Association Between Nonalcoholic Fatty Liver Disease and Severe Male Reproductive Organ Impairment (Germinal Epithelial Loss): Study on a Mouse Model and on Human Patients

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Abstract
Metabolic syndrome (MS) has been associated with testicular damage. Nonalcoholic fatty liver disease (NAFLD) is a multisystemic disease that affects different organs, but its effect on the testes is unknown. A study analyzing germ cell involvement on BALB/c mice was carried out. A parallel comparative study was conducted that investigated alterations in the germinal epithelium of male humans that died from an unrelated acute event. The complete medical histories and histologic samples of the thoracic aorta, liver tissue, and testicular tissue from the deceased subjects were collected. The degree of germinal epithelial loss (DGEL) was evaluated and the clinical and histologic data were compared between individuals with and without NAFLD. The only metabolic or morphologic variable that caused a significant difference in the DGEL, in both the animal model and humans, was the presence of liver steatosis. The percentage of steatosis was also correlated with the percentage of the DGEL. In humans, steatosis (greater than 20%) increased the risk 12-fold for presenting with a severe DGEL (OR: 12.5; 95% CI [1.2, 128.9]; \( p = .03 \)). There was no association with age above 50 years or MS components. Steatosis grade was also correlated with atherosclerosis grade. NAFLD was a strongly associated factor implicated in severe DGEL, as well as the testis was identified as a probable target organ for damage caused by the disease. This finding could result in the search for new approach strategies in the management of men with fertility problems. Further studies are required to confirm these results.

Keywords
germin cells, testis, steatosis, atherosclerosis, metabolic syndrome

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Diseases related to poor alimentary habits and a sedentary lifestyle, such as metabolic syndrome (MS) and nonalcoholic fatty liver disease (NAFLD), have become a worldwide public health problem (Murillo-Zamora et al., 2016). Both conditions are risk factors for cardiovascular disease. MS consists of the presence of three or more comorbidities, including high fasting glucose (insulin resistance), high blood pressure, hypertriglyceridemia, hypercholesterolemia, or abdominal obesity. Its prevalence varies from 10% to 30% of the world population, with a greater prevalence in Western countries (Han & Lean, 2016). NAFLD is the most common cause of
chronic liver disease in the developed world and it encompasses a spectrum that ranges from simple steato-
sis to steatohepatitis, fibrosis, or cirrhosis. According to
the United States National Health and Nutrition Exami-
nation Survey (1988–1994) study (Lazo et al., 2013), the prevalence of NAFLD in men <30 years old
was about 7.4% and for men 30–40 years old was 13.3%.
The overall prevalence of NAFLD varies between 20% and 50% in Western countries (Fotbolcu & Zorlu, 2016).

NAFLD has traditionally been considered the hepatic
manifestation of metabolic syndrome, due to the close
association between NAFLD and the various component
features of MS. Many epidemiologic studies have demon-
strated an association between NAFLD and metabolic
syndrome (Wainwright et al., 2016). Approximately 90% of
NAFLD patients present with more than one compo-
nent of MS, and about 33% of the patients meet the crite-
rion for MS (Masarone, Federico, Abenavoli, Loguercio, &
Persico, 2014). MS and NAFLD are beginning to be
thought of as separate entities, in which insulin resistance
is the central pathophysiologic process common to both
conditions. There is growing evidence that the relation-
ship between NAFLD and metabolic syndrome is bidirec-
tional, in that NAFLD can predispose to metabolic
syndrome features, which, in turn, can exacerbate NAFLD
or increase the risk for its development in those patients
without a preexisting diagnosis (Masarone et al., 2014). It
has recently been postulated that NAFLD should be con-
sidered not only a liver-specific disease, but also an early
mediator of systemic diseases (Fotbolcu & Zorlu, 2016).

Most of the studies on the general population that search
for causes of male reproduction disorders attempt to asso-
ciate testicular alterations with MS or its components.
There are reports that MS is associated with decreased fer-
tility parameters in males (Leisegang et al., 2016). Obesity
is associated with low levels of testosterone and with sub-
fertility or infertility (Fan et al., 2015). Despite all these
data, the association between MS or its comorbidities with
spermatogenic disorders has not been fully established. A
large part of the experimental or morphologic information
comes from animal models that do not necessarily accu-
rate reflect what may occur in humans. Epidemiologic
association studies have produced controversial results.

Ventimiglia et al. (2016) recently carried out an analysis
with statistical adjustments to avoid interpretation confu-
sion (multivariate logistic regression analysis) and con-
cluded that semen parameters and the rate of either
obstructive or nonobstructive azoospermia had no associa-
tion with MS.

Perhaps the presence or not of MS or any of its com-
ponents is not the determining factor for presenting with
sperm production disorders. NAFLD has recently been
suggested to be a multisystemic disease that affects dif-
ferent organs. It most likely affects the testes, becoming a
determining factor for germinal epithelial damage. To
analyze this, testicular damage in a mouse NAFLD model
was evaluated, as well as the clinical data (MS compo-
nents) and histologic aspects of the liver, arteries, and tes-
tes in deceased human patients. This was done to
determine which clinical or morphologic characteristic
was conclusively correlated and associated with testicu-
lar germinal epithelial loss.

Materials and Methods

Mouse Model Study

An experimental study on a mouse model to determine
the relation between nonalcoholic fatty liver disease and
germinal epithelial damage was carried out.

Two groups of 6- to 8-week-old male BALB/c mice
(Harlan, Mexico), weighing 25–30 g, were included in
the experiment. The control group of mice (n = 16) was
fed a standard diet (the SD group) (2018S Tekl and Global
18% Protein Rodent Diet, Harlan®, KY) and the NAFLD
group (n = 13) was fed a high-fat diet (Atherogenic
Rodent Diet, TD.02028, Harlan, KY). This model has
been reported to be capable of the simultaneous produc-
tion of steatohepatitis (NAFLD) and preclinical ather-
sclerosis within a period of 6 months (Garcia-Rivera
et al., 2014; Madrigal-Perez et al., 2015).

The mice were kept in cages, with a maximum of 5
mice per cage. Light and temperature were controlled and
the animals had access to food and water ad libitum. The
mice were fed the diet for a period of 6 months, after
which they were decapitated. Samples of the testis, liver,
and thoracic aorta were extracted and processed for their analysis. Whole blood samples were collected and biochemical analyses were performed. The protocols for experiments on animals were approved by the Research Ethics Committee of the School of Medicine of the Universidad de Colima, Mexico. The animals were manipulated according to institutional guidelines, in addition to the Mexican Official Norm regulating laboratory animal use (NOM-062-ZOO-1999) and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences.

**Human Study**

A parallel study was conducted to carry out the association analysis. Deceased patients that underwent autopsy at the Pathology Department of the Hospital Universitario “Dr. José Eleuterio González” in Monterrey, Mexico, within the time frame of January 2014 to December 2015 were studied. The simultaneous analysis included 29 men with the following inclusion criteria: age >30 years old with completed medical records, especially those patients with analysis of histologic tissue samples of the thoracic aorta, right lobe of the liver, testis, as well as the cause of death related to an acute event that did not affect the morphology of the tissues analyzed. Sixty-nine percent of the patients died from complications from acute cerebral edema secondary to trauma, 21% from acute infection of the central nervous system or lung, and 10% from hypovolemic and hemorrhagic shock. Patients with cancer, alcoholism, drugs uptake, autoimmune diseases, chronic kidney disease, liver trauma, acute or chronic infections affecting the liver were excluded. The clinical data of the patients were obtained from their medical records. All the subjects were having outpatient family medicine, internal medicine, or geriatric consultations before presenting with the event causing their deaths. The diagnoses of type 2 diabetes mellitus (DM), high blood pressure, and obesity (body mass index ≥30) were confirmed by medical specialists from those departments. The main clinical characteristics of the studied patients are shown in Table 1. The individuals were divided into two groups, depending on the presence or absence of one of the vascular comorbidities that make up MS or on the histologic alterations of NAFLD or aortic atherosclerosis (see Table 2). The Health Research Ethics Board of the School of Medicine of the Universidad Autónoma de Nuevo León approved that part of the study.

**Histopathologic Analysis**

All of the tissue specimens were fixed, processed, and embedded in paraffin to form blocks. Histologic sections of 5 microns were prepared and stained with hematoxylin and eosin, according to the standard staining technique. The Masson trichrome stain was used for evaluating fibrosis. Tissue samples of the liver, thoracic aorta, and testis from both the animal model and human patients were included and analyzed. The evaluations of the slices were carried out through images taken with an Axiocam MRC-5 model digital camera (Zeiss®, Germany) attached to an AxioPlan 2 M model bright field optical microscope (Zeiss, Germany) with a motorized stage and A-plan ×5 and ×20 objective (total magnification ×50 for the aorta and ×200 for the liver). Images of the entire sample surfaces were scanned using MosaicX and

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**Table 1. Main Clinical Characteristics of Human Patients.**

| Clinical characteristic          | N = 29          |
|----------------------------------|-----------------|
| Age mean (years)                 | 45.3 ± 9.4      |
| Age range (years)                | 31–60           |
| BMI                              | 29.0 ± 8.9      |
| Healthy weight                   | 34.5%           |
| Overweight                       | 34.5%           |
| Obesity                          | 31.0            |
| Diabetes                         | 41.4            |
| High blood pressure              | 20.7            |
| Hyperlipidemia                   | 17.2            |
| Metabolic syndrome               | 31.0            |
| Smoking (%)                      | 13.8            |

Note. Percentages or averages and standard deviation are shown. *Diagnosed in accordance with the guidelines of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults “ATP III.” BMI = body mass index.

**Table 2. DGEL According to the Presence or Absence of Relevant Clinical or Morphologic Characteristics in Human Patients and According to the Group in Mice.**

| Characteristic (n) | DGEL | Human patients | Absence | Presence | p  |
|-------------------|------|----------------|---------|----------|----|
| Type 2 diabetes (12) |     | 63.1±16 | 64.7±15.2 | .79 |
| High blood pressure (6) |     | 66.0±14 | 51.2±16.1 | .08 |
| Obesity (9)         |     | 59.1±17 | 66.2±14.9 | .27 |
| Steatosis (14)      |     | 55.0±15.5 | 71.0±14.6 | .02c |
| Liver inflammation (12) | | 62.7±17.0 | 60.6±13.9 | .76 |
| Atherosclerosis (15) |     | 60.0±14.1 | 72.9±14.0 | .03c |
| Age above 50 years (12) | | 65.7±11.0 | 60.7±21.8 | .43 |
| MS* (9)             |     | 63.3±15.3 | 65.3±17.7 | .78 |
| Mouse model group   |     | Control | NAFLD | 12.3±8.2 | 32.2±14.1 | .000c |

Note. *(n): Individuals with presence of the characteristic of a total sample of 29 human patients. *Steatosis in more than 20% of the liver tissue. *Statistically significant. *Liver inflammation in 33% or more of the liver tissue. *Grade IV atherosclerosis, or higher. *Metabolic syndrome. DGEL = degree of germinal epithelial loss; MS = metabolic syndrome.
Autofocus modules. All the shots were taken under the same conditions of light and exposure. The analyses were done in a blinded manner by one pathologist.

The evaluation of germinal epithelial loss was carried out in the previously described manner. Ten randomly selected, essentially round cross sections of seminiferous tubules from each sample were classified into one of eight different grades, indicating the relative severity of seminiferous epithelial abnormalities. Briefly, grade 0—normal intact seminiferous epithelium; grade 1—seminiferous epithelium with pyknotic germ cells and desquamation or focal vacuolation; grade 2—seminiferous epithelium between grades 1 and 3; grade 3—seminiferous epithelium with pre-meiotic germ cells and Sertoli cells; grade 4—Sertoli cells only; grade 5—no seminiferous epithelium, leaving only the basement membrane; grade 6—seminiferous tubule with sperm stasis, sperm granuloma, or mineralization; grade 7—fibrosis of the seminiferous tubule. A weight between 0 and 1, to reflect the relative absence of germ cells, was assigned to each grade—0, 1/4, 2/4, 3/4 to grades 0, 1, 2, 3, respectively, and 4/4 to grades 4–7. The degree of germinal epithelial loss (DGEL) was calculated by multiplying the percentage of tubules in each grade by the respective assigned weight and summing the products (Higuchi, Palmer, Gray, & Veeramachaneni, 2003).

Steatosis was considered in relation to the percentage of liver tissue with fat accumulation. Three cross-sectional liver slices were analyzed, two from the right lobe (central and external third region). The percentage of liver tissue with inflammation was evaluated by functional histologic zones, according to the oxygen supply (zone 1 encircles the portal tracts where the oxygenated blood from the hepatic arteries enters; zone 3 is located around the central veins, where oxygenation is poor; zone 2 is located between zones 1 and 3). There were four categories with respect to the percentage of tissue presenting with inflammatory infiltrate: none, mild, moderate, and severe (0%, up to 33%, 34%–66%, and more than 66%, respectively) (Haddad, Vallerand, Brault, Spenard, & Haddad, 2011).

To evaluate atherosclerosis, only the slice qualitatively showing the lesion with the highest grade of disease, according to the Stary classification (grades I–VI) (Stary, 2000), was selected and a blinded analysis was carried out by one pathologist. The same slice was quantitatively evaluated by measuring the intima-media thickness (from the interior edge of the endothelium to the exterior edge of the middle layer). This was done at eight equidistant sites per section, selected through systematic uniform random sampling, regardless of the presence or absence of atherosclerotic lesions at the measuring site (Garcia-Rivera et al., 2014; Madrigal-Perez et al., 2015).

Data Analysis

The comparative analysis of continuous clinical data between groups was carried out using the Student’s t-test for the numerical data with normal distribution and the Mann–Whitney U test for the qualitative data or the numerical data that did not have normal distribution. The categorical variables were compared using the Fisher’s exact test. A Pearson product-moment correlation test was done to evaluate the correlation between pairs of clinical or histologic variables in the study groups. The association between any of the MS vascular comorbidities, age, steatosis, liver inflammation, or atherosclerosis, and the risk for severe germinal epithelial loss (equal to or greater than a 66% loss) was estimated by odds ratios (OR) and 95% confidence intervals (CI) (Crosstabs procedure) using the Mantel–Haenszel test. Statistical analysis was performed with SPSS v. 20 software (IBM, Armonk, NY) and the statistical power of the case-control human study (Table 5) was computed by an on-line power calculator (http://sampsize.sourceforge.net/iface/s3.html#ccp) developed by Philippe Glaziou. The estimated statistical power for the association between steatosis (≥20% of liver cells with fat deposition) and severe degree of germinal epithelial loss (≥66%) was 85.3%, using the following parameters: OR = 8; exposed controls = 27%; one-sided alpha risk =5%; controls/case ratio = 1.07; number of cases = 14 and number of controls = 15. p values < .05 were considered statistically significant.

Results

Table 2 reports that the presence of liver steatosis was the only metabolic or morphologic variable that caused a significant difference in the DGEL, in both the animal model and in humans (see Figures 1 and 2). In humans, having advanced atherosclerosis (Stary scale grade IV or higher) was a factor that significantly increased the DGEL. Age above 50 years or having MS, diabetes mellitus, or high blood pressure did not significantly increase the DGEL.

Tables 3 and 4 report that liver steatosis, again, was the only factor statistically correlated with DGEL in the animal model, as well as in humans. Other variables correlated with the DGEL were the grade of atherosclerosis in humans, and liver inflammation in the animal model. Other variables in humans that were correlated with each other were: aortic wall thickness with liver inflammation; atherosclerosis grade with age; and diabetes mellitus with the percentage of steatosis and with high blood pressure. Aortic wall thickness was correlated with the percentage of steatosis and liver inflammation in the animal model.

To estimate the degree of association between the risk factors and germinal epithelial loss, the human patients were divided into two groups: cases (severe germinal
**Figure 1.** Histologic images of human liver and testicular tissues. A and C are tissues from a patient with seminiferous tubules with conserved germinal epithelium (mild germinal epithelial loss -A-) showing the liver tissue with normal characteristics, and C) the absence of steatosis and inflammatory cells. B and D are images from another patient showing B) a seminiferous tubule that has severe germinal epithelial loss, and D) severe liver steatosis and inflammatory cell foci.

**Figure 2.** Histologic images of liver and testicular tissues from the mouse model. A and B are tissues from a mouse fed a balanced diet showing A) undamaged germinal epithelium in the seminiferous tubules and B) normal liver tissue characteristics. C and D are tissues from a mouse fed a high-fat diet showing C) a seminiferous tube with moderate germinal epithelial loss and reduced thickness, and D) a liver with severe steatosis (micro and macrodrip pattern) and inflammatory cell foci.
epithelial loss equal to or greater than 66%) and controls (germinal epithelial loss below that of the cases). Table 5 reports that in a raw analysis, steatosis and atherosclerosis increased the risk for presenting with severe germinal epithelial loss 8 and 6-fold, respectively. Moderate-to-severe inflammation of the liver, age above 50 years, or MS or any of its components (obesity, DM, or HBP) were not associated factors. When the analysis adjusting the variable of atherosclerosis or steatosis was performed, steatosis prevailed as the only significant risk factor, increasing the risk 12-fold for a severe DGEL.

**Discussion**

NAFLD was identified to be the main factor associated with severe germinal epithelial loss. This was demonstrated in an animal model and in a data analysis of human patients. It is striking that age and MS or its components were not
-associated or correlated with germinal epithelial damage. This last finding concurs with results from a study by Ventimiglia et al. (2016), in which they concluded that different anomalies related to germinal epithelium function were not associated with MS. Nevertheless, there are controversial reports on this (Leisegang et al., 2016).

It has recently been suggested that NAFLD is not only a liver-specific disease, but also an early mediator of systemic diseases (Fotbolcu & Zorlu, 2016). Compared with patients without NAFLD, those with the disease exhibit increased liver-related complications and liver-related mortality, as well as an increased risk for developing type 2 diabetes, cardiovascular disease (CVD), chronic kidney disease, an increased risk for postoperative complications after major liver surgery, and an increased risk for developing certain malignancies, including primary liver cancer and colorectal cancer (Lonardo, Sookoian, Chonchol, Loria, & Targher, 2013; Adams, Anstee, Tilg, & Targher, 2017). Ours is the first study to report that the testes (germinal epithelium) are one of the organs damaged by NAFLD’s multisystemic effect. Previous studies on animal models reported that certain liver alterations affected semen parameters. It was reported that paternal NAFLD in rats did not influence sperm morphology by decreasing testicular testosterone synthesis (Li et al., 2013). A study on the European eel (Anguilla anguilla) stated that the liver appears to play a role in determining sperm volume, through the production of certain fatty acids (Baesa et al., 2015).

NAFLD could cause damage to the germinal epithelium in numerous ways. In a recent meta-analysis of 27 studies, Polyzos et al. (2011) reported that lower circulating adiponectin levels were observed in patients with simple steatosis, compared with controls. Adiponectin has anti-inflammatory properties and its deficiency can trigger an increase in the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 (Kaser et al., 2015), and interleukin-1 alpha (IL-1 α) (Lonardo et al., 2013), among others. Physiologically, the immunosuppressive testicular microenvironment protects germ cells from immune attack, whereas under inflammatory conditions, tolerance is disrupted and immune cells and their mediators respond to germ cell self-antigens, inducing damage of the germinal epithelium (Pérez et al., 2012; Pérez et al., 2013). TNF-α and IL-6, both of which are elevated in NAFLD, have an essential role in testicular damage under conditions of inflammation. In vitro experiments reported that IL-6 induced germ cell apoptosis (Rival, Theas, Guazzone, & Lustig, 2015) and in vivo experiments induced focal inflammatory cell infiltration and germ cell sloughing in adjacent seminiferous tubules associated with alterations of tight junction protein expression and distribution (Pérez et al., 2012; Pérez et al., 2013). TNF-α, IL-1 α, and IL-1 β cytokines in the male reproductive tract (testis, epididymis, and sperm) may have certain physiologic functions. When the levels of these cytokines are higher than normal, as seen in conditions of inflammation, TNF-α, IL-1 α, and IL-1 β cytokines levels become very harmful to sperm production (Azenabor, Ekun, & Akinloye, 2015). High levels of TNF-α induced germ cell apoptosis in vitro. Neutralization of TNF-α with etanercept, a protein that mimics the inhibitory effects of soluble TNF receptors, reverted this apoptotic effect (Scallon et al., 2002). Concomitant with high levels of proinflammatory cytokines in the testicular microenvironment, nitric oxide (NO) production by macrophages increases. Ex vivo experiments have reported that NO released by testicular macrophages in rats with orchitis induced germ cell apoptosis. The presence of a competitive NOS inhibitor, L-NAME, reduced NO production by these cells and the percentage of seminiferous tubules with apoptotic germ cells (Pérez et al., 2012; Pérez et al., 2013).

Table 5. Relevant Clinical or Morphologic Characteristics and Their Associations with a Severe DGEL in Human Patients.

| Variable     | OR     | Raw 95% CI | p     | OR     | 95% CI | p     | OR     | 95% CI | p     |
|--------------|--------|------------|-------|--------|--------|-------|--------|--------|-------|
| Atherosclerosis-adjusteda |        |            |       |        |        |       |        |        |       |
| Steatosis    | 8.0    | [1.2, 51.5]| .03   | 12.5   | [1.2, 128.9]| .03   | -      | -      | -     |
| Athero       | 6.0    | [1.0, 35.3]| .04   | -      | -      | -     | 2.5    | [0.3, 21.4]| .38   |
| MS           | 1.3    | [0.2, 7.5]| .74   | 0.7    | [0.1, 6.8]| .78   | 0.2    | [0.01, 6.0]| .24   |
| Age ≥50 years| 1.6    | [0.3, 8.2]| .52   | 0.8    | [0.1, 6.6]| .86   | 1.8    | [0.3, 12.8]| .54   |
| Obesity      | 0.8    | [0.2, 3.9]| .78   | 1.5    | [0.2, 12.1]| .67   | 0.5    | [0.1, 3.8]| .53   |
| DM           | 3.0    | [0.6, 14.6]| .17  | 1.2    | [0.1, 9.1]| .86   | 0.5    | [0.1, 4.9]| .58   |
| HBP          | 0.25   | [0.02, 2.8]| .26  | 0.1    | [0.01, 2.2]| .15   | 0.1    | [0.01, 2.9]| .21   |
| Inflam       | 1.4    | [0.3, 7.7]| .67   | 0.9    | [0.1, 7.7]| .97   | 0.3    | [0.03, 3.6]| .37   |
| Note.        | 645    |            |       |        |        |       |        |        |       |

- Steat: ≥20% of liver cells with fat deposition
- Athero: degree of atherosclerosis ≥IV, according to Stary’s classification
- Obesity: body mass index ≥30
- Controlling other risk factors using a Mantel–Haenszel test
- DM = type 2 diabetes mellitus
- DGEL = degree of germinal epithelial loss
- Inflam: >33% of liver tissue with inflammation
- MS = metabolic syndrome
- HBP = high blood pressure
- OR = odds ratios
- 95% CI: confidence intervals
Inflammation is also associated with oxidative stress. In NAFLD, histopathologic disease severity significantly correlated with oxidative stress parameters (Koroglu et al., 2016). In addition, epidemiologic studies regarding male infertility have revealed that more infertile men suffer from acute or chronic inflammation of the genitourinary tract, which often occurs without any symptoms. The inflammatory reactions within the male genital tract are inevitably connected to oxidative stress. Oxidative stress is harmful, especially to spermatozoa, because it damages sperm DNA and causes sperm apoptosis (Azenabor et al., 2015). In animal models, drugs that reduce oxidative stress, such as doxycycline, have been reported to prevent testicular damage caused by a high-fat diet (Delgado-Enciso et al., 2014). Elevated oxidative stress parameters at the systemic level caused by NAFLD could be another cause of damage to the germinal epithelium. It is striking that in the analysis of the histologic alterations of the liver, steatosis was more relevant for presenting with germinal epithelial loss than the liver inflammation itself. The pathophysiology of NAFLD is complex and other mechanisms could be involved in testicular damage.

Age is an important factor in testicular health. However, the present study results did not correlate age with the DGEL. Studies on animals have reported that individuals with short life expectancies present with inflammatory, oxidative, and apoptotic testicular processes. In contrast, longevity confers anti-inflammatory, anti-oxidant, and anti-apoptotic capacities on the adult testis. Studies on humans have also reported that comorbidities and obesity strongly contribute to late-onset hypogonadism (Basaria, 2013). This concurs with the results of this study, in the sense that patients with NAFLD have a shorter life expectancy and a systemic inflammatory state (Matzkin et al., 2016), accompanied with accelerated testicular damage. This suggests that testicular damage is more related to the state of health of the liver and/or systemic inflammation, than to patient age.

In the present study, atherosclerosis was also a parameter associated with severe germinal epithelial loss. That association was not statistically significant when the adjustments for the presence of steatosis were made, supporting the idea that NAFLD simultaneously participates in the genesis of atherosclerosis, as well as in testicular damage, and that as a result, there is a noncausal association between atherosclerosis and testicular damage. The current study identified a significant correlation between steatosis/liver inflammation and the grade of atherosclerosis and/or aortic wall thickness. Specifically related to this, there is evidence to support the association of NAFLD with subclinical atherosclerosis that is unrelated to traditional risk factors and metabolic syndrome (Oni et al., 2013; Ampuero, Gallego-Durán, & Romero-Gómez, 2015). The findings of the present study could be useful in the study of male fertility. Evaluating liver health is not a traditional approach in the management of patients presenting with fertility problems. Nevertheless, the first strategies recommended for improving fertility correctly include measures that reduce NAFLD symptoms. Weight reduction through diet and exercise and the taking supplements, such as glutathione, omega-3, selenium, and zinc, are supported in the medical literature and have positive effects on male fertility (Yao & Mills, 2016), as well as on NAFLD (Cave et al., 2007; Guo, Liong, So, Fung, & Tiope, 2015; de Castro & Calder, 2017; Chen et al., 2013; Trappoliere et al., 2005). Some experimental therapies that have been independently tested for NAFLD (Wong et al., 2016) or fertility have also reported improvement benefits for both pathologies. These include vitamin E (Salas-Huetos, Bulló, & Salas-Salvadó, 2016) pioglitazone (Meneses et al., 2016), and pentoxifylline (Feyli, Ghanbari, & Keshtmand, 2017) therapies, but their results are still limited. New preventive and therapeutic perspectives in relation to fertility could involve strategies used in the approach to NAFLD, and vice versa. At this point, it is important to take into consideration that the degree of germinal epithelial loss that could be associated with clinical infertility remains unknown. Studies that associate testicular morphological variables with the fertility of individuals are limited, even in animal models. A previous report in rats in which dipentyl phthalate (toxic substance for the germinal epithelium) was administrated showed that it is required >50% of the seminiferous tubules are degenerated to cause a significant loss of fertility (reduction in pregnancies in their partners) compared to a control group (Lindström, Harris, Ross, Lamb, & Chapin, 1988). The degeneration of 20%–50% of the seminiferous tubules did not reduce the ability of the animals to generate pregnancies in their partners (Lindström et al., 1988). We believe that very important germinal epithelial losses are required to generate affectations in male fertility, without being able to determine a loss percentage associated with such affection. Undoubtedly, further studies are required to generate more data about these observations, as well as assessing its clinical implications in patients.

A limitation of the present study was its small sample size, which could be the reason age or MS or its components were not associated with germinal epithelial damage. Damage to the germinal epithelium undoubtedly has a multifactorial origin and it is likely that MS and age participate to some degree in its pathophysiology. Nevertheless, these findings suggest that NAFLD could play an important role, because despite our small sample, it was strongly associated with DGEL in both the human and animal model studies. Future studies are needed to confirm these findings. Another characteristic of the present
study was that patients with severe germinal epithelial damage were evaluated and this should be taken into account in future comparisons.

In conclusion, the results presented herein suggest that NAFLD was a strongly associated factor with the severity of germinal epithelial damage. Additionally, the testis was identified as a probable target organ for damage caused by the disease. These findings suggest that NAFLD interferes more with testicular damage than other characteristics, such as diabetes, MS or age >50 years old, therefore, these results could lead for developing new strategies focus on the management of men with fertility problems. Further studies are necessary to confirm these findings.

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Study Concept and Design: Ivan Delgado-Enciso, Alejandra Rodriguez-Hernandez, Laura E. Garcia-Labastida; Data collection: Uriel A. Lopez-Lemus, Alejandro Garcia-Rivera, Violeta M. Madrigal-Perez, Alejandro D. Soriano-Hernandez, Iram P. Rodriguez-Sanchez, Elizabeth Sanchez-Duarte, Raquel Garza-Guajardo, Oralia Barboza-Quintana, Ariana Cabrera-Licona; Statistical analyses and data interpretation: Margarita L. Martinez-Fierro, Jose Guzman-Esquivel; Critical revision of the manuscript: Jose Guzman-Esquivel, Gabriel Ceja-Espiritu.

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