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Vitamin D3 Nanoemulsion Ameliorates Testicular Dysfunction in High-Fat Diet-Induced Obese Rat Model

HANY A. EL KATTAWY, M.D.*,**,*** and EMAN R. ABOZAID, M.D.*

The Department of Medical Physiology, Faculty of Medicine, Zagazig University, Egypt, Member in Zagazig Obesity Management and Research Unit, Faculty of Medicine, Zagazig University Hospitals, Egypt** and On Leave to Physiology Department, Faculty of Medicine, Almaarefa University, Saudi Arabia****

Abstract

Background: Obesity is a major health problem which may lead to infertility, vitamin D has anti-inflammatory, anti-oxidant, anti-apoptotic properties and metabolic activities Nano emulsions are very effective delivery system for medicines and nutrients. Nano vitamin D has a greater water solubility and stability compared to the conventional vitamin D.

Aim of Study: The study aims to evaluate the potential protective effects of vitamin D3nanoemulsionintervention against some altered testicular, metabolic and hormonal changes in high-fat diet (HFD)-induced obese rats.

Material and Methods: 32 adult male albino rats were equally and randomly divided into four groups: Group I (control group on standard diet), group II (untreated HFD), group III (HFD + conventional vitamin D) and group IV (HFD + Nano vitamin D3). Vitamin D3 administration was begun along with the start of introduction of the high fat diet. After eight weeks, body weight (BW) was measured and body mass index (BMI) was calculated then rats were euthanized. Serum testosterone, insulin and glucose levels were measured; homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. In addition, testicular levels of malondialdhyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), TNF-a and IL-6 were measured. The changes in the histopathological aspects of the testis were examined. The testicular weight, testicular coefficient, epididymal sperm count and motility all were investigated.

Results: Nano vitamin D3 treatment showed significant reductions in BMI, fasting serum glucose, insulin resistance, total cholesterol, LDL-c, with significant elevations in serum HDL-c, final testicular weight, testicular coefficient, epididymal sperm count, sperm motility, and serum testosterone. Moreover, Nano vitamin D3 significantly increased the testicular SOD and GSH with significant decline in the lipid peroxidation contributed to MDA, testicular TNF- a and IL-6 content. Improvement of the metabolic and biochemical measurements induced by Nano vitamin D3 is linked toimprovement of testicular histology in obese rats. Interestingly, Nano vitamin D3 has more significant protective effects against HFD-induced testicular dysfunction compared to the conventional vitamin D3.

Conclusions: Nano vitamin D3 has a potential protective role in improving the adverse effects of HFD induced obesity on testicular functions, withmore prominent protective effects than conventional vitamin D3.

Key Words: *Vitamin D3 – Nanoemulsion – High -fat diet – Testicular – Obesity – Rats.*

Correspondence to: Dr. Eman R. Abozaid,
E-Mail: eman.zaher2005@gmail.com

Introduction

OBESITY is a prevalent increasing major health problem leading to numerous diseases and even death. It is a leading risk factor for dyslipidemia, insulin resistance, type 2 diabetes, metabolic dysfunction, cardiovascular diseases, endocrine disorders and infertility **[1,2]**. Obesity inducedhormonal dysregulation and metabolic dysfunction may result in a decline in semen quality and spermatogenesis **[3,4]**. Moreover, visceral obesity and insulin resistance are associated with low serum testosterone concentrations and poor sperm quality **[5,6]**. Additionally, in high-fat diet fed animals, sperm energy metabolism is impaired and sperm quality is declined as well. Hence, the model of high fat diet-induced obesity has been used to investigate sperm dysfunction in rats **[7]**. Vitamin D is a fat-soluble steroid produced from 7-dehydrocholesterol in the skin or ingested from the dietary sources. It enhances the intestinal absorption of calcium, phosphate, zinc and magnesium playing a central role in bone physiology and calcium homeostasis. Calcitriol (1, 25(OH) 2VD3) is the active form of vitamin D traditionally known for regulating calcium and phosphorus homeostasis. Interestingly, vitamin D has anti-inflammatory, anti-oxidant, anti-apoptotic properties and immune modulating activities as well **[8-10]**. Vitamin D deficiency may be implicated in the development of abdominal adiposity, insulin resistance, hypertension, dyslipidemia, cardiovascular diseases, cancer, and autoimmune diseases **[11-13]**. Specifically, vitamin D

receptor and vitamin D metabolizing enzymes are expressed in the human ejaculatory duct, germ cells and mature spermatozoa [14]. Therefore, its deficiency may result in some fertility problems [15]. Nanoemulsions are very effective delivery system for medicines and nutrients. They are liquefied dispersions where the dropletssize range from 50 to 500nm [16]. This delivery systemhasgreater bioavailability and larger absorptive capability of hydrophobic complexes compared to other systems [17]. Nano vitamin D has a greater water solubility and stability upon exposure to light or elevated temperature as compared to the conventional vitamin D. Hence, the therapeutic potential of vitamin D Nano emulsion may be maximized [18]. The current study was designed to evaluate the potential impact of Nano vitamin D and traditional vitamin Don high-fat diet induced testicular dysfunction.

Material and Methods

Animals and obesity induction: This study was done from June to September 2019. The experimental procedures were approved by the animal research Ethical Committee (IACUC), Faculty of Medicine, Zagazig University, Egypt following the international guidelines set by National Institutes of Health guide for the care and use of Laboratory animals [19]. Healthy thirty two adult male albino rats weighing 250-270 gmselected from the animal house of Faculty of Veterinary Medicine, Zagazig University. Animals were kept in steel wire cages, 5 rats per cage; they were kept under comfortable temperature (20-24ºC) and were maintained on a normal light/dark cycle. After acclimatization for one week, the rats were equally and randomly divided into a control group (standard diet), non-treated HFD group, conventional vitamin D-treated group and vitamin D nanoemulsion-treated group. The control group (group I) (n=8 rats) was fed on a commercial rat standard chow that consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat [20]. The controls were given 0.9% saline only as a vehicle. For induction of a rat obese model, rats inthe other three groups were fed onHFD that consisted of high amounts of corn oil, containing >98% ω-6 poly unsaturated fat acid (PUFA) (21.4% fat, 17.5% protein, 50% carbohydrate, 3.5% fiber, and 4.1% ash) for 8 weeks [21]. HFD-fed rats were further classified into three groups: Group II (untreated HFD), group III (HFD + conventional vitamin D3 (cholecalciferol) from (Sigma Aldrich), by a daily oral dose of 1 µg (40IU)/kg) [22] and group IV (HFD + Nano vitamin D3) by daily oral dose of 40 IU/Kg/day orally [23], where a dose of 9 µg of Nano-vitamin D powder dissolved in 1ml distilled deionized water producing 3.24IU/ml.Vitamin D3 administrationwas begun along with the start of introduction of the high fat diet.

Vitamin D nanoemulsion: In the current study, the used vitamin D3 (cholecalciferol) containing nanoemulsion (VDN) was formed by sonication and pH-Shifting of pea protein isolate and canola oil (Pea Protein Nano-Particles (VD) powder (Sigma Aldrich). Pea protein isolate (PPI, NUTRALYS, S85F, 85% pea protein based on dry basis) was formedbythe wet extraction method from dry yellow peas. Soluble pea protein wasinvestigated for the particle size, water solubility, surface hydrophobicity, solution turbidity, free sulfhydryl group content, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). To formulate nanoemulsions (0.25% oil) and nano complexes loaded with vitamin D3, the pea protein isolate samples (10mg/ml) were treated by pH-shifting at pH 12 combinedtothe ultrasound (pH 12 + U 5) with a greatest solubility. The loading capacity of pea protein isolate-based nanoparticles was 1.5 ± 0.2 µg/mg pea proteins [24].

Calculation of body mass index (BMI): The total body weight (gm) was measuredby a digital scale; the rats were weighed the day before the experiments, then twice a week and at the final day of experiment. The nose to anus length (cm) and the weight (gm) were measured at the end of the experiments for calculation of body mass index (BMI) (gm/cm^2) by dividing the body weight (gm)/ $length^2$ (cm^2) [25].

Blood samplingand biochemical analyses: Rats were sacrificed by large dose of ether anesthesia and then blood samples were immediately obtaineddirectly from cardiac puncture, blood allowed to clot and then centrifuged at approximately 3000 rpm for 10 minutes. The separated serum was used to measure free testosterone levels according to the method of Tietz [26] by using rat kits (Sigma Aldrich). Moreover, serum levels of glucose (mg/dl) [26] and insulin (µIU/ml) [27]. Insulin resistance (IR)wascalculated by homeostasis model assessment of insulin resistance (HOMA-IR)using the following formula: HOMA-IR = Fasting serum glucose (mg/dL) x fasting serum insulin (µIU/mL)/ 405 [28]. Moreover, serum levels of total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TGs) were assayed following the manufacturer's protocols as described by various previous studies [26,29]. However, serum low density lipoprotein (LDL) levels were calculated by Friedewaldequation [30] as follows: LDL = TC - HDL - TG/5. The

kits used for estimation of serum glucose, insulin, cholesterol, TG and HDL levels were purchased from Biosource (Europe S.A. Belgium). All steps were performed following the manufacturer's instructions.

Gonadal extraction: Laparotomy was conducted. Testes and epididymis were extracted and dissected cautiously [31]. The weights of the testes were measured using an electronic balance.Then, the testicular coefficient was calculated according to the formula ofYan et al. [32]: Testicular coefficient (gm/Kg) = The weight of the testes (the mean of the weight of the two testes in gm)/final body weight (kg).

Sperm analysis: The right epididymis of each rat was dissected, removed and minced in 2ml of Hank's buffer salt solution (HBSS) at 37°C [33]. After 5 minutes incubation at 37°C, the caudal epididymis sperm was determined using the standard haemocytometric method [34]. The samples placed on the glass slides were evaluated for motility and total sperm counts. The epididymal fluid was drawn up to the 0.5 mark of White Blood Cell pipette and the semen diluting fluid (sodium bicarbonate 5 grams, formalin 1ml, distilled water 99.0ml) was drawn up to ' 11' mark, and then mixed well. One drop was added to the haemocytometer chamber and allowed the sperms to settle by keeping haemocytometer in wet humid chamber for 1 hour after incubation, the number of spermatozoa in the appropriate squares of the haemocytometer was counted under the light microscope at 400X [34]. The sperm concentration refers to the number of spermatozoa/ml fluid, and calculated using the following formula. Sperm count=No. of spermatozoa counted x dilution factor x volume factor/No. of areas counted. For motility evaluation, at least 200 static sperm were recorded. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells [35]. Moreover, sperm samples from each group were smeared on glass slides, and examined for malformations using a light microscope at 200 sperm/smear.

Evaluationof testicular oxidative and antioxidants factors, and inflammatory markers: Specimens from testis were collected from all experimental and control groups. The tissues were homogenized in 50mM potassium phosphate (pH 7.4). The samples were centrifuged at 4000 rpm for 15min, at 4ºC and the supernatants were stored at –80ºC until analysis. Testicular superoxide dismutase (SOD) activity, reduced glutathione (GSH) and malondialdhyde (MDA) were assayed by using spectrophotometer (spectronic 3000 Array, Germany) following the manufacturer's protocols. Moreover, the inflammatory markers (tissue IL-6 and TNF-α) were measured by ELISA using kits purchased from Sigma Aldrich following the manufacturer's instructions.

Histopathological examination: Testes were dissected out and cleaned with cold physiological saline to remove blood and the adhering tissues. One of samples were then fixed in 10% formaldehyde in fresh alcoholic bouin's fluid for 8 hours, and then processed and embedded in paraffin wax, sectioned at 5 µm thicknesses and stained in hematoxylin & eosin. The sections were examined or observed under a light microscope and the general histological appearance was assessed [36].

Statistical analysis: The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (19) (SPSS Inc. Chicago, IL, USA). ANOVA with [Post hoc (LSD)] test was used to compare means among all studied groups, p-value <0.05 was considered statistically significant [37].

Results

Obesity induction and testes gross features: Body weights and BMI were significantly higher in the untreated HFD group (group II) than in the control rats in group I. However,a significant decline in body weights and BMI was observed in HFD fedrats supplemented with conventional vitamin D (group III). Surprisingly, HFD fed rats treated with nano-vitamin D (group IV) showed higher significant reductions in body weights and BMI when compared to conventional vitamin D treated rats (p<0.001) (Table 1). However, the opposite was observed as regard to the testes weights. The testes of control ratswere opaque white, oval, smooth, elastic and vascular. They were larger in size in comparison to the testes of untreated HFD fed rats. In addition, the present study showed that obesity significantly reduced testis weight and testis coefficient in HFD fed rats compared with the control group (p<0.001). Interestingly, with conventional vitamin D intervention, the testis weight was significantly higher compared with the untreated HFD fed rats (p<0.001). However, nano-vitamin D group showed more significant increase in the testis weight andtestis coefficient than conventional vitamin D (p<0.001) indicating thatits protective value against the testicular atrophy observed in the untreated HFD group.

Effect of HFD and vitamin D on sperm examination and serum testosterone levels: Sperm concentration, motility and morphology in addition to serumtestosterone levels play central roles in assessment of male fertility. Serum Testosterone, sperm density and motility rate in HFD group were significantly reduced in comparison to the controls (p<0.001, as summarized in Table (1). They improved with vitamin D intervention, however, these parameters significantly increased in response to nano-vitamin D treatment compared to those treated by conventional vitamin D (p>0.001). Moreover, HFD group had higher abnormal sperm ratio than that of the control rats and vitamin D-treated rats as well (p<0.001). Nano-vitamin D significantly cut down the abnormal sperm ratio in comparison to conventional vitamin D-treatment (p<0.001).

Assessment oftesticular oxidative stress and inflammatory markers: The testicular MDA content as a marker of lipid peroxidation and the inflammatory markers IL-6 and TNF-α, all are elevated significantly in non-treated obese rats compared to the controls (p<0.001), in association withsignificant reductions of testicular SOD and the content of GSH (p<0.001), while these parameters were significantly reversed in vitamin D-treated rats with more significant values in nano-vitamin D treated group when compared with conventional vitamin D treated group as shown in Table (2) (p<0.001).

Effect of vitamin D on metabolic parameters: The total cholesterol level, LDL-cholesterol and triglyceride levels were significantly higher and HDL-cholesterol was significantly lower in non-treated obese rats than in the controls (p<0.001), these disturbances were reversed by vitamin D intervention. Nano-vitamin D-treated group showed more significant improvement than conventional vitamin D-treated group (p<0.001). Moreover, non-treated obese rats showed significantly higher glycaemia, insulin levels and insulin resistance compared with control rats (p<0.001).

Interestingly, all rats treated with vitamin D had normal glycaemia values with significant decrease in insulin levels and insulin resistance when compared with non-treated obese rats (p<0.05, p<0.001) with more significant improvement observed in nano-vitamin D group than conventional vitamin D group (p<0.001).

Table (1): Body weights, BMI, testicular coefficient, sperm parameters and serum testosterone in all the studied groups (mean ± SD).

Parameters		Group I (Controls)	Group II (HFD)	Group III (HFD + conventional vit. D)	Group IV (HFD + nano-vit. D)
Final body weight (gm)	Mean ± SD p-value of LSD	300.4± 12.5	451.3±21.5 p<0.001 **a**	351.7±20.1 p<0.001 **b**	320.2± 18.5 p<0.001 **a,b,c**
BMI (g/cm^2)	Mean ± SD p-value of LSD	0.48±0.04	0.72±0.1 p<0.001 **a**	0.56±0.04 p<0.001 **b**	0.51 ±0.06 p<0.001 **a,b,c**
Testicular weight (gm)	Mean ± SD p-value of LSD	3.36±0.23	2.46±0.13 p<0.001 **a**	2.82±0.19 p<0.001 **b**	3.22±0.2 p<0.001 **a,b,c**
Testicle coefficient (%)	Mean ± SD p-value of LSD	11.2±0.13	6.15±0.13 p<0.001 **a**	8.1±0.13 p<0.001 **b**	9.75±0.13 p<0.001 **a,b,c**
Sperm count (x10^9 spermatozoa/ml)	Mean ± SD p-value of LSD	1.61 ±0.60	1.36±0.51 p<0.001 **a**	1.41 ±0.39 p<0.001 **b**	1.58±0.52 p<0.05 **a**, p<0.001 **b**,**c**
Sperm motility rate (%)	Mean ± SD p-value of LSD	71.5±9.7	47±7.11 p<0.001 **a**	54.6±7.71 p<0.001 **b**	62.5±6.51 p<0.001 **a,b,c**
Sperm abnormality rate (%)	Mean ± SD p-value of LSD	0.31 ±0.06	0.49±0.1 p<0.001 **a**	0.39±0.05 p<0.001 **b**	0.34±0.04 p<0.001 **a,b,c**
Free Testosterone (ng/ml)	Mean ± SD p-value of LSD	4.16±0.71	2.92±0.51 p<0.001 **a**	3.48±0.72 p<0.001 **b**	3.88±0.64 p<0.001 **a,b,c**

a Significant versus group I.
b Significant versus group II.
c Significant versus group III.

Table (2): Testicular oxidative stress and inflammatory markers in all studied groups (mean ± SD).

Parameters		Group I (Controls)	Group II (HFD)	Group III (HFD + conventional vit. D)	Group IV (HFD + nano-vit. D)
MDA (pmol/mg protein)	Mean ± SD *p*-value of LSD	0.32±0.05	0.86±0.06 $p<0.001$ **a**	0.7±0.06 $p<0.001$ **b**	0.45±0.07 $p<0.001$ **a,b,c**
SOD (u/mg protein)	Mean ± SD *p*-value of LSD	42±9.1	26±7.5 $p<0.001$ **a**	29±7.9 $p<0.01$ **b**	40±8.5 $p<0.05$ **a** $p<0.001$ **b'c**
GSH (nmol/g protien	Mean ± SD *p*-value of LSD	61±10.9	25±9.7 $p<0.001$ **a**	29±9.6 $p<0.05$ **b**	55±5.7 $p<0.01$ **a** $p<0.001$ **b,c**
IL-6 (ng/ml)	Mean ± SD *p*-value of LSD	67±16.9	120±15.4 $p<0.001$ **a**	100±14.2 $p<0.001$ **b**	81±14.3 $p<0.001$ **a,b,c**
TNF-α (ng/g)	Mean ± SD *p*-value of LSD	33±4.9	60±4.6 $p<0.001$ **a**	57±6.3 $p<0.05$ **b**	40±5.2 $p<0.001$ **a,b,c**

a Significant versus group I.
b Significant versus group II.
c Significant versus group III.

Table (3): Metabolic profile and HOMA-IR in all studied groups (mean ± SD).

Parameters		Group I (Controls)	Group II (HFD)	Group III (HFD + conventional vit. D3)	Group IV (HFD + nano-vit. D3)
Total cholesterol (mmol/L)	Mean ± SD *p*-value of LSD	1.55±0.15	1.96±0.11 $p<0.001$ **a**	1.87±0.18 $p<0.05$ **b**	1.61±0.15 $p<0.01$ **a**, $p<0.001$ **b,c**
HDL-cholesterol (mmol/L)	Mean ± SD *p*-value of LSD	0.82±0.04	0.59±0.06 $p<0.001$ **a**	0.76±0.04 $p<0.001$ **b**	0.80±0.06 $p<0.01$ **a**, $p<0.001$ **b,c**
LDL- cholesterol (mmol/L)	Mean ± SD *p*-value of LSD	0.49±0.02	0.78±0.04 $p<0.001$ **a**	0.56±0.07 $p<0.001$ **b**	0.51±0.05 $p<0.01$ **a**, $p<0.001$ **b,c**
Triglyceride (mmol/L)	Mean ± SD *p*-value of LSD	0.53±0.08	1.28±0.1 $p<0.001$ **a**	1.24±0.06 $p<0.001$ **b**	0.68±0.03 $p<0.001$ **a,b,c**
Serum glucose (mg/dl)	Mean ± SD *p*-value of LSD	91.9±5.9	140.6±7.6 $p<0.001$ **a**	128.7±9.5 $p<0.05$ **b**	100.2±5.1 $p>0.05$ **a**, $p< 0.001$ **b'c**
Insulin (μIU/mL)	Mean ± SD *p*-value of LSD	27.3±9.1	129.1±10.1 $p<0.001$ **a**	99.3±6.7 $p<0.05$ **b**	44.7±7.8 $p<0.05$ **a**, $p<0.001$ **b'c**
HOMA-IR	Mean ± SD *p*-value of LSD	6.2±1.30	44.80±8.89 $p<0.001$ **a**	31.6±5.25 $p<0.001$ **b**	11.1±4.25 $p<0.001$ **a,b,c**

a Significant versus group I.
b Significant versus group II.
c Significant versus group III.

Histopathologic results: Spermsare produced in the testis and stored in the epididymis, so we observed the morphological structure of the testicular spermatogenic epithelium stained with Hematoxylin & eosin (HE). The control group showed normal structure of testis with normal tubular pattern of spermatids and spermatogonia maturation (Fig. 1A). However, in obese rat testes, the seminiferous tubules were deformed and atrophied, decrease in spermatogonia lining the seminiferous tubules and interstitial compartment filled with extracellular matrix (Fig. 1B). Moderate damage was observed in ratstreated withconventional vitamin D3 (Fig. 1 C). Meanwhile, ratstreated with Nano vitamin D3 (Fig. 1D) exhibited nearly normal tubule diameter andshape with nearly normal number of cells in each seminiferous tubule when compared with group treated with conventional vitamin D.

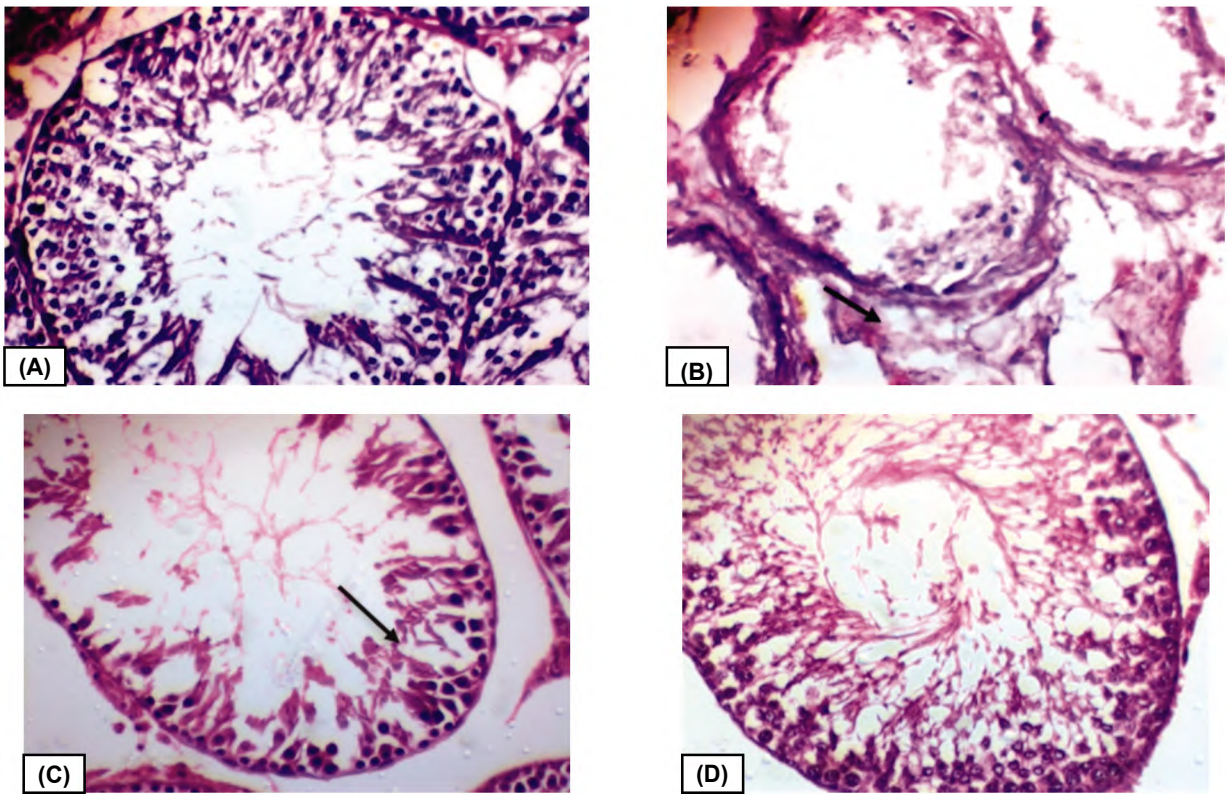

Fig. (1): Histological morphology of testis from the four groups; A: Control group, having normal structure of testis with normal tubular pattern of spermatids and spermatogonial maturation. B: HFD-induced obese rat testes showing atrophy of tubule with decrease in spermatoginia lining the seminiferous tubules and interstitial compartment filled with extracellular matrix (arrow). C: Conventional vitamin. D-treated testes show intact tubular basal compartment with presence of some spermatogonia (arrow), decrease in extracellular matrix in interstitial space. D: Nano vitaminD-treated testes showing increase in spermatidewith nearly normal tubular pattern (H&E staining magnification x200).

Discussion

In the current study, a rat model of the HFD-induced obesitywas used to investigatetesticular dysfunction as Ferramosca et al. [7] documented that sperm quality and function are affected by dietary fatty acids. In addition, abdominal obesity, dyslipidemia, insulin resistance,metabolic syndrome and type 2 diabetes mellitusare linked to male infertility [38-40]. Interestingly, it has been suggested that vitamin D synthesis declines in obese males and vitamin D deficiency is 35% higher in obese individuals, its deficiency resulting in semen quality worsening [41-43].

The present study revealed that HFD cause adiposity and worsened the lipid profile resulting in dyslipidemia characterized by significant higher levels of triglycerides, total cholesterol and LDL-c and lower levels of HDL c compared to controls. As previously mentioned, long lasting HFD results in altered lipid profile [44].

In our study, Nano vitamin D3 protect against adiposity and dyslipidemia induced by HFD as it significantly reduced final body weights, BMI and corrected dyslipidemia by decreasing triglycerides, total cholesterol and LDL-c levels and elevating HDL-c levels. These findings were in coincidence with other studies [8,23,45]. Interestingly Nano viitamin D3 more effectively improved the BMI and lipid profile more than the conventional one. The findings were in agreement with the results of El-Sherbiny et al. [23] who implied the superiority of Nano vitamin D3 over the conventional one as an effective protective agent against the diet-induced dyslipidemia by modulating lipid metabolism. This Nano vitamin D3 superiority may be explained by findings of Almajwal et al. [46] who reported that the bioavailability and intestinal absorption of Nano vitamin D3 were enhanced compared to oral conventional vitamin D3. The antiadipogenic property of vitamin D may be due to elevation of the intracellular ionized calcium enhancing the fat cells apoptosis via activating sympathetic nervous system, furthermore, it augments the diet-induced thermogenesis and fat oxidation resulting in increasing the energy expenditure [47]. Moreover, it enhances the release of

gastrointestinal hormones controlling appetite and excretion of fecal fat [48]. In addition, it might increase the lipolysis controlling genes at the expense of lipogenesis controlling genes [49]. Therefore, it was recommended that vitamin D restoration may enhance weight loss improving many altered metabolic markers [50].

In addition, this work showed that Nano vitamin D3 significantly reduced the elevated fasting serum glucose, insulin and the HOMA values compared to the non-treated HFD group. This indicates that Nano vitamin D3 might have a role in glycemic control, insulin sensitivity and protection against type 2 diabetes in comparison with the conventional one as well. These findings are supported by the findings of Chiu et al. [51]. Interestingly, pancreatic β-cells have vitamin D receptors and 1-α-hydroxylase enzymes [52]. Moreover, 1, 25(OH)2 D was found to enhance insulin gene transcription, glucose transporter 4 upregulation, and glucose utilization in fat cells [53].

As regard to the testicular function parameters, the present study revealed that HFD-induced obesity resulted in a significant decline in the values of final testicular weight, testicular coefficient, epidydimal sperm count, sperm motility and serum testosterone, In accordance with other studiesexplaining that long lasting HFD intake could induce apoptosis and declined sperm viability [3,54]. In addition, semen quality is closely correlated to BMI [4,55]. Obesity induced hormonal disorders and metabolic dysfunction leading to altered spermatogenesis. Therefore, insulin resistance and metabolic syndrome could be predicted by low serum testosterone in men [56]. Fortunately, the values of final testicular weight, testicular coefficient, epidydmal sperm count, sperm motility and serum testosterone could be compensated by vitamin D3 treatment. Our findings were supported by the studies that found out a positive association between serum vitamin D level and sperm motility via elevation of the intracellular calcium stored in the neck of human sperms indicating that vitamin D may share in sperm motility induction [57,58]. In contrary to our results, Jensen et al. [59] found that serum vitamin D level was not associated with the sperm count. However, we are in agreement with Sood et al. [60] who documented that vitamin D deficient and vitamin D receptors knock out mice have declined sperm count. The enhancement of these testicular function parameters might follow the reductions in body weight, BMI in HFD fed rats treated by vitamin D especially the nano particles form. This can be supported by findings of Hakonsen et al. [61] who confirmed that weight loss could recover sperm motility and count in obese men. Interestingly, in accordance with our findings, Tirabassiet al. [62] concluded that vitamin D administration improves the sexual function, they observed a slight significant rise in testosterone levels after vitamin D administration indicating a direct association between vitamin D and testosterone levels.

Further, in HFD fed rats, the accumulated fat stimulates adipocyte to release several adipokines including TNF-α establishing a chronic inflammatory state [63,64]. We found elevated testicular TNF-α and IL-6 levels in HFD fed rats. Moreover, vitamin D3 diminished the elevated tissue TNF-α and IL-6 suggesting an anti-inflammatory roleof vitamin D. These results are consistent with other findings [65,66]. Anti-inflammatory effect of vitamin D may be explained by declining IL-6 and inhibiting the nuclear factor κB pathway [67] Vitamin D3 nanoemulsion augmented the anti-inflammatory effect of vitamin D.

While HFD-induced obesity resulted in a significant rise of the testicular MDA levels with a decline of testicular GSH and SOD activities, exogenous administration of nanoparticles of vitamin D to these HFD-fed rats resulted in a significant recovery of all these parameters. The pro-inflammatory state induced by obesity results in oxidative stress through whichthe mitochondria produced reactive oxygen species (ROS) damaging the testicular environment and alteringthe reproductive functions [68]. Of note, the antioxidant effect of vitamin D was described by Kuz'menko et al. [69]. Furthermore, vitamin D supplementation could up-regulate some of the testis-specific genes in mice [70]. The present study also investigated the effect of vitamin D on testicular histological structures and the H&E staining showed that the testes of untreated HFD fed ratshadreduced sperms with apparent vacuoles, declined seminiferous epithelium height and seminiferous tubular diameter affecting sperms production when compared with the testes of controls. However, all these adverse effects were decreased with conventional vitamin D3 intervention and nearly almost reversed by Nano vitamin D3 treatment. Finally, we recommended evaluation of vitamin D on the fertilization capability in obese rats and comparing the pregnancy and abortion rates. Furthermore, more cellular and molecular studies are required. In addition, larger prospective case-controlled studies are required to clarify the effects of vitamin D on male reproductive function in obese patients.

Conclusion:

The present study confirmed the bad effect of HFD-induced obesity on testicular function in rats. In addition, we concluded for the first time that Nano vitamin D3 improves testicular dysfunction in obese male rats, possibly due to its beneficial effects on body weight loss, biochemical and metabolic status in addition to its antioxidant and anti-inflammatory properties. Furthermore, the superiority of Nano vitamin D effect above the traditional vitamin D support the use of nanotechnology for vitamin D delivery as an approach in managing and protecting against obesity-induced testicular dysfunction and hence other therapeutic purposes.

Conflict of interest the authors declare that they have no conflict of interest.

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References

1- BARTH R.J.: Insulin resistance, obesity and the metabolic syndrome. SD Med. Spec., No. 22-7, 2011.

2- Pi-SUNYER F.X. The obesity epidemic: Pathophysiology and consequences of obesity. Obes. Res., 10: 97S-104, 2002.

3- JENSEN T.K., HEITMANN B.L., BLOMBERG JENSEN M., et al.: High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. Am. J. Clin Nutr., 97: 411-8, 2013.

4- HAMMOUD A.O., GIBSON M., PETERSON C.M., et al. Impact of male obesity on infertility: A critical review of the current literature. Fertil Steril., 90: 897-904, 2008.

5- ALLAN C.A. and McLACHLAN R.I.: Androgens and obesity. Curr. Opin. Endocrinol. Diab. Obes., 17: 224-32, 2010.

6- KAPOOR D., CHANNER K.S. and JONES T.H.: Rosiglitazone increases bioactive testosterone and reduces waist circumference in hypogonadal men with type 2 diabetes. Diab. Vasc. Dis. Res., 5: 135-7, 2008.

7- FERRAMOSCA A., MOSCATELLI N., DI GIACOMO M. and ZARA V.: Dietary fatty acids influence sperm quality and function. Andrology, 1-8. American Society of Andrology and European Academy of Andrologydoi: 10.1111/andr.12348, 2017.

8- BARCHETTA F.A. and CIMINI M.G. CAVALLO: Vitamin D supplementation and non-alcoholic fatty liver disease: Present and future, Nutrients, 9 (9): 1015, 2017.

9- HAN H., CUI M., YOUX., CHEN M., PIAO X. and JIN G.: A role of 1, 25 (OH) 2D3 supplementation in rats with nonalcoholic steatohepatitis induced by choline-deficient diet, Nutr. Metabol. Cardiovasc. Dis., 25 (6): 556-561, 2015.

10- Determinants of vitamin D status in Caucasian adults: Influence of sun exposure, dietary intake, sociodemographic, lifestyle, anthropometric, and genetic factors. Ouvier M., Deschasaux M., Montourcy M., Sutton A., Charnaux N., Kesse-Guyot E., Assmann K.E., Fezeu L., Latino-Martel P., Druesne-Pecollo N., Guinot C., Latreille J., Malvy D., Galan P, Hercberg S., Le Clerc S., Souberbielle J.C., Ezzedine K.J. Invest Dermatol. Feb., 135 (2): 378-388, 2015.

11- STRANGE R.C., SHIPMAN K.E. and RAMACHANDRAN S.: Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome. World J. Diabetes, 6: 896-911, 2015.

12- FUNG G.J., STEFFEN L.M., ZHOU X., HARNACK L., TANG W., LUTSEY P.L., et al.: Vitamin D intake is inversely related to risk of developing metabolic syndrome in African American and white men and women over 20y: The Coronary Artery Risk Development in Young Adults study. Am. J. Clin. Nutr., 96: 24-9, 2012.

13- NAKANO T., CHENG Y.F., LAI C.Y., HSU L.W., CHANG Y.C., DENG J.Y., HUANG Y.Z., HONDA H., CHEN K.D., WANG C.C., CHIU K.W., JAWAN B., ENG H.L., GOTO S. and CHIU K.W.: Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats, J. Hepatol. 55 (2): 415-425, 2011.

14- BLOMBERG JENSEN M., NIELSEN J.E., JORGENSEN A., RAJPERT-DE MEYTS E., KRISTENSEN D.M., JORGENSEN N., SKAKKEBAEK N.E., JUUL A. and LEFFERS H.: Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. Hum. Reprod, 25: 1303-1311, 2010.

15- MARTIN BLOMBERG JENSEN, POUL J. BJERRUM, TORBEN E. JESSEN, JOHN E. NIELSEN, ULLA N. JOENSEN, INGE A. OLESEN, JØRGEN H. PETERSEN, ANDERS JUUL, STEEN DISSING and NIELSJØRGENSEN: Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. Human Reproduction, 26: 1307-1317, 2011.

16- ABOALNAJA K.O., YAGHMOOR S., KUMOSANI T.A. and McCLEMENTS D.J.: Utilization of nanoemulsions to enhance bioactivity of pharmaceuticals, supplements, and nutraceuticals: Nanoemulsion delivery systems and nanoemulsion excipient systems, Expet. Opin. Drug Deliv., 13 (9): 1327-1336, 2016.

17- WALIA N., DASGUPTA N., RANJAN S., CHEN L. and RAMALINGAM C.: Fish oil based Vitamin D nanoencapsulation by ultrasonication and bioaccessibility analysis in simulated gastro-intestinal tract, Ultrason. Sonochem., 39: 623-635, 2017.

18- GUTTOFF M., SABERI A.H. and Mc CLEMENTS D.J.: Formation of vitamin D nanoemulsion based delivery systems by spontaneous emulsification: Factors affecting particle size and stability, Food Chem., 171: 117-122, 2015.

19- Institute of Laboratory Animal Resources, Commission on life sciences and National research council: Guide for

the care and use of laboratory animals, 8 th Edition. National academy press, Washington DC, 21-55, 1996.

20- AHREN B. and SCHEURINK A.J.: Marked hyperleptinaemia after high fat diet associated with severe glucose intolerance in mice. Eur. J. Endocrinal, 139 (4): 461-7, 1998.

21- LOZANO I., VAN DER WERF R., BIETIGER W., SEYFRITZ E., PERONET C., PINGET M., JEANDIDIER N., MAILLARD E., MARCHIONI E., SIGRIST S. and DAL S.: High-fructose and high fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications, Nutr. Metabol., 13 (1) 15, 2016.

22- MALSAWMHRIATZUALA JEREMY, GURUSWAMI GURUSUBRAMANIAN and VIKAS KUMAR ROY: Vitamin D3 regulates apoptosis and proliferation in the testis of D-galactose-induced aged rat model, Scientific Reports, 9: 14103, 2019.

23- MOHAMED EL-SHERBINY, MOHAMED ELDOSOKY, MOHAMED EL-SHAFEY, GAMAL OTHMAN, HANY A. ELKATTAWY, TAMER BEDIR and NEHAL MOHSEN ELSHERBINY: Vitamin D nanoemulsion enhances hepatoprotective effect of conventional vitamin D in rats fed with a high-fat diet. Chemico-Biological Interactions, 288: 65-75, 2018.

24- JIANG S.: Enhanced Physicochemical and Functional Properties of Pea (PisumSativum) Protein by PH-shifting and Ultrasonication Combined Process (Master dissertation). Retrieved from Graduate Dissertations and Theses at Illinois, http://hdl.handle.net/2142/88229, 2015.

25- NASCIMENTO A., SUGIZAKI M., LEOPOLDO S., LIMA-LEOPOLDO A., NOGUEIR C., NOVELLI E., PADOVANI C. and CICOGNA A.: Misclassification probability as obese or lean in hypercaloric and normocaloric diet. Biol. Res., 41: 253-259, 2008.

26- TIETZ N.W.: Clinical Guide to Laboratory Tests, 3 rd Ed., pbl: W.B. Saunders Company, Philadelphia, pp 509-580, 1995.

27- REAVEN G.M.: Insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypertension. Parallel between human disease. Diab. Care, 14 (3): 195-202, 1991.

28- BONORA E., TARGHER G., ALBERICHE M., BONADONNA R.C., SAGGIANI F., ZENERE M.B., MONAUNI T. and MUGGEO M.: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care, 23 (1): 57-63, 2000.

29- FOSSATI P. and PRENCIPE L.: Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28 (10): 2077-2080, 1982.

30- FRIEDEWALD W.T., LEVY R.I. and FREDRICKSON D.S.: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18 (6): 499-502, 1972.

31- CASTEILLA L., PENICAUD L. and COUSIN B.: Choosing an adipose tissue depot for sampling. Factors in selection and depot specificity. Methods. Mol. Biol., 155: 1-19, 2001.

32- YAN W.J., MU Y., YU N., YI T.L., ZHANG Y., PANG X.L., CHENG D. and YANG J.: Protective effects of metformin on reproductive function in obese male rats induced by high-fat diet. J. Assist Reprod Genet, 32 (7): 1097-104, 2015.

33- IDRIS M.H., BUDIN S.B., OSMAN M. and MOHAMED J.: Protective role of hibiscus sabdariffa calyx extract against streptozotocin induced sperm damage in diabetic rats. EXCLI Journal, 11: 659-669, 2012.

34- BELSEY M.A., MOSHISSI K.S., ELIASSON R., PAULSEN C.A., CALLEGOS A.J. and PRASAD M.R.: Laboratory manual for the examination of human semen and semen cervical mucus interaction. J. R. Soc. Med., 75 (1): 58, 1982.

35- KHAKI A., NOURI M., FATHIAZAD F., AHMADIASHTIANI H.R., RASTGAR H. and REZAZADE H.: Protective Effects of Quercetin on Spermatogenesis in Streptozotocin induced Diabetic Rat. Journal of Medicinal Plans, 8 (5), 2009.

36- MURTHY N.V., WRAY S.R., MELVILLE G.N., WYNTER H.H., RAM N.V. and HARAN N.V.: Testicular function in rats following immobilization stress. Int. J. Gynaecol. Obstet., 26 (2): 297-299, 1988.

37- KIRKWOOD B.R.: Essential medical statistics. Statistics in Medicine, 8 (5): 636, 1989.

38- KASTURI S.S., TANNIR J. and BRANNIGAN R.E.: The metabolic syndrome and male infertility. J. Androl., 29: 251-259, 2008.

39- MORRISON C.D. and BRANNIGAN R.E.: Metabolic syndrome and infertility in men. Best Pract Res. Clin. Obstet. Gynaecol., 29: 507-515, 2015.

40- O'NEILL S. and O'DRISCOLL L.: Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies, Obes. Rev., 16 (1): 1-12, 2015.

41- TANGPRICHA V., PEARCE E.N., CHEN T.C. and HOLICK M.F.: Vitamin D insufficiency among free-living healthy young adults. Am. J. Med., 112: 659-662, 2002.

42- ARUNABH S., POLLACK S., YEH J. and ALOIA J.F.: Body fat content and 25 hydroxy vitamin D levels in healthy women. J. Clin. Endocrinol. Metab., 88: 157-161, 2003.

43- WORSTMAN J., MATSUOKA L.Y., CHEN T.C., LU Z. and HOLICK M.F.: Decreased bioavailability of vitamin D in obesity. Am. J. Clin. Nutr., 72: 690-693, 2000.

44- ZHANG Q.Q. and LU L.G.: Nonalcoholic fatty liver disease: Dyslipidemia, risk for cardiovascular complications, and treatment strategy, J. Clin. Transl. Hepatol., 3 (1): 78, 2015.

45- ELIADES M. and SPYROU E.: Vitamin D: A new player in non-alcoholic fatty liver disease? World J. Gastroenterol. WJG, 21 (6): 1718, 2015.

46- ALMAJWAL A.M., ABULMEATY M.M.A., ANDRADE J.E., ELSADEK M.F. and RAZAK S.: Mon-LB273: Novel vitamin d-nanoemulsion developed by sonication and PH-shifting of pea protein. Isolate enhances intestinal absorption of vitamin D in rats, Clin. Nutr., 35: S253-S254, 2016.

47- SLUSHER A.L., McALLISTER M.J. and HUANG C.J.: A therapeutic role for vitamin D on obesity associated

inflammation and weight loss intervention. Inflamm. Res., 64: 565-575, 2015.

48- RAYALAM S., DELLA-FERA M.A., AMBATI S., et al.: Enhanced effects of 1,25 (OH)(2)D(3) plus genistein on adipogenesis and apoptosis in 3T3-L1 adipocytes. Obesity (Silver Spring), 16: 539-546, 2008.

49- SOARES M.J., PATHAK K. and CALTON E.K.: Calcium and vitamin D in the regulation of energy balance: Where do we stand? Int. J. Mol. Sci., 15: 4938-4945, 2014.

50- ZITTERMANN A., FRISCH S., BERTHOLD H.K., et al.: Vitamin D supplementation enhances the beneficial effects of weight loss on car-diovascular disease risk markers. Am. J. Clin. Nutr., 89: 1321-1327, 2009.

51- CHIU K.C., CHU A., GO V. and SAAD M.F.: Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. Am. J. Clin. Nutr., 79: 820-825, 2004.

52- BLAND R., MARKOVIC D., HILLS C.E., et al.: Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J. Steroid Biochem. Mol. Biol., 89-90: 121-125, 2004.

53- GIRGIS C.M., CLIFTON-BLIGH R.J., HAMRICK M.W., HOLICK M.F. and GUNTON J.E.: The roles of vitamin D in skeletal muscle: Form, function, and metabolism. Endocr. Rev., 34: 33-83, 2013.

54- FERRAMOSCA A., CONTE A., MOSCATELLI N. and ZARA V.: A high-fat diet negatively affects rat sperm mitochondrial respiration. Andrology, 4: 520-525, 2016.

55- RAMLAU-HANSEN C.H., HANSEN M., JENSEN C.R., et al.: Semen quality and reproductive hormones according to birthweight and body mass index in childhood and adult life: Two decades of follow-up. Fertil Steril., 94: 610-8, 2010.

56- LAAKSONEN D.E., NISKANEN L., PUNNONEN K., et al.: Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care., 27: 1036-41, 2004.

57- AQUILA S., GUIDO C., MIDDEA E., PERROTTA I., BRUNO R., PELLEGRINO M. and ANDO S.: Human male gamete endocrinology: 1 alpha, 25-dihydroxyvitamin D3 (1,25(OH)2D3) regulates different aspects of human sperm biology and metabolism. Reprod Biol. Endocrinol., 7: 140, 2009.

58- BEDU-ADDO K., COSTELLO S., HARPER C., HADO-OLIVEIRA G., LEFIEVRE L., FORD C., BARRATT C. and PUBLICOVER S.: Mobilisation of stored calcium in the neck region of human sperm-a mechanism for regulation of flagellar activity. Int. J. Dev. Biol., 52: 615-626, 2008.

59- MARTIN BLOMBERG JENSEN, POUL J. BJERRUM, TORBEN E. JESSEN, JOHN E. NIELSEN, ULLA N. JOENSEN, INGE A. OLESEN, JØRGEN H. PETERSEN, ANDERS JUUL, STEEN DISSING and NIELS JØRGENSEN: "Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa". Human Reproduction, Vol. 26, No. 6, pp. 1307-1317, 2011.

60- SOOD S., REGHUNANDANAN R., REGHUNANDANAN V., MARYA R.K. and SINGH P.I.: Effect of vitamin D repletion on testicular function in vitamin D-deficient rats. Ann. Nutr. Metab., 39: 95-98, 1995.

61- HAKONSEN L.B., THULSTRUP A.M., AGGERHOLM A.S., OLSEN J., BONDE J.P., ANDERSEN C.Y., BUNGUM M., ERNST E.H., HANSEN M.L. and RAMLAU-HANSEN C.H.: Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. Reprod Health, 8: 24, 2011.

62- GIACOMOTIRABASSI, MAURIZIO SUDANO, GIANMARIA SALVIO, MELISSA CUTINI, GIOVANNA MUSCOGIURI, GIOVANNI CORONA and GIANCARLO BALERCIA: Vitamin D and Male Sexual Function: A Transversal and Longitudinal Study. International Journal of Endocrinology, 3720813, 8 pages, 2018.

63- SCHÄFFLER A., SCHÖLMERICH J. and BÜCHLER C.: Mechanisms of disease: Adipocytokines and visceral adipose tissue-emerging role in nonalcoholic fatty liver disease, Nat. Rev. Gastroenterol. Hepatol., 2 (6): 273, 2005.

64- SONG L., QU D., ZHANG Q., ZHOU H., JIANG R., LI Y., ZHANG Y. and YAN H.: Phytosterol esters attenuate hepatic steatosis in rats with non-alcoholic fatty liver disease rats fed a high-fat diet, Sci. Rep., 7: 41604, 2017.

65- FARHANGI M.A., MESGARI-ABBASI M., NAMENI G., HAJILUIAN G. and SHAHABI P.: The effects of vitamin D administration on brain inflammatory markers in high fat diet induced obese rats, BMC Neurosci., 18 (1): 81, 2017.

66- YUAN Y., DAS S.K. and LI M.: Vitamin D ameliorates impaired wound healing in streptozotocin induced diabetic mice by suppressing NF-ɪcB mediated inflammatory genes expression, Biosci. Rep., 38 (2): 1294, 2018.

67- WÖBKE T.K., SORG B.L. and STEINHILBER D.: Vitamin D in inflammatory diseases. Front Physiol., 5: 244-67, 2014.

68- MORIELLI T. and O'FLAHERTY C.: Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. Reproduction, 149: 113-123, 2015.

69- KUZ'MENKO A.I., MOROZOVA R.P., NIKOLENKO A. and DONCHENKO G.V.: "Vitamin D3 and 20-hydroxyecdysone-inhibitors of free radical lipid oxidation during D-hypervitaminosis". Ukr Biokhim Zh., 73 (3): 44-50, 1999.

70- TOSHIAKI HIRAI, AKIRA TSUJIMURA, TOMOHIRO UEDA, KAZUTOSHI FUJITA, YASUHIRO MATSUOKA, TETSUYA TAKAO, YASUSHI MIYAGAWA, NOBUO KOIKE and AKIHIKO OKUYAMA: Effect of 1, 25-dihydroxyvitamin D on testicular morphology and gene expression in experimental cryptorchid mouse: Testis specific cDNA microarray analysis and potential implication in male infertility. J. Urol., 181: 1487-1492, 2009.

مستحلب نانو فيتامين د٣ يخفف من ضعف الخصية فى حالات السمنة الناجمة عن ارتفاع نسبة الدهون فى النظام الغذائى المحدثه فى الفئران

تعتبر السمنة مشكلة صحية كبيرة قد تؤدى إلى العقم، وفيتا مين (د) له خصائص مضادة للالتهابات ومضادة للأكسدة وله دور فى تقليل السمنة، وتعتبر مستحلبات النانو نظام إيصال فعال للغاية للأدوية والمواد المغذية. يحتوى نانو فيتا مين (د) على قابلية للذوبان فى الماء وثبات أكبر مقارنة بفيتا مين د التقليدى.

\الهدف من الدراسة: تهدف الدراسة إلى تقييم الآثار الوقائية المحتملة للتدخل النانوى لفيتامين د٣ ضد بعض التغيرات التى تمت فى الخصية والتمثيل الغذائى والهرمونى فى الفئران المحدث لها سمنهعن طريق نظام غذائى غنى بالدهون.

\الطريقة: تم عشوائياً تقسيم ٣٢ من ذكور الفئران البيض بالتساوى إلى أربع مجموعات: المجموعة الأولى (المجموعة الضابطة فى النظام الغذائى القياسى)، المجموعة الثانية (نظام غذائى عالى الدهون وغير المعالجة)، المجموعة الثالثة (نظام غذائى عالى الدهون + فيتامين د٣ التقليدى) والمجموعة الرابعة (نظام غذائى عالى الدهون + نانو فيتامين د٣). بدأ اعطاء فيتامين (د) جنباً إلى جنب مع بداية إدخال نظام غذائى عالى الدهون. بعد ثمانية أسابيع تم قياس وزن الجسم وحساب مؤشر كتلة الجسم. وتم قتل الفئران وقياس مستويات هرمون تستوستيرون والأنسولين والجلوكوز فى الدم. تم حساب قيم المقاومة للأنسولين. بالإضافة إلى ذلك، تم قياس مستويات عوامل ومضادات الأكسدة ودلائل الالتهابات فى نسيج الخصية. كما تم فحص التغيرات فى الجوانب النسيجية للخصية. تم فحص وزن الخصية، ومعامل الخصية، وعدد الحيوانات المنوية ونسبة الحركة ونسبة الحيوانات المنوية الغير طبيعية.

\النتائج: أظهر العلاج بالنانو بفيتامين د٣ تخفيضات كبيرة فى مؤشر كتلة الجسم، الجلوكوز فى مصل الدم، مقاومة الأنسولين، الكوليسترول الكلى، الكوليسترول منخفض الكثافة، مع ارتفاعات ذات قيمة معنوية فى الكوليسترول عالى الكثافة، وزن الخصية، معامل الخصية، عدد الحيوانات المنوية، حركية الحيوانات المنوية، وهرمون التستوستيرون فى المصل. علاوة على ذلك، زاد نانو فيتامين د٣ بشكل كبير من مضادات الأكسدة وقلل من دلالات الأكسدة والإلتهابات. وقد ارتبط تحسين قياسات التمثيل الغذائى والكيمياء الحيوية التى سببها نانو فيتامين د٣ بتحسين التغيرات الناتجة عن السمنة فى أنسجة الخصية فى الفئران. ومن المثير للاهتمام، أن نانو فيتامين د٣ قد أظهر تأثيرات أكثر قيمة ضد خلل الخصية الناجم مقارنة بفيتامين د٣ التقليدى.

\الإستنتاج: يلعب دواء نانو فيتامين د٣ دوراً و قائياً محتملاً فى تحسين التأثيرات الضارة للسمنة الناجمة عن نظام غذائى عالى الدهون على وظائف الخصية، مع تأثيرات وقائية أكثر وضوحاً مقارنة بفيتامين د٣ التقليدى.