels, and (C) serum testosterone levels in Control, Sham, 10 nM Metrnl, and 100 nM Metrnl groups. Data were analyzed using the Kruskal–Wallis H test, followed by the Mann–Whitney U test with Bonferroni correction for multiple comparisons. Values are expressed as mean ± standard deviation (SD). Different letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$).

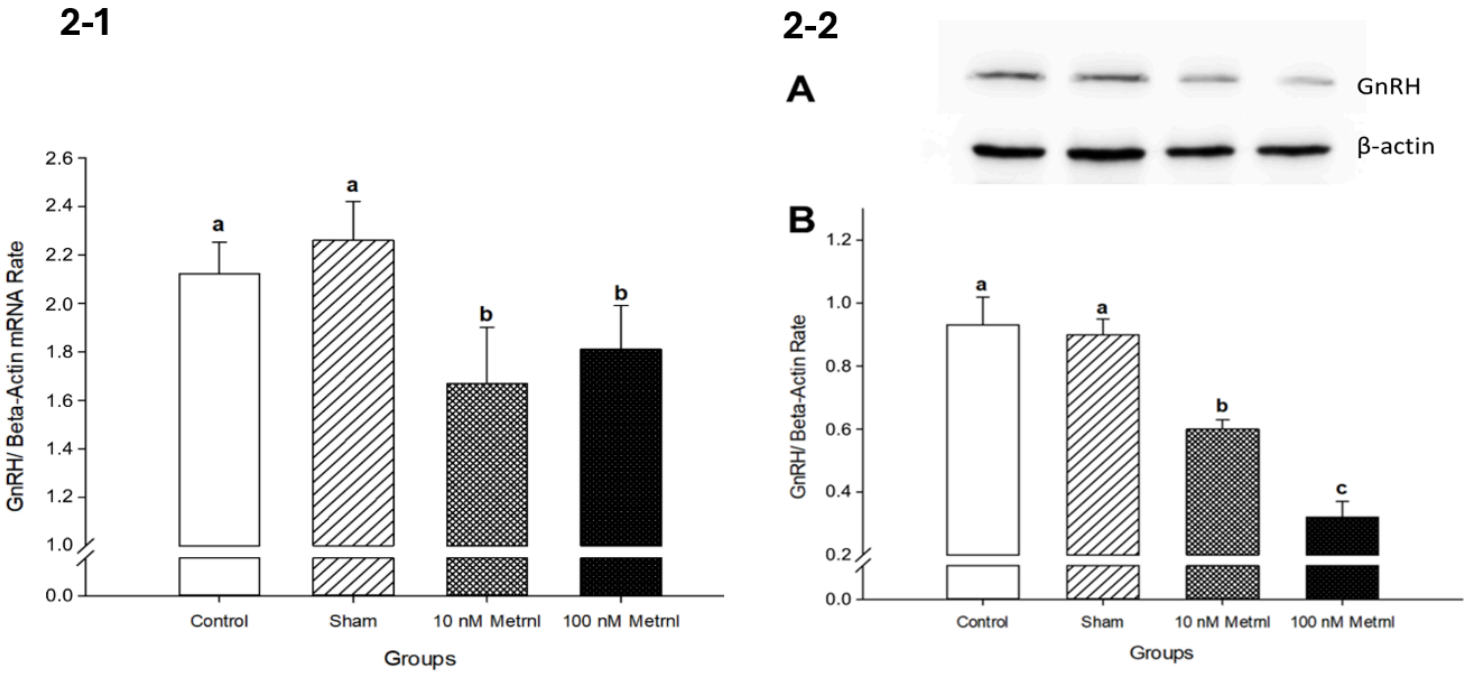


Figure 2

Effects of central Metrnl administration on hypothalamic GnRH expression levels.

2-1: Graphical representation of GnRH mRNA expression levels normalized to β-actin. **2-2:** Effects of Metrnl on GnRH protein levels; **(A)** representative Western blot image of GnRH and β-actin bands, **(B)** graphical representation of densitometric analysis of GnRH protein levels normalized to β-actin. Different letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$).

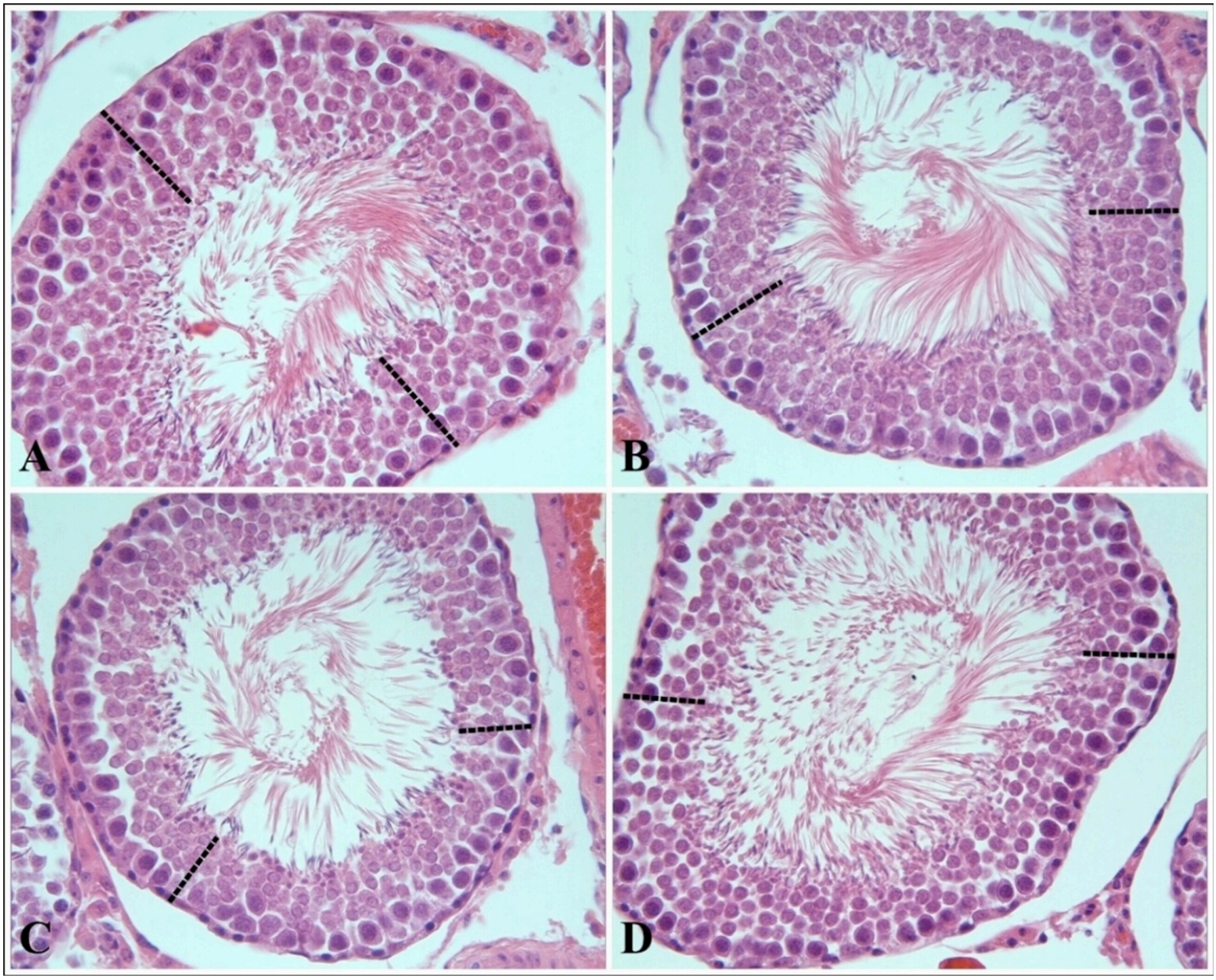


Figure 3

Histological evaluation of testicular tissue (H&E staining, x400).

Germinal epithelium (dashed line) is shown in the control (A), sham (B), 10 nM Metrnl (C), and 100 nM Metrnl (D) groups. Metrnl administration resulted in a significant reduction in germinal epithelium thickness in both treatment groups compared to controls.

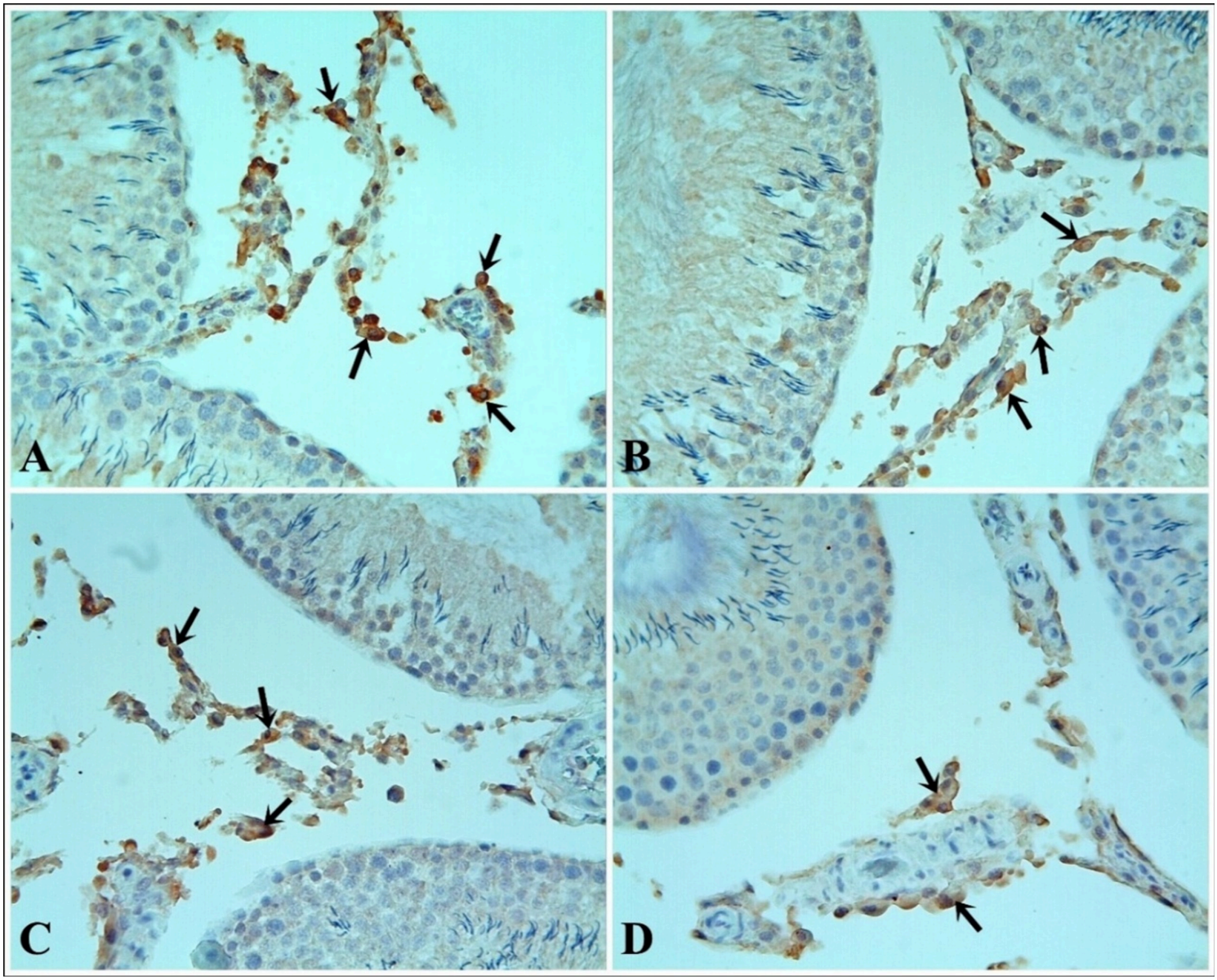


Figure 4

Illustrates the HSD3B1 immunoreactivity in Leydig cells across different experimental groups (x400).

In the Control **(A)**group, strong immunoreactivity for HSD3B1 is observed. Conversely, a significant decrease in immunoreactivity is noticeable in the Sham **(B)** and 10 nM Metrnl **(C)** groups compared to the Control group. The lowest HSD3B1 immunoreactivity is seen in the 100 nM Metrnl (D) group.

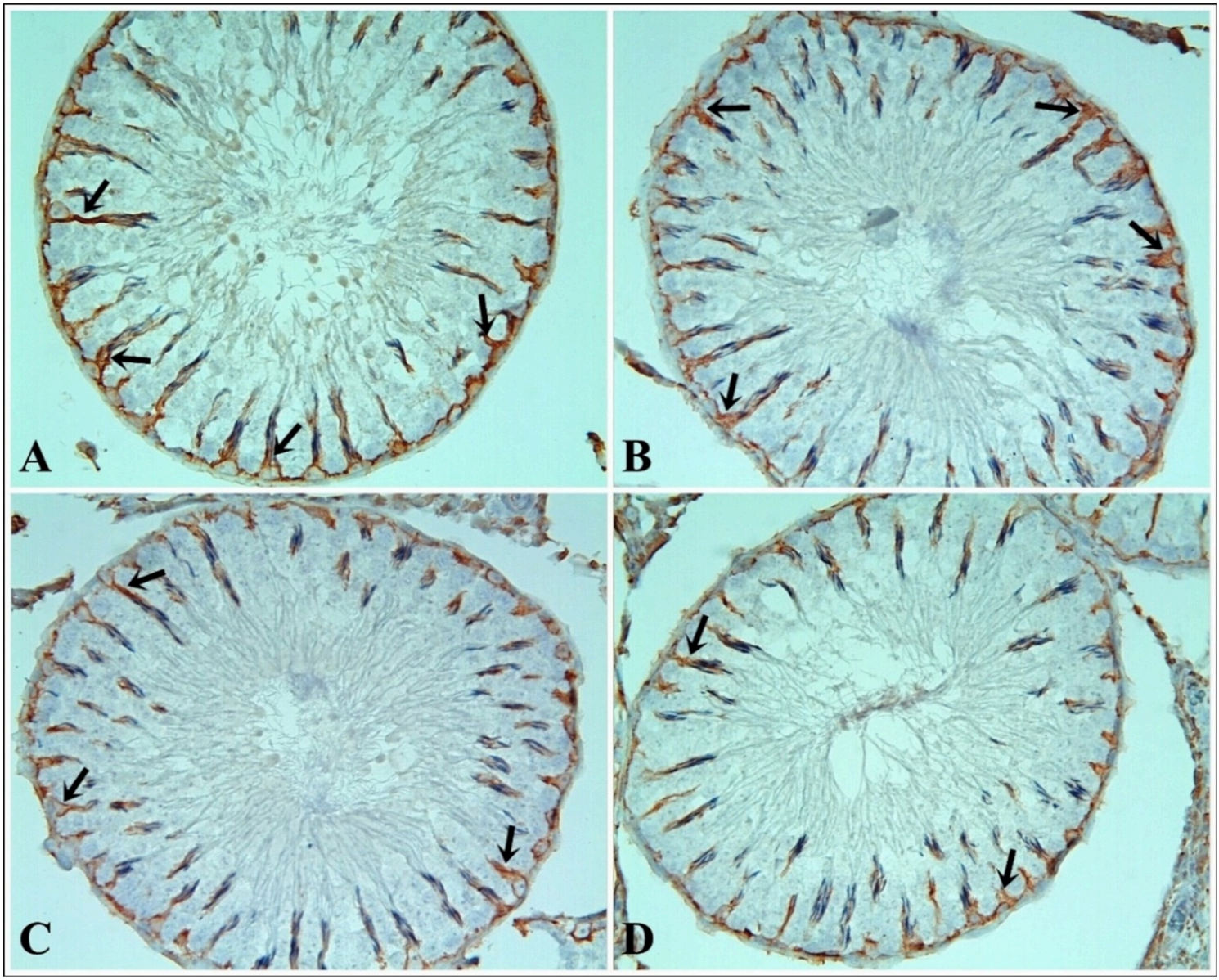


Figure 5

Displays the vimentin immunoreactivity within Sertoli cells across various experimental groups (x400).

A strong immunoreaction for vimentin was observed in the Control **(A)** and Sham **(B)** groups. Conversely, a weak reaction was noted in the Metrnl 10 **(C)** and Metrnl 100 **(D)** groups.)