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Effects of Methionine Chronic Administration on Testicular Tissue in Rats: A Histopathological and Immunohistochemical Study

Efectos de la Administración Crónica de Metionina en el Tejido Testicular de Ratas: Un Estudio Histopatológico e Inmunohistoquímico

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SUMMARY: Studies have shown an association between homocysteine (Hcy) and several pathological conditions, including cardiovascular disease. However, the relationship between Hcy and testicular disorders is still unclear. The present study investigated the effects of Hcy on testicular structure. Thirteen adult Wistar albino rats were divided into two groups. The control group and the methionine group received an oral solution of L-methionine 200 mg /kg per day for six months. Blood samples and testes were collected for subsequent experiments. Serum levels of Hcy and testosterone were analyzed. We examined the histopathological and immunohistochemical localization of GATA-4, vimentin (VIM), connexin-43 (Cx-43), occludin (OCLN), androgen receptor (AR), estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2) in the rat testis. Results showed that rats receiving methionine showed a reduction in body and testicular weights. Histological and immunohistochemical examination of the testicular tissues of methionine group showed altered structure of seminiferous tubules with decreased immunoexpression of Cx-43, GATA-4, OCLN,VIM, AR, ESR1 and ESR2 compared to control. The results suggest that Hcy has a negative effect on the testes with a probable disruption of spermatogenesis.

KEY WORDS: Methionine; Homocysteine; Testis; Tight and gap junctions; Receptors.

INTRODUCTION

Homocysteine (Hcy) is a sulfur amino acid formed during the metabolism of methionine (Met) to cysteine. Levels of Hcy can be increased by genetic defects in homocysteine metabolism enzymes or by deficiencies of important cofactors for these enzymes, including vitamins B6, B12 and folate. In addition, excessive methionine intake, various diseases , and lifestyle changes are known to increase Hcy levels (Selhub & Miller, 1992). Moderate elevation of plasma Hcy, or hyperhomocysteinemia, is associated with an increased risk of cardiovascular disease as well as a number of other diseases, such as diabetes, Alzheimer's disease, and other dementias (Chaouad *et al*., 2019). In addition, some recent studies have shown that high Hcy is significant parameter in the COVID-19 follow-up (Oner *et al*., 2023). In addition, Hcy is associated with reproductive problems such as recurrent pregnancy loss, and some studies suggest that Hcy in men affects the sperm quality and DNA methylation, both of which are associated with male infertility (Clement *et al*., 2023). In addition, our previous research has shown that Hcy affects the extracellular matrix of seminal vesicle (Ghoul *et al*., 2017). It is therefore important to investigate the effects of Hcy on the male reproductive system, particularly on testicular tissue structure and spermatogenesis. We therefore hypothesized that Hcy might affect the blood-testis barrier (BTB) and lead to spermatogenesis defects. The BTB is a complex cell structure consisting of tight junctions (Tjs), gap junctions (Gjs), and ectoplasmic specializations. It divides the seminiferous tubule into basal and adluminal (apical) compartments, and is required for the development and maturation of germ cells. Adhesion proteins and Tjs expression levels are primarily responsible for maintaining homeostasis and enabling normal BTB function (Tarulli *et al*., 2008). Defects in these proteins can disrupt the BTB and increase the passage of toxic substrates across the BTB, which is associated with a decrease in spermatogenesis and male fertility (Lu & Liu, 2023). First, we investigated whether Hcy affects the expression of Tjs in the BTB, such as occludin, and connexin-

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43; which is an important gap junction for BTB dynamics, formation and regulation. In addition, a number of factors, including vimentin and transcription factor GATA-4 (GATA binding protein 4), are essential for spermatogenesis and testis development. Indeed, the development and function of the mammalian testis has been linked to the transcription factor GATA-4. It is required for normal steroid production in males and a key regulator of BTB function and spermatogenesis (Schrade *et al*., 2016). Similarly, vimentin is an essential filament that is upregulated at different stages of spermatogenesis and is required for testicular germ cells (Tabar *et al*., 2022).

Therefore, this study was designed to analyze the effect of chronic Met administration on testicular structure and spermatogenesis dysfunction. The distribution and expression of OCLN, Cx43, GATA-4 and VIM were analyzed. ESR1, ESR2, AR and testosterone were also analyzed to determine the effect of Met on testicular structure.

MATERIAL AND METHOD

Animals and experimental protocol. For this study, 13 adult male Wistar rats, weighing 246-25 g and aged between 8 and 9 weeks were purchased from the Institut Pasteur (Algiers). The rats were housed in standard cages (50 × 25 × 25 cm) at 22 ± 2 °C and a 12/12 h light/dark cycle, with *ad libitum* access to food and water. The rats were randomly divided into two groups as follows: group 1 (n= 6), the control, was fed standard diet and water; group 2 (n= 7), the experimental, was fed standard diet and water supplemented with methionine. The dose of 200 mg L-methionine / kg bw per day (Sigma-Aldrich, France) was administered orally for six months. All experimental protocols were carried out according to the guidelines of the Local Institutional Animal Care Committee of USTHB University with decision number of CEEA-USTHB-2023/11119 and were supported by the Algerian Association of Experimental Animal Sciences (Agreement number 45/DGLPAG/DVA.SDA.14).

Weight monitoring. Rats were weighed before and at the end of the study. The index of body weight (IBW) was measured based from the initial and final body weights using the formula: IBW= final body weight (g) - initial body weight (g)/ initial body weight ×100. The testes were removed and weighed, and the gonadosomatic index (GI %) was determined using the following formula: Gonadosomatic index = testes weight (g)/ body weight (g) × 100.

Serum analysis. Throughout the experimental period, blood samples were taken from the retro-orbital plexus using capillary tubes. Blood was collected in dry tubes, centrifuged at 3000 rpm for 10 min, and stored at -20 °C until biochemical and hormonal analyses were performed. Total Hcy was determined by fluorescence polarization immunoassay using the Abbot IMx analyzer (AxSYM system, Abbott, Abbott Park, IL, USA). Testosterone levels were determined by radioimmunoassay using a commercially available kit; TESTO CT2 (Cis Bio, International, France).

Histology and immunohistochemistry of testes. Testes were fixed by immersion in Bouin-Hollande's solution or in 10 % formalin immediately after removal, for 48 h and 24 h, respectively, for histological and immunohistochemical (IHC) studies. The testes were then dehydrated in an ascending series of ethanol, cleared in two changes of xylene and embedded in paraffin at 58 °C. The testes were then sectioned transversely at 5 µm thickness using a rotary microtome (Leica RM 2125RT, Wetzlar, Germany). For histological studies, sections were mounted on basic glass slides and stained with periodic acid-Schiffs (PAS) and Masson's trichrome to examine structural changes. For IHC studies, the sections were mounted on positively charged slides (Superfrost plus, Menzel Gläser, Thermo Scientific, Braunschweig, Germany). The slides were then deparaffinized in cyclohexane, washed with distilled water, and treated with 0.3 % hydrogen peroxide (SP-6000) to quench endogenous peroxidase activity, followed by blocking with 10 % normal horse serum in PBS for 1hour at room temperature (RT) to block non-specific binding sites. Slides were then incubated with primary antibodies against rabbit anti-androgen receptor (AR) (1 :50 dilution ; overnight at +4 °C, ab 74272, Abcam plc, Cambridge,UK), rabbit anti- estrogen receptor 1 (ESR10) (1:100 dilution ; overnight at +4 °C, H-184 :sc-7207, Santa Cruz Biotechnology, USA), rabbit anti- estrogen receptor 2 (ERS2) (1:50 dilution ; overnight at +4 °C, H-150 :sc-8974, Santa Cruz Biotechnology, USA), rabbit anti-occludin (1:100 dilution ; 2h at RT, Thermo Fisher Scientific, Invitrogen, 71-1500), rabbits anti-connexin-43 (1:100 dilution ; 2 h at RT, Sigma-Aldrich ; C6219), rabbit anti-vimentin (1:50 dilution ; 1 h at RT, Abcam, 137321) and mouse anti-GATA-4 (1:50 dilution, 1 h at RT, Santa Cruz-25310). All sections were then incubated with biotinylated secondary antibodies (anti-mouse IgG/rabbit IgG, Vectastain Elite Universal PK-6200, Vector Laboratories, Burlingame, California, USA) for 1 hour in a wet chamber. After washing in PBS, slides were revealed with diaminobenzidine (SK-4100, DAB substrate kit for peroxidase; Vector Laboratories) and nuclei were counterstained with hematoxylin QS (H-3404; Vector laboratories). Slides were dehydrated, mounted, and observed using a Nikon Eclipse E400 light microscope (Nikon, Tokyo, Japan), and digitized images were taken using a Nikon DXM 1200 digital camera.

Statistical analysis: Data were analyzed by Student's t-test using Graph Pad Prism 8.01.The results are presented as mean ± standard error of the mean (SEM). Differences were considered statistically significant at $P \leq 0.05$.

RESULTS

Body and testicular weights: At the end of the study, weight gain was highest in the control group and was and lower in the Met group (Table I; P<0.05). Absolute and relative weights of the testes were slightly altered by Met administration.

Biochemical analysis. The plasma Hcy levels of the two groups are shown in Figure 1. The results of the study showed that the level of plasma Hcy increased significantly (P<0.0001) in the Met group compared to the control group. Meanwhile, testosterone levels were lower in the Met group than in the control group, and this decrease was statistically significant (<0.001, Fig. 2).

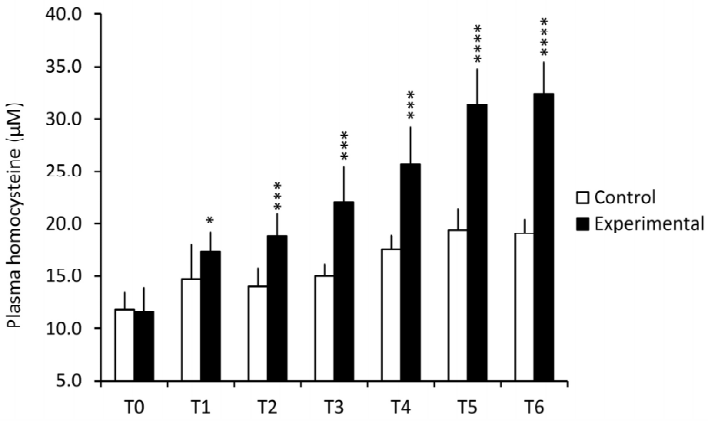


Fig. 1. Effect of Met supplementation on plasma homocysteine concentration. Results are means ± SD compared to control. Statistically significant data (*P < 0.05, **P< 0.001, ***P<0.0001, ****P< 0.00001); (Met *vs* control); T: time (month)

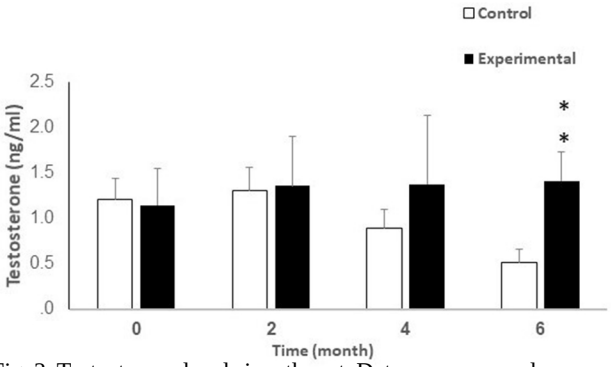


Fig. 2. Testosterone levels in n the rat. Data are expressed as mean ± SEM; *p<0.05; **p<0.001 (Met *vs* control); ns= not significant.

Histopathological findings. Histopathological examination of the testicular tissue of the control group (Fig. 3a-3b) showed normal spermatogenesis, spermatogonia (sg) and sustentacular cells (Sertoli cells) (Sc) resting on an intact thin basement membrane (bm). Each testis is consist of regular seminiferous tubules (ST) separated by thin interstitial tissue (Ti) which containing interstitial cells (Leydig cells) (Lc) and blood vessels (Bv). However, in all experimental rats, most tubules show loss of germ cells and cell-cell interactions (Fig. 3d-3e-3f) and vacuolation of spermatogenic cells and Sustentacular cells (Fig. 3d-3e-3f), resulting in increased lumen (Fig. 3c-3d), and significant sloughing of spermatogenic cells into the lumen of the seminiferous tubules (Fig. 3c-3j). Many giant cells in mitosis were seen among the other germ cells (Fig.3c, red arrowhead). In addition, few or no spermatozoa were seen in the lumen of most tubules, indicating arrest of spermatogenesis (Fig. 3c-3d-3e). These altered tubules showed that germ cells were detached from the basement membrane (Fig. 3i-3j), and many tubules appeared with a thick and irregular basement membrane (Fig. 3i-3j). In addition, a large area of interstitial tissue was found in the parenchyma of altered tubules (Fig. 3d-3f).

Table I. Values of body weight gain (BWG, g), index body weight (IBW, %) and gonadosomatic index (GI %) in control and Met rats.

Parameter	Control group	Methionine group	p-value
Initial body weight (g)	251.83±8.17	246.14±11.31	0.42 ns
Final body weight (g)	317.71±7.86	298.18±6.01	**0.001**
BWG (g) at the end of experiment	65.88	52.00	-
Absolutes testes weight (g)	1.62±0.11	1.54±0.13	0.19 ns
Relative testes weight (mg/g)	0.53±0.03	0.49±0.04	**0.05**
IBW (%)	26.23	21.14	-
GI (%)	0.18	0.15	-

Data are expressed as the mean ± SEM; *p<0.05 (Met *vs* control); ns= not significant.

PAS-stained sections of control group testes showed the normal distribution of PAS material. Indeed, PAS-positive reaction was detected in the regular basement membrane of seminiferous tubules, spermatids, spermatozoa, blood vessels, and interstitial spaces (Fig. 3g-3h, black arrowhead). In the Met group, PAS staining showed very strong PAS-positive reaction in the thick irregular basement membrane of seminiferous tubules and interstitial spaces (Fig. 3i-3j).

Immunohistochemical findings. Immunohistochemical staining for OCLN, GATA-4, VIM,Cx-43, AR, ESR1 and ESR2 (Figs. 4 and 5) revealed the following results: OCLN was localized in a linear pattern at the basolateral membrane region of the adjacent Sustentacular cells in the control group (Fig. 4a). In the Met group (Fig. 4c), OCLN was more irregular and its intensity decreased in damaged seminiferous tubules. With the regard to GTATA-4, the immunohistochemical results showed that GATA-4 immunoreactivity was stronger in the testes of the control group than in the Met group. Indeed, we observed that the GATA-4 antibody strongly stained the somatic cells, i.e., sustentacular and Interstitial cells (nucleus and cytoplasm), spermatogonia, and spermatids (nucleus) of the seminiferous tubules of the control group (Fig. 4e-4f). In the Met group (Fig. 4g-4h),

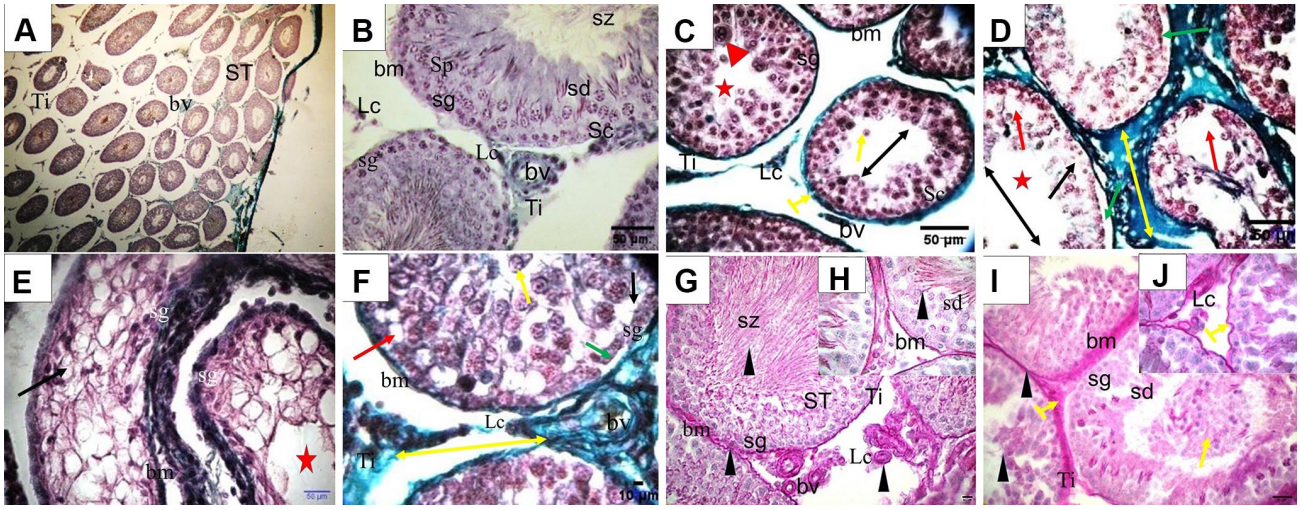
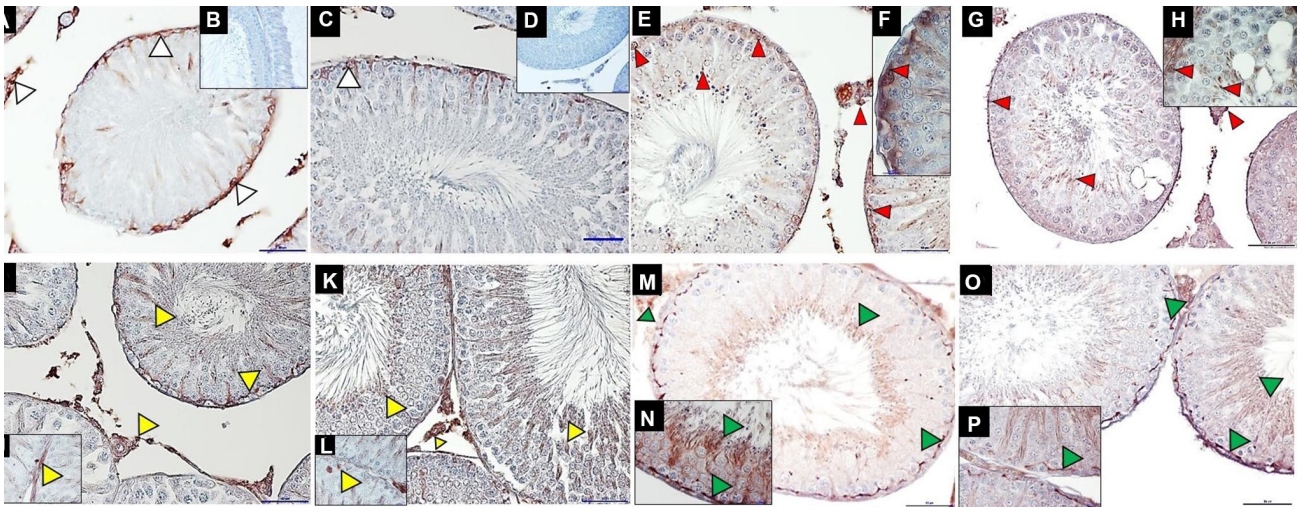


Fig. 3. Representative images of Masson's trichrome and PAS staining in the testis. (Masson's trichrome: Control: a and b, scale bar = 50 µm; Met: c, scale bar =10 µm, and d, e, and f scale bar = 100 µm) (PAS: Control g and h, scale bar = 50 µm; Met: I and j, scale bar = 50 µm); Bv: lood vessel; Lc : Interstitial cells; Sp : Spermatocytes; Sc : Sustentacular cells; Sd : Spermatids; Sg : Spermatogonia; Sz : Spermatozoa.



Germ cells loss, Vacuolation, Desquamation, Giant cells, Lumen,
No SPZ, Germ cells detachment, Basement membranes, Interstitial tissue,
PAS.

Fig. 4. Immunohistochemical localization of OCLN, GATA-4, VIM, Cx-43. OCLN (a and c, bar: 50 µm), GATA-4 (e, f, g, and h; bar: e and g 50 µm, bar: f and h: 10 µm), VIM (i, j, k and l; bar: i and k 50 µm, bar: j and l 10 µm), Cx-43 (m, n, o, and p; bar m and o 50 µm, bar n and p 10 µm). Negative controls prepared without primary antibody are also shown in Figure 3b-d (bar: 50 µm);
OCLN, GATA-4, VIM, Cx-43.

immunostaining showed that GATA-4 was localized in Leydig, Sustentacular cells and spermatogonia. Within Interstitial cells, GATA-4 was predominantly detected in the cytoplasm, whereas in Sustentacular cells, spermatogonia, and spermatids GATA-4 immunoreactivity was mainly localized in the nucleus. VIM (Fig. 4) immunoreactivity was observed in the cytoplasm of Sustentacular cells, mainly perinuclear in basal compartment and extended in thin linear columns towards the tubular lumen in the control group (Fig. 4i-4j). In addition, IHC analysis revealed VIM expression in Interstitial cells and blood vessels. In the Met group, VIM immunoreactivity (Fig. 4k-4l) in some seminiferous tubules showed a decrease in expression in the perinuclear cytoplasm of Sustentacular cells in the basal compartment, whereas in the apical compartment the reaction was intense in the cytoplasm of Sustentacular cells. In addition, the Interstitial cells and blood vessels were positive for VIM in the Met group (Fig. 4j). With regard to Cx43 IHC analysis (Fig. 4m-4n), the control group showed that Cx43 immunoreactivity was located in the basal and apical compartments of the seminiferous tubules. In the basal compartment, Cx43 immunoreactivity was observed in linear array between Sustentacular cells or spermatogonia in the region of the BTB. In the apical compartment, Cx43 immunoreactivity was detected between Sustentacular cells and elongated spermatids. In addition, blood vessels showed strong immunoreactivity for Cx43 in the interstitial tissue (Fig. 4m-4n). The Met group (Fig. 4o-4p), showed weak positive immunoreactivity for Cx43 in the basal compartment, while the positive signal remained almost unchanged in the apical compartment and in the blood vessels.

AR was observed in the nuclei of Leydig and Sustentacular cells in the control group (Fig. 5a). In Met testes (Fig. 5b), AR immunoreactivity was detected mainly in the cytoplasm of Interstitial cells. The staining was weaker in the Interstitial cells of Met group compared to the control group testicular tissue. ESR1 immunoexpression (Fig. 5c-5d) showed strong or moderate immunoreactivity of ESR1 in Interstitial cells (nuclear and cytoplasmic) and spermatids (nuclear) in the control group. A slight decrease in ERS1 immunoreactivity was observed in the testicular sections of Met group (Fig. 5e-5f). ESR2 immunoreactivity in the control group (Fig. 5g-5h) was observed in the nuclei and cytoplasm of Interstitial cells, Sustentacular cells and germ cells, including spermatogonia and spermatids. In addition, ESR2 was detected in a perinuclear region in Sustentacular cells and spermatogonia. In the Met group, ESR2 immunolocalization (Fig.5i-5j) was similar to that observed in the control group with the exception of the Interstitial cells where ERS2 immunoreactivity was seen only in the cytoplasm.

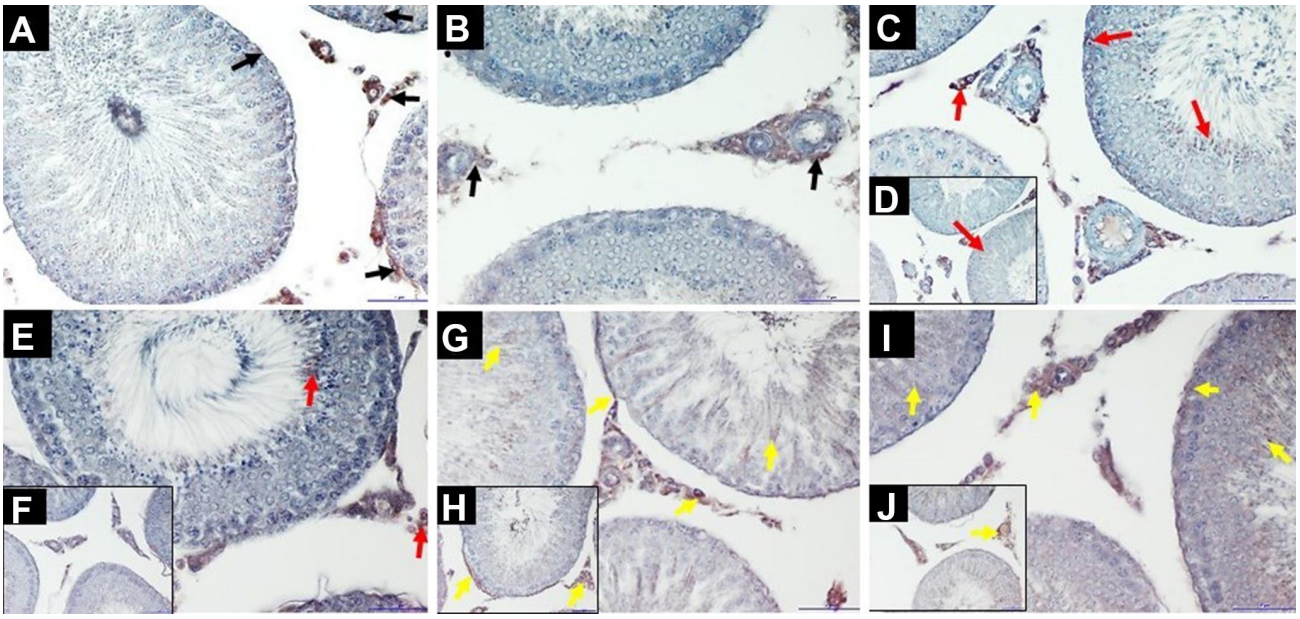


Fig. 5. Immunohistochemical localization of AR, ESR1 and ESR2 in rat testis. AR (a and b, bar: 50 µm), ESR1 (c,d,e, and f, bar 50 µm) and ESR2 (g,h,i, and j, bar: 50 µm). ➔ AR, ➔ ERS1, ➔ ERS2.

DISCUSSION

The impact of the Hcy on fertility has gradually attracted researchers' attention over the last decade. According to previous research, male fertility and sperm quality are negatively associated with Hcy concentration

(Clement *et al*., 2023). However, Hcy in relation to changes in testicular structure has not been investigated. Therefore, the aim of the present study was to elucidate the effect of Hcy on testicular structure in adult male Wistar rats. First, we evaluated the effect of oral Met supplementation on Hcy levels. Our data clearly show that Met supplementation was associated with an increase in total plasma Hcy plasma levels. These results are consistent with our previous findings (Ghoul *et al*. 2017), which demonstrated that Met excess induced a significant disturbance in Hcy metabolism.

In the present study, the Met group showed minimal reduction of body weight compared to the control group. The present results are in agreement with the study by Zhang *et al*. (2021), who suggested that the reduction in weight gain induced by high methionine diets may be due to a reduction in feed intake. We also observed lower absolute and relative testes weights in the Met group compared to the control group. This is consistent with previous results (Chin *et al*., 2015), which found that Met supplementation in rats induced a decrease in testicular weight. It has been reported (Akhigbe & Ajayi, 2020), that reduced testosterone levels and structural deterioration of the testes are the main causes of reduced testes weight. The results of our study demonstrated that the Met rats had significantly lower testosterone levels than the control rats.

Histomorphometric analysis of Met group in the present study revealed various degenerative changes in seminiferous tubules such as disorganization, vacuolization, desquamation and fibrosis. This result is in agreement with the previous study reported by Chin *et al*. (2015), which showed various changes in the seminiferous tubules and atrophy of accessory reproductive organs in Met rats. Testosterone is the most abundant androgen produced in the testes, and is the main regulator of male reproductive function. In addition, estrogen and androgen are known to be important for many aspects of spermatogenesis (Cacciola *et al*., 2013). These hormones act primarily through nuclear receptors such as AR, ERs (ESR1 and ESR2). Estrogen is synthetized by interstitial, sustentacular and germ cells through aromatization of testosterone (Akingbemi 2005). In adults rat testis, AR is present on sustentacular, interstitial and peritubular myoid cells (Shan *et al*., 1997). Although both ESR1 and ESR2 are found in germ cells, only ESR2 is found in spermatogonia and Sustentacular cells (Bois *et al*., 2010). Here, we investigated the effect of Hcy on the expression of AR and ERs in rat testis. In our control rat, AR was strong and localized in the nuclei of Leydig and Sustentacular cells. In the Met rat, it was modest and mainly detected in the cytoplasm of Interstitial cells. According to our study, testosterone levels were significantly reduced in the Met group. We hypothesized that this reduction may contribute to a decrease in AR expression in hyperhomocysteinemic rats. Studies have shown demonstrate that nuclear AR levels in adult rat Sustentacular cells are dependent on circulating testosterone levels (Shan *et al*., 1997). In addition, according to the study by Bruschi *et al*. (2005), there is an increase in Hcy levels in elderly women with low estrogen. Similarly, several studies have shown that hormone therapy can significantly reduce these levels according de certain studies (Mijatovic & Van Der Mooren, 2001; Lakryc *et al*., 2015). In addition, we investigated the effect of Hcy on ERs expression in testis of Wistar rats and showed that these rats showed a reduction in ERs expression. These results suggest that Hcy has a remarkable effect on the expression and localization of ERs. According to Liu & Zhang (2008), elevated levels of Hcy downregulate ESR2 expression in the aorta of ovariectomized rats through methylation modification. In our work, we suggest that elevated blood Hcy levels may lower blood levels of estrogen, which in turn may affect the expression of ERs. On the other hand, androgen and estrogen are known to be essential regulators of Tjs and Gjs of BTB during the remodeling of the seminiferous epithelium cycle (Tarulli *et al*., 2008). Kolasa *et al*. (2011), reported that hormonal imbalance by finasteride induced changes in the expression of Tjs and Gjs in rat seminiferous epithelium. Therefore, we propose that the BTB may be indirectly affected by the low testosterone found in this study. To investigate this possibility, immunostaining was performed to identify Hcy-induced damage to cell junctions (Cx-43), cytoskeleton (VIM), BTB (OCLN), and function (GATA-4).IHC analysis of OCLN also showed that it was expressed at low levels on BTB in the Met group. In addition, Hcy is known to activate matrix metalloproteinases (MMPs involved in pathological remodeling of seminal vesicles (Ghoul *et al*., 2017). This suggests that changes in Tjs occur by disrupting the balance between MMPs and tissue inhibitors of metalloproteinases. Indeed, Mu *et al*. (2022), showed that OCLN was degraded by MMP-8 in testes of mice fed a high fat diet. IHC analysis of the Met group of the Cx43 signal showed a loss of signal in Sustentacular cells at the BTB. Several studies shown that Cx43 is crucial for maintaining homeostasis for Tjs reassembly at the BTB (Li *et al*., 2010). Our results are consistent with previous in vitro results, showing that Hcy affects C43 expression in various cells (Chen *et al*., 2020). In addition, Sustentacular cells VIM filaments have been reported to be important for normal testicular morphology and spermatogenesis. Therefore, any alteration in the distribution of VIM filaments correlates with the exfoliation of spermatogenic cells into the lumen (Wang & Stamenovic, 2002). Therefore, the degradation of vimentin could promote the shedding of germ cells from the seminiferous. In the present study VIM expression was reduced in the Met group.

We studied another factor that is important in the regulation of spermatogenesis and is involved in the Sustentacular cells and BTB function. This is the transcription factor GATA-4 (Kyrönlahti *et al*., 2011). In this study, GATA-4 expression was reduced in sustentacular and Interstitial cells in the Met group. This result suggests that Hcy downregulates GATA-4 expression, which contributes to testicular tissue damage. Indeed, a previous study in mouse heart tissue showed that Hcy reduced GATA-4 activity through homocysteinylation (Zhao *et al*., 2023).

CONCLUSION

The results of our study have shown that Hcy has a negative effect on the structure of testis via the loss of cell-cell adherens in the seminiferous epithelium, which will be the consequence of the impairment of the integrity of seminiferous tubules. Our data converge towards an alteration in BTB integrity contributing to the alteration of spermatogenesis.

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RESUMEN: Estudios han demostrado una asociación entre la homocisteína (Hcy) y varias afecciones patológicas, incluidas las enfermedades cardiovasculares. Sin embargo, la relación entre Hcy y los trastornos testiculares aún no está clara. El presente estudio investigó los efectos de la Hcy sobre la estructura testicular. En este trabajo se dividieron trece ratas albinas Wistar adultas en dos grupos. El grupo de control y el grupo de metionina recibieron una solución oral de L-metionina 200 mg/kg por día durante seis meses. Se recogieron muestras de sangre y testículos para experimentos posteriores. Se analizaron los niveles séricos de Hcy y testosterona. Examinamos la localización histopatológica e inmunohistoquímica de GATA-4, vimentina (VIM), conexina-43 (Cx-43), ocludina (OCLN), receptor de andrógenos (AR), receptor de estrógeno 1 (ESR1) y receptor de estrógeno 2 (ESR2) en el testículo de la rata. Los resultados mostraron que las ratas que recibieron metionina presentaron una reducción en el peso corporal y testicular. El examen histológico e inmunohistoquímico de los tejidos testiculares del grupo de metionina mostró una estructura alterada de los túbulos seminíferos con una inmunoexpresión disminuida de Cx-43, GATA-4, OCLN, VIM, AR, ESR1 y ESR2 en comparación con el control. Los resultados sugieren que la Hcy tiene un efecto negativo sobre los testículos con una probable alteración de la espermatogénesis.

PALABRAS CLAVE: Metionina; Homocisteína; Testículos; Uniones estrechas y separadas; Receptores.

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