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Spermatogenesis and steroidogenesis disruption in a model of metabolic syndrome rats

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ABSTRACT

Context: Metabolic syndrome (MetS) is a clustering of several physiological alterations.
Objective: This study was designed to evaluate the effects of MetS on rats spermatogenesis and steroidogenesis.
Materials and methods: We developed a MetS rodent model using high-sugar and high-fat diet.
Results: MetS rats showed severe disorders in sperm parameters. Interestingly, a significant increase in malondialdehyde level and a decrease in the antioxidant activities were observed. Moreover, qRT-PCR analysis showed Bax down-regulation and Bcl-2 up-regulation. A decrease in testosterone level was identified, correlated with the CYP11A1, CYP17A1 and 17β HSD testicular marker down-regulation. Finally, MetS rats showed an up-regulation of pro-inflammatory cytokines receptors IL-1R and IL-6R.
Conclusion: MetS induced severe testis toxicity in male rats. Mets markedly distorted sperm parameters, inhibited the transcription of steroidogenic enzymes and led to oxidative stress, inflammation, and alteration of Bax/Bcl-2 ratio in testicular tissues.

Introduction

Metabolic syndrome (MetS) encompasses cluster of risk factors for cardiovascular diseases which include abdominal obesity, hyperglycemia, dyslipidemia and hypertension (Ford 2005). Various definitions of MetS have been designed by diverse organisations. But later, these organisations combined and developed a new definition known as “harmonized criteria” which included central obesity, increased blood pressure, high concentration of triglycerides, low concentration of HDL cholesterol (high-density lipoprotein) and presence of diabetes or insulin resistance (Alberti et al. 2009). MetS is diagnosed when a patient has at least three of the above-mentioned conditions.

Factors that play a part in the development of MetS are high-carbohydrate and high-fat diet, as well as sedentary lifestyles. Statistics reveal 20–25% of adult population in the world being diagnosed as MetS (Mamikutty et al. 2014). One of the consequences of the excessive fat accumulation due to overfeeding and less exercise is male infertility (Hammoud et al. 2008a).

In fact, previous studies suggested a close correlation between body mass index (BMI) and semen quality (Sallmen et al. 2006). Accumulating evidence demonstrate that obesity impairs male fertility (Hammoud et al. 2008b, Hofny et al. 2010), manifesting as poor semen quality by affecting spermatogenesis (Esener et al. 2017), erectile dysfunction, or hormonal disturbances (Abiad et al. 2017). Moreover, obesity leads to insulin resistance (IR), resulting in a series of obesity-related diseases (Palmer et al. 2012).

Interestingly, low serum testosterone was demonstrated to be predictive of IR, type 2 diabetes and MetS in men (Muraleedharan and Jones 2010). These results imply that IR plays a vital role in reduced semen quality (La Vignera et al. 2012) and spermatogenic dysfunction induced by obesity (Palmer et al. 2012). However, the full relationship between MetS and reproductive dysfunction remains to be defined. Overall, evidence suggests that maintaining a proper weight may help to maintain reproductive capacity.

Research using animal models that develop a complete case of MetS, including all of the above-mentioned risk factors are rare. However, studies have demonstrated that two strains of rats, namely Wistar rats and Sprague-Dawely rats were able to present an increase in body weight, dyslipidemia, hyperinsulinemia and glucose intolerance (Ghezzi et al. 2012). Despite previous studies investigating the effect of MetS on spermatogenesis (Leisegang et al. 2014), morphological change of testis and steroidogenesis in humans (Rodriguez et al. 2007, Winter et al. 2014), the causes and the mechanisms underlying MetS-associated infertility are complex and remain unclear.

Therefore, we designed the present study to reveal the changes in semen quality, abnormalities in testicular tissue,
and to understand the possible biochemical and molecular mechanisms underlying the MetS effects using a high-sugar and high-fat fed rats model.

Materials and methods

Animal and experimental procedure

Animals and experimental design
Adult male Wistar rats weighing (175 ± 5 g) were obtained from Pasteur Institute of Tunis (Tunisia). They were allowed two weeks for accommodation to the new environment before starting the experiment. Rats were housed under well-controlled conditions of temperature (22 ± 2 °C), relative humidity (70 ± 4%), and a 12/12 h light-dark cycle and were given standard (control group) or modified rodent chow (MetS group) and drinking water ad libitum. Procedures involving the animals and their care followed the Guidelines for Ethical Control and Supervision in the Care and Use of Animals.

Sixteen Rats were randomised into two groups (eight per group) assessed for corresponding nutritional diet: Groupe I: Normal Control group (CTR): each rat in this group was fed with a standard pellet diet during 10 weeks. Groupe II: Metabolic syndrome group (MetS): each rat in this group was fed with a high-fat high-sugar diet during 10 weeks.

The animals received the indicated treatments every day for more than 56 days (10 weeks) that was chosen based on the time necessary for 4–5 successive cycles for the seminal epithelium to pass from the state of spermatogonia to the state of spermatozoa in Wistar rats (Russell et al. 1990).

Experimental diets
Standard rodent pellets containing as energy contribution: 4% fat, 75% carbohydrate, 18% protein and 3% fibre were purchased from El Bader (Bizerte, Tunisia). High-fat and high-sugar diet was prepared by soaking standard diet pellets in warmed (100 °C), liquefied fat of animal origin for 15 min, and then supplemented with 15% of sucrose. The mixture was made into pellet form and dried at room temperature. As energy contribution, high-fat high-sugar diet contains 30% fat, 15% sugar, 41.2% carbohydrate, 9.9% protein and 1.65% fibre.

Evaluation of metabolic syndrome parameters

Anthropometric parameters
The Body mass index (BMI), abdominal circumference and percentage of body weight gain were determined as indicators of obesity. Body mass index and abdominal circumference were measured at baseline, 4th and at the end of experiment (10th weeks). The BMI was calculated by dividing the weight (g) by the length (cm²) (Novelli et al. 2007). The body length was measured between nasal and anal region (Poudyal et al. 2010). The abdominal circumference was determined using the measuring tape around the anterior abdomen in centimeter (Novelli et al. 2007).

Body weight was recorded every week using an electronic weighing scale (Mettler Toledo, AB 135 S). The changes of body weight were calculated by subtracting the final weight from the initial weight and then the percentage was calculated.

Determination of biochemical parameters and plasmatic level of insulin
Blood samples for estimation of blood glucose (in mg/dL) were collected at the baseline, 4th and 10th weeks via the orbital vein. In addition, after completion of the treatment schedule, rats were sacrificed by decapitation under mild ether anaesthesia; the arteriovenous blood was quickly collected in heparin tubes and centrifuged at 1000 g for 10 min at 4 °C. The resulting supernatant (plasma) was immediately transferred into clean polypropylene tubes and plasma aliquots were then stored at −80 °C until use.

Triglyceride, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) concentrations (in mg/dL) were measured using commercially available diagnostic kits supplied by SPINREACT laboratory (Girona, SPAIN). The Atherogenic index (AI) was calculated as divided LDL per HDL (Millan et al. 2009). The plasmatic level of insulin (in μU/mL) was determinate by ELISA kit supplied by ALPCO (Windham, NH, USA) and the Homeostasis model assessment for insulin resistance (HOMA-IR) and Pancreatic β-cell function (HOMA-β) were calculated as previously described (Matthews et al. 1985): HOMA-IR = [Insulin (µmol/L) × Glucose (mmol/L)/2.25], HOMA-β = [20 × Insulin (µmol/L)/ [Glucose (mmol/L) – 3.5]. Metabolic syndrome and diabetic rats were identified with blood glucose levels >200mg/dL, total cholesterol >110mg/dL, triglyceride >150mg/dL, change in body weight = 8% of initial weight and HDL levels <35 mg/dL (Suman et al. 2016).

Determination of the plasmatic level of testosterone.
Plasmatic level of testosterone (in ng/mL) was determinate by ELISA kit, specific for rat, supplied from Demeditec (Germany, DEV9911) according to the manufacturer’s instructions.

Tissue preparation and sample analysis. Pairs of testis and epididymis were removed, cleared from adhering tissue and weighed immediately using an electronic analytical balance (Mettler Toledo, AB 135 S). The gonadosomatic index (GSI) was subsequently calculated: GSI = [testis weight/body weight] × 100 (Bello et al. 2014). The right epididymis was immediately placed in Roswell Park Memorial Institute (RPMI) medium (ensuring the survival of sperm) and was used for sperm parameters evaluation, while the left one was stored at −80 °C and used for biochemical analysis. The right testis was kept in 10% formal saline, while the left testis was stored at −80 °C until biochemical molecular analysis.

Evaluation of sperm parameters

Epididymal sperm concentration and motility. One single cauda epididymis was placed in Roswell Park Memorial
Institute (RPMI) medium in a Petri dish and then shredded using a needle in a glass cup. After 10 min incubation at ambient temperature, well-dispersed sperm was recovered as the stock solution. The cauda epididymal sperm count was performed according to the method of Vega (Vega et al. 1988). Sperm solution was diluted to 1/5th, and then counted using Neubauer counting chamber light microscope (Olympus, type CX23). Only spermatozoa heads found on the grid were counted. Each sample was counted twice and averaged. Sperm concentration was expressed as number $10^6$/mL.

Motility was evaluated under light microscope at 400x magnification within 4–6 min of sperm isolation from the cauda. Estimations were performed from three different fields in each sample.

Sperm motility was presented as percentage of progressive (rapid and slow) and no progressive spermatozoa.

**Sperm vitality.** Sperm vitality study was assessed using the Vita-Eosin kit purchased from RAL diagnostics laboratories (France, ref. 380420). The staining was performed with one drop of fresh semen into two drops of staining solution on a microscope slide. Using another slide, a smear was made and allowed to dry. Unstained (intact) and red-colored (with damaged membranes) spermatozoa were counted under the light microscope at 100x magnification. Sperm viability was defined as the percentage of intact cells (Eliasson 1977).

Counting was performed from three different slides in each samples, on a total of 150 spermatozoa and was averaged.

**Sperm morphology.** Determination of abnormal sperm morphology was performed by the Spermo-scan staining kit provided by RAL diagnostics laboratories (France, ref. 366510–0000).

Sperm morphological alterations were evaluated and examined under a light microscope (100x objective). Sperm samples were obtained from the cauda of one single epididymis of the two groups of rats. One drop of the solution was smeared onto a glass slide and allowed to air-dry. The smears were stained by the spermo-EOSINE-RAL and spermo-FIX-RAL and fixed by spermo-EOSINE-RAL and spermo-FIX-RAL. Sperm morphological alterations were evaluated and examined under light microscope and the percentage of abnormal spermatozoa was obtained. The classification of morphological abnormalities consisting of the head and tail of the spermatoza was performed according to the modified descriptions, based on (Filler 1993) and (Miranda-Spooner et al. 2016) adapted for the experimental model used. The abnormal characteristics considered were as follows: (1) shape of spermatozoa head; (2) defects in tails; (3) multiple abnormalities in head and tails.

**Markers enzymes for functional evaluation of testes.** An aliquot of supernatant of testis homogenised in phosphate buffer (Ice-cold; 0.1 M; pH 7.4) was used for determining the activities of acid phosphatase (ACP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) using kits supplied from Bio Maghreb (Tunis), lactate dehydrogenase (LDH) by a kit supplied from Biosystems (France), all according to the manufacturer's instructions. Enzyme activities were expressed in International Units per Litre (U/L).

**Oxidative stress parameters.** Supernatants of testis homogenised in phosphate buffer (Ice-cold; 0.1 M; pH 7.4) were taken for the analysis of malondialdehyde (MDA) and oxidative related parameters.

Lipid peroxidation (LPO) was evidenced by measuring the formation of malondialdehyde (MDA) using the method of Buege and Aust (Buege and Aust 1978), and expressed in nmol per mg protein. Superoxide dismutase (SOD) activity was determined by the inhibition of epinephrine auto-oxidation in an alkaline medium (pH = 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD according to Misra and Fridovich (Misra and Fridovich 1972). Catalase (CAT) activity was estimated from the ability of the tissue to decompose H$_2$O$_2$, whose concentration can be followed at 240 nm (Aebi 1984). Total protein content was determined spectrophotometrically by Biuret reaction according to Lowry (Lowry et al. 1951), using bovine serum albumin (BSA) as the standard (Bio Maghreb).
### Primers sequences, temperatures, PCR efficiency and correlation coefficients $r^2$ determined for real time quantitative PCR.

<table>
<thead>
<tr>
<th>Gene Full name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>PCR efficiency (%)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>5'-CAGGAAGGCTGGTCCATAGG-3'</td>
<td>5'-ATGAAGGGGTCGTTGATGG-3'</td>
<td>104.00</td>
<td>0.998</td>
</tr>
<tr>
<td>HPRT1</td>
<td>5'-TTTCTCCTGGCTGTCTCTGAA-3'</td>
<td>5'-CATATTTGTTTGGGGCAGGT-3'</td>
<td>96.00</td>
<td>0.995</td>
</tr>
<tr>
<td>Bax</td>
<td>5'-TTTCTCCTGGCTGTCTCTGAA-3'</td>
<td>5'-CATATTTGTTTGGGGCAGGT-3'</td>
<td>104.33</td>
<td>0.992</td>
</tr>
<tr>
<td>Bcl2</td>
<td>5'-TTTCTCCTGGCTGTCTCTGAA-3'</td>
<td>5'-CATATTTGTTTGGGGCAGGT-3'</td>
<td>99.25</td>
<td>0.902</td>
</tr>
<tr>
<td>CYP11A1</td>
<td>5'-GTCGTCAATCTCTGGGCACT-3'</td>
<td>5'-CTCTGCGTGGGTGTAATGAG-3'</td>
<td>96.84</td>
<td>0.999</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>5'-ACCGCCGATGAGTTTGTT-3'</td>
<td>5'-GGGTGGTGCTGCTGTAGA-3'</td>
<td>100.28</td>
<td>0.995</td>
</tr>
<tr>
<td>17$\beta$HSD</td>
<td>5'-ACGGAATGAGATGGAAG-3'</td>
<td>5'-CCAGTAGACAAGGTCGAA-3'</td>
<td>102.97</td>
<td>0.984</td>
</tr>
</tbody>
</table>

### Results

#### Metabolic syndrome parameters

**Determination of anthropometric parameters and blood glucose level**

Blood glucose level in MetS group was significantly higher, as compared to control group rats at 4th (155.30 ± 5.48 mg/dL vs 104.00 ± 4.71 mg/dL) and 10th week (206.00 ± 3.27 mg/dL vs 105.70 ± 3.88 mg/dL, $p < .001$).

The MetS group showed significant increase in Body Mass Index on 4th (1.23 ± 0.06g/cm$^2$ vs 0.88 ± 0.03g/cm$^2$, $p < .001$) and 10th (1.34 ± 0.04g/cm$^2$ vs 0.94 ± 0.06g/cm$^2$, $p < .001$) week, as compared with control group. Similarly, the Body Weight Gain of the MetS group also increased significantly at 4th (28.11 ± 3.74% vs 8.57 ± 1.41%, $p < .001$) and 10th (31.56 ± 2.25% vs 12.04 ± 1.06%, $p < .001$) week, as compared to control rats. Abdominal circumference was significantly increased in MetS rats, compared to control rats at similar times points 4th (19.75 ± 0.92 cm vs 16.90 ± 0.35 cm, $p < .001$) and 10th (20.50 ± 1.02 cm vs 17.13 ± 0.44 cm, $p < .01$).

**Determination of biochemical parameters**

Triglyceride (162.00 ± 5.15 mg/dL vs 100.80 ± 7.56 mg/dL, $p < .001$), low-density lipoprotein (63.00 ± 6.48 mg/dL vs 41.94 ± 3.05 mg/dL, $p < .05$), and atherogenic index (1.91 ± 0.29 vs 1.11 ± 0.12, $p < .05$) were significantly increased in MetS group, as compared with control group at the end of the 10 weeks (Table 3).

Plasmatic insulin (16.72 ± 0.97 mU/mL vs 5.99 ± 0.72 mU/mL, $p < .001$), HOMA-IR (7.03 ± 0.99 vs 1.59 ± 0.36, $p < .001$) and HOMA-β (38.41 ± 2.57 vs 24.95 ± 0.86, $p < .05$) were significantly increased in rats fed with high-sugar and high-fat diet for 10 weeks, while high-density lipoprotein (32.76 ± 2.32 mg/dL vs 41.68 ± 0.97 mg/dL, $p < .001$) was significantly reduced in MetS group compared to control group rats (Table 3).

**Testicular weights and sperm function**

MetS caused a decrease in testicular weight (2.53 ± 0.05g vs 2.81 ± 0.06g $p < .01$), and gonad somatic index (0.54 ± 0.02% vs 0.77 ± 0.02%, $p < .001$) compared with the control group (Table 4).
Analysis of spermatozoa extracted from the cauda epididymis revealed that sperm concentration in MetS group was significantly decreased (26.45 ± 2.97 × 10^6/mL vs 46.77 ± 5.27 × 10^6/mL, p < .01, Table 5). Percentages of sperm viability (30.57 ± 5.36% vs 100.00 ± 21.32%, p < .01) and motility (32.65 ± 1.65% vs 48.80 ± 3.75% p < .01), were also significantly lower in the MetS group than in the control group (Table 5).

Abnormalities of the sperm head and tail were significantly higher in the MetS rats compared to control group (32.17 ± 3.86% vs 22.08 ± 1.53%, p < .05, Table 5).

Metabolic Syndrome rats showed head and tails abnormalities (Figure 2): Banana-shaped head spermatozoa (Figure 2A), spermatozoa head detachment from its tail (Figure 2D), coiled head (Figure 2B) and angular, bent and broken tails (Figure 2E). Thus, MetS impaired sperm quality and quantity.

Effects of metabolic syndrome on testicular marker enzymes

All testicular marker enzymes activities are shown in Table 6. MetS reduced the testicular activity of LDH (290.60 ± 91.74 U/L vs 641.30 ± 109.70U/L, p < .05), ALP (211.40 ± 40.05 U/L vs 380.00 ± 58.04 U/L, p < .05), ACP (58.09 ± 9.04 U/L vs 149.30 ± 29.73 U/L p < .05) but increased activity of AST (13.53 ± 1.15 U/L vs 7.26 ± 2.10 U/L < p < .05), ALT (140.70 ± 15.55 U/L vs 69.77 ± 13.91 U/L < p < .01), as compared to the control group.

Table 3. Metabolic parameters in the experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR</th>
<th>MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>100.80 ± 7.56</td>
<td>162.00 ± 5.15***</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.68 ± 0.97</td>
<td>32.76 ± 2.32**</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>41.94 ± 3.05</td>
<td>63.00 ± 6.48*</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>5.99 ± 0.72</td>
<td>16.72 ± 0.97***</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.56 ± 0.36</td>
<td>7.03 ± 0.99***</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>24.95 ± 0.86</td>
<td>38.41 ± 2.57*</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>1.11 ± 0.12</td>
<td>1.91 ± 0.29*</td>
</tr>
</tbody>
</table>

Effects of metabolic syndrome on oxidative stress parameters

Table 7 showed that MetS group presented a reduction in catalase (0.20 ± 0.02 U/mg of proteins vs 0.33 ± 0.03 U/mg of proteins, p < .05) and SOD activities (0.60 ± 0.14 nmol/min/mg of proteins vs 1.54 ± 0.18 nmol/min/mg of proteins, p < .01) but the level of MDA increased (1.49 ± 0.09 nmol/mg of proteins vs 0.80 ± 0.12 nmol/mg of proteins, p < .001) in rat testis, as compared to control rats.

Effects of metabolic syndrome on mitochondria-dependent apoptotic pathway

Bcl-2 mRNA expression level in the testis of MetS rats was significantly lower (p < .05), while Bax mRNA expression was significantly higher compared with the control group (p < .05) (Figure 3).

Table 4. Testis and epididymal weights of the experimental rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR</th>
<th>MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular weight (g)</td>
<td>2.81 ± 0.06</td>
<td>2.53 ± 0.05**</td>
</tr>
<tr>
<td>Gonadosomatic index of testis (%)</td>
<td>0.77 ± 0.02</td>
<td>0.54 ± 0.02***</td>
</tr>
<tr>
<td>Epididymal weight (g)</td>
<td>0.67 ± 0.03</td>
<td>0.53 ± 0.04*</td>
</tr>
<tr>
<td>Gonadosomatic index of epididymis (%)</td>
<td>0.18 ± 0.01</td>
<td>0.11 ± 0.01***</td>
</tr>
</tbody>
</table>

Table 5. Effects of Metabolic Syndrome on sperm quality.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR</th>
<th>MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatozoa count (×10^6/mL)</td>
<td>46.77 ± 5.27</td>
<td>26.45 ± 2.97***</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>48.80 ± 3.75</td>
<td>32.65 ± 1.65**</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>27.98 ± 3.16</td>
<td>16.75 ± 3.54*</td>
</tr>
<tr>
<td>No progressive motility (%)</td>
<td>20.81 ± 2.09</td>
<td>15.90 ± 3.61</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>100.00 ± 21.32</td>
<td>30.57 ± 5.36***</td>
</tr>
<tr>
<td>Abnormal sperm (%)</td>
<td>22.08 ± 1.53</td>
<td>32.17 ± 3.86*</td>
</tr>
</tbody>
</table>

Table 2. Time course of changes in anthropometric parameters in the experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>CTR 4 weeks</th>
<th>CTR 10 weeks</th>
<th>MetS 4 weeks</th>
<th>MetS 10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (g/cm²)</td>
<td>0.87 ± 0.03</td>
<td>0.88 ± 0.03</td>
<td>0.94 ± 0.06</td>
<td>0.88 ± 0.04</td>
<td>1.23 ± 0.06***</td>
</tr>
<tr>
<td>BWG (%)</td>
<td></td>
<td>8.57 ± 1.41</td>
<td>12.04 ± 1.06</td>
<td>28.11 ± 3.74***</td>
<td>31.56 ± 2.25***</td>
</tr>
<tr>
<td>Abd. C. (cm)</td>
<td>16.63 ± 0.37</td>
<td>16.90 ± 0.35</td>
<td>17.13 ± 0.44</td>
<td>16.25 ± 0.67</td>
<td>19.75 ± 0.92*</td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± Std. Error. **p < .01, ***p < .001 control group (CTR) versus Metabolic Syndrome group (MetS).
Effects of metabolic syndrome on testosterone level and testicular steroidogenic enzymes gene expression

MetS rats presented a considerable reduction in plasmatic testosterone level, as compared to the control group (\( p < .05 \), Figure 4). Moreover, a marked decline in the mRNA expression of CYP11A1 (\( p < .05 \) Figure 5), CYP17A1 (\( p < .05 \) Figure 5) and 17\( \beta \)HSD (\( p < .05 \) Figure 5) was observed in the testis tissues of MetS rats, compared to the control group.

**Effects of metabolic syndrome on interleukin 1 and 6 receptors gene expression**

Interestingly, MetS promoted an up-regulation of IL-1R (\( p < .05 \)) and IL-6R mRNA expression (\( p < .001 \) Figure 6).

**Discussion**

In recent decades, MetS has become a public health problem across the world (Torgerson et al. 2004). MetS can be seen as a cause of adverse health outcomes that include male
infertility. The causes of MetS-impaired male reproduction remain unclear. Several animal models were directed to develop a suitable experimental model that would mimic all the symptoms of human Metabolic syndrome and to screen the potential pathogenesis compounds. Although, to our knowledge, no experimental model investigated the effects of MetS on male fertility.

In order to bring new insights regarding male reproduction impairments due to MetS, we successfully established an animal MetS model using a high-sugar, high-fat diet, which closely reflects the natural history and characteristic of MetS. While developing the model, it was kept in mind that it should be less expensive, easily available, taking short periods for development and displaying the various components.

In fact, MetS rats treated with a high-sugar and high-fat diet possess most components of MetS such as central obesity, hyperglycemia, insulin resistance, an increase of triglyceride and a decrease of HDL-Cholesterol. Metabolic Syndrome and diabetic rats were identified with blood glucose level >200mg/dL, total cholesterol >110mg/dL, triglyceride >150mg/dL, change in body weight = 8% of initial weight and HDL levels <35 mg/dL (Suman et al. 2016).

The present study demonstrated that MetS caused abnormalities in sperm quality, testicular marker enzymes activities, associated with a decrease in testosterone level and a downregulation of specific steroidogenic enzymes mRNA in the testis of MetS rats. We also highlighted an increase in oxidative stress and inflammation.

First of all, we found that sperm count, viability and motility decreased, and abnormal sperm count increased in the Mets group. Previous studies in obesity and diabetes mellitus are consistent with our findings (Belhan et al. 2020, Jia et al. 2018) and a recently human study firmly established that MetS and its components exhibited deleterious effects on semen quality (Chen et al. 2020).

Endocrine disorders are key features of spermatogenesis dysfunction (Plant and Marshall 2001), testosterone playing a pivotal role in testicular development, normal masculinisation and the maintenance of spermatogenesis (Arver et al. 1996, Sharpe et al. 1992).
In our findings, testosterone levels in the MetS group were lower than in the control group. Our results regarding the testosterone level in the MetS group are compatible with previous studies in obese and diabetic male rats (Fatani et al. 2015, Xiang et al. 2018). The decrease in the testosterone levels may be due to injury in the Leydig cells, where it is secreted. A previous mouse studies reported that obesity (Haffner et al. 1993) and diabetes mellitus (Saad 2009, Traish et al. 2009) had harmful effects on pituitary-testis axis and led to excessive oxidative stress and the degeneration of Leydig cells (Ballester et al. 2004). As oxidative stress affects Leydig cells (Zhou et al. 2013, Shen et al. 2014), it may be presumed that MetS induced an early depression of testosterone level in our study.

In the present investigation, it was needed to ascertain the possible mechanism of the disturbance testosterone biosynthesis following the MetS treatment. However, the decline in testosterone levels formed a basis for the investigation of genes expressions of enzymes involved in steroidogenesis. Nevertheless, to our knowledge, there is no study evaluating the effects of MetS on testicular steroidogenesis at a molecular level. We highlighted a marked decrease in the activities of the key testicular androgenesis enzymes, CYP11A1 (involved in breakdown of cholesterol into pregnenolone), CYP17A1 (involved in the conversion of progesterone into androstenedione) and HSD17B6 (involved in the conversion of androstenedione into testosterone), which may explain the machinery underlying the decreased of testosterone production in the testis of MetS rats. Inhibition of these genes might downregulated testosterone biosynthesis. Additionally, testicular testosterone is essential for the

Figure 6. Effect of Metabolic Syndrome on the gene expression of inflammation factors in the testis determined by qRT-PCR. Data are expressed as the mean ± Std. Error (n = 8). Gene expressions were normalised to the house-keeping genes. The control value for each gene was considered 1 (100%), and the expression in the MetS group was normalised to the control value and expressed as fold change. *p < .05, **p < .01, control group (CTR) versus Metabolic Syndrome group (MetS).

Figure 7. Hypothetical mechanisms explaining MetS disruption of spermatogenesis and steroidogenesis.
normal spermatogenesis, as well as for the maintenance of the normal structural morphology and physiology of seminiferous tubules.

To our knowledge, our study is the first to reveal the effect of MetS on testicular marker enzymes. Our findings revealed a remarkable decline in testicular ACP, ALP and LDH activities in rats feeding with a high-glucidic and hyperlipidic diet. ACP is one of the specific markers of dyszoosperma associated with the alteration of seminiferous epithelium and phagocytosis of Sertoli cells (Samarth and Samarth 2009, El-Kashlan et al. 2015). LDH is involved in the maturation and energy metabolism of spermatogenic cells and spermatozoa (Rizk et al. 2014). ALP plays critical role in spermatogenic cells glucose uptake and division (Yan et al. 2010). Thus, MetS induced reduction in these enzymes’ activity indicating an alteration in the testicular physiology. A parallel finding demonstrating that the decrease of these activities in testicular homogenate is directly associated with oxidative stress in rat testis (Olayinka and Ore 2015). Also, MetS induced rising of AST and ALT activities that is usually associated with rat testis (Olayinka and Ore 2015). ALP plays critical role in spermatogenic cells glucose uptake and division (Yan et al. 2010). Thus, MetS induced reduction in these enzymes’ activity indicating an alteration in the testicular physiology. A parallel finding demonstrating that the decrease of these activities in testicular homogenate is directly associated with oxidative stress in rat testis (Olayinka and Ore 2015). Also, MetS induced rising of AST and ALT activities that is usually associated with reduced integrity of spermatozoa membrane and frequency of intact acrosome spermatozoa (Yang et al. 2010).

Our results showed that MetS induced a drop of the antioxidant capacity (decreased CAT and SOD activities) and a higher lipid peroxidation (MDA, as an indicator of lipid per-oxidation LPO) in the testis tissues of MetS rats. It is noteworthy that oxidative stress has a tremendous role in the pathophysiology of reproductive dysfunction (Sahoo et al. 2005, Sahoo et al. 2008) and it has been reported that testicular oxidative stress is a key features of spermatogenesis abnormality (Giribabu et al. 2014). Reactive oxygen species (ROS) play a vital role in maintaining a number of physiologic process in male reproductive system (Riley and Behrman 1991) and are essential for detoxification, energy supply, and immune function (Aruoma 1998). Although, over production of ROS leads to oxidation of cellular biomolecules such as lipids, proteins and DNA (De Lamirande and Gagnon 1995, Aruoma 1998). The harmful effects of MetS may result in the generation of free radicals and the suppression of antioxidant enzyme activities. There is more evidence that obesity, metabolic disorders, and diabetes mellitus increase the reactive oxygen species formation (Tunc et al. 2009, Fang et al. 2012, Pomjunta et al. 2017).

Defective spermatogenesis detected in MetS group can be attributed to ROS increase. Indeed, spermatozoa are vulnerable to oxidative stress (Rashid and Sil 2015). The elevated MDA level may be partly attributed to the high content of polyunsaturated fatty acids in the male germ cells membranes (Sahoo et al. 2008, Ben Abdallah et al. 2013), thus, rendering them particularly susceptible to LPO. So, alterations of the membrane structure, decrease motility, morphological abnormalities and efficiency of spermatozoa production and lower viability may be caused by LPO. According to a previous study (Yeh et al. 2005), the decrease in the antioxidant defense molecules is due to LPO accumulation. SOD is a major antioxidant enzyme that can eliminate harmful ROS in male reproductive organ. The alteration in the activity of testicular SOD might lead to growth arrest and impaired function of the testis and spermatogenesis, which might contribute to severe oxidative damage of the spermatozoa cellular membrane resulting in the impaired motility and the increased abnormal sperm rates.

The oxidative stress status induced by MetS may result in considerable testicular DNA damage and apoptosis that may trigger further testicular dysfunctions. In addition, it is known that spermatogenesis is a complex process that relies on coordinated cell proliferation and apoptosis (Han et al. 2008). MetS rats presented increased pro-apoptotic Bax and decreased anti-apoptotic Bcl-2 expression in their testis tissues. In the view of our results, it was hypothesised that MetS induced reproductive damage by apoptosis (Excessive apoptosis is reported to be a prevalent phenomenon in defective spermatogenesis) in testis, underlying oxidative stress mechanisms.

Notably, it has been reported that the impairment of the sperm parameters in obese and diabetes models is associated with inflammatory reactions (Wang et al. 2018), which play an important role in male infertility (Mogaddami et al. 2018). In this model, MetS led to inflammatory responses in the testis involving increase of expression IL-6R mRNA and IL-1R mRNA (the receptors of IL-1 and IL-6 pro-inflammatory cytokines). Data from many studies indicate that IL-1 (Hink et al. 2018) and IL-6 (Li et al. 2018, Wang et al. 2018) are integrated in a complex network of endocrine and local regulatory mechanisms within the seminiferous epithelium. The results of the present study suggest that MetS promotes inflammation in testis and elevates IL-6R and IL-1R level leading to germ cells apoptosis. We hypothesise that higher expression of these receptors ultimately led to poor sperm quality and limited sperm fertility.

Hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote reactive oxygen species (ROS) accumulation which, in turn, promote cellular damage (Faria and Persaud 2017). ROS can directly damage lipids, proteins or DNA and modulate intracellular signalling pathways (Fiorentino et al. 2013). Conversely, increased levels of ROS can be detrimental to testicular function. Spermatogenesis and steroidogenesis changes as seen in this study are supported by the significant changes seen in the enzymes antioxidant defense systems as well as lipid peroxidation, which reflect increased ROS production.

We proposed this model (Figure 7), suggesting the potential relations between oxidative stress, apoptosis, inflammation and spermatogenesis and steroidogenesis disruption.

Conclusion

MetS induced severe testis toxicity in male rats. MetS markedly distorted sperm parameters, inhibited the transcription of steroidogenic enzymes and led to oxidative stress, inflammation and dysfunction of Bax/Bcl-2 mediated cell death pathway.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, N.G.

References


