Original Paper

Protective Effects of Fluvastatin on Reproductive Function in Obese Male Rats Induced by High-Fat Diet through Enhanced Signaling of mTOR

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Key Words

Obesity • Spermatogenesis • Fluvastatin • mTOR • Proliferation

Abstract

Background: Statins can reduce reproductive damage induced by obesity or high-fat diet (HFD), but the specific regulatory mechanisms are largely unknown. Since mTOR/p70s6k signaling promotes spermatogonia proliferation and spermatogenesis, we hypothesized that this pathway will be involved in the protective effects of statin in HFD-induced reproductive dysfunction. **Methods:** Male Sprague Dawley rats (3 weeks old) were randomly divided into a control group (standard diet), HFD group, and a fluvastatin group (HFD + fluvastatin at 6mg/kg, once daily by oral gavage). After 8 weeks, body weight was obtained and rats were sacrificed. Weights of the testes, gross morphology, sperm parameters, circulating levels of sex hormones, lipid levels, and tissue mTOR, p-P70s6k were measured. Another set of male rats were treated with rapamycin or vehicle. Flow cytometry was used to detect the spermatogonia marker c-kit and cell cycle. p-P70s6k expression was analyzed by Western blot. **Results:** HFD not only results in rat obesity but also leads to spermatogenetic damage and fluvastatin was able to partially block the effects of HFD. Fluvastatin also partially reversed the suppression of mTOR and p-p70s6k expression. **Conclusion:** Our data suggest that fluvastatin has protective effects on reproductive function in obese male rats most probably through enhanced signaling of mTOR.

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Published by S. Karger AG, Basel**Introduction**

Obesity, as a consequence of high-fat diet (HFD) exposure, is a fast growing problem reaching epidemic proportions worldwide [1]. This is not only associated with a variety of chronic diseases, such as hyperlipidemia, diabetes mellitus, hypertension, but has also

been demonstrated to have hazardous effects on the reproductive system and fertility [2-5]. Moreover, obesity can affect male infertility by affecting spermatogenesis, thus reducing fertilization rate [5-9]. Spermatogenesis is highly complex and critical process for maintaining male fertility. The continuous self-renewal and differentiation of spermatogonial stem cells (SSC) is the most important step for maintaining sperm production, and this process involves numerous molecules and pathways [10, 11].

Previous studies have found that the mammalian target of rapamycin (mTOR) signaling pathway plays a vital role in spermatogenesis [12]. mTOR is a master signaling molecular in mammalian cells which regulates an array of cellular events, such as protein synthesis, cytoskeleton reorganization, and cell survival [12]. As a master regulator, mTOR regulates the downstream translation inhibitory molecule ribosomal protein S6 kinase p70s6k by interacting with growth factors, nutrient molecules, hormones and other proteins to control protein synthesis [12, 13]. However the role of mTOR in regulating spermatogenesis in diet-induced obesity has not been studied in great detail. Statins, important cholesterol lowering agents for patients at risk of cardiovascular disease [14] are known to offer protection against many of the deleterious effects of HFD. However, it is not clear if the same protection is extended to male reproductive functions in diet-induced obese rats. In the present study, we examined the effects of fluvastatin on spermatogenesis impaired by HFD and the possible role of the mTOR signaling pathway in mediating its protective effect.

Materials and Methods

Animals and diets

Male Sprague Dawley rats (3 weeks and 8 days old) were purchased from the Experimental Animal Center of Shanxi Medical University. All rats were maintained under standard laboratory conditions (12 : 12 light-dark cycle with humidity of 30-40% at 25±2°C). The noise was kept below 85 decibels, and the ammonia concentration was maintained at 20 parts per million. After acclimatization, 24 rats were divided into three groups of eight animals each and exposed to different diets ways. Rats in the control group received a standard diet (total energy = 420 kcal/100g diet; total protein = 18% of energy; total fat = 9% of energy), while the high-fat group received a high-fat diet (total energy = 515 kcal/100g diet; total protein = 18% of energy; total fat = 39% of energy). The full compositions of the standard and high-fat diet have been reported previously [15]. The third group (high-fat diet with fluvastatin) received high-fat food plus fluvastatin (Novartis, Basel, Switzerland) treatment (6mg/kg, once daily by oral gavage for 8 weeks); the control and high-fat groups received saline by oral gavage. Intake of energy, fatty acid, protein, and carbohydrate in rats was calculated during the study. Animals' weight was measured once weekly during the study. Another sixteen 8-week-old SD rats were randomly divided into two groups (blank and rapamycin group), with 8 rats in each group. The rapamycin group received intraperitoneal injections of 2mg/kg/day for 4 weeks. Rapamycin was purchased from Fujian Kerui Pharmaceutical Co., Ltd (Fujian, China). Ten 8-day-old male rats were used to isolate spermatogonia.

Acquisition of serum and tissue

Rats were sacrificed by cervical dislocation and blood was collected from the abdominal aorta through a syringe. After centrifugation (500×g, 4°C, 10min), serum and buffy coat were separated. The serum was stored at -80°C until further analysis. The testes of each rat were harvested and weighed. The testicle coefficient (g/kg) was defined as the weight ratio of the testes to the whole body.

Sperm analysis

The epididymides cauda were quickly placed in a Petri dish with 2ml of physiological saline and cut into pieces. The mixture was incubated for 15min at 37°C to release the sperm. A total of 20μl of suspension was transferred into 4ml of fresh physiological saline (diluted 200-fold) and incubated in a 37°C water bath for 1h. Sperm number was evaluated using a microscope Olympus CX41 (Olympus, Tokyo, Japan) with a Neubauer chamber at 1000× magnification. Sperm motility was expressed as a percentage of motile

spermatozoa of the total sperm counted under a microscopic field for 200 cells in the Neubauer chamber. Viability of sperm was determined with eosin staining. Sperms with normal (intact and typical rat sperms) and abnormal morphology (without head or tail coiled tail, and pinhead) were calculated to survey sperm morphology.

Isolation, culture and treatment of Spermatogonia

8-day-old male SD rats were sacrificed and the testes harvested. Rat spermatogonia were prepared and cultured as described previously [12]. Testes were excised, decapsulated and then transferred into 50 ml sterile tubes containing 35 ml of glycine buffer. Seminiferous epithelial cells were dispersed and separated by the method of Bellvé with minor modifications. Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 containing 5% horse serum (Hyclone, Logan, UT, USA), 10% fetal bovine serum, basic fibroblast growth factor and epidermal growth factor (Peprotech, Rocky Hill, CT, USA) was added to the cells which were cultured in a CO₂ incubator (3°C, 5% CO₂) for 48 h. The dispersed cells were washed twice and plated in rat tail collagen (type I)-coated six-well plates (Millipore, Billerica, MA, USA), successively. The cells were cultured in a CO₂ incubator (34°C, 5% CO₂) for 2 h. The non-adherent cells were transferred to new rat tail collagen (type I)-coated six-well plates and cultured 2h as above conditions. Spermatogonia were identified by flow cytometry as described below. Spermatogonial cells were treated with 50nM rapamycin (a specific mTOR inhibitor) for 24h, 0.5μM okadaic acid (Sigma-Aldrich, USA : phosphatase inhibitor, which can increase global cellular protein phosphorylation levels) for 12 or 24h [16]. Spermatogonia cells were harvested and washed twice with PBS, and protein was extracted as described below.

Flow cytometry

Spermatogonia cells were washed twice with PBS, and a fluorescein isothiocyanate labeled anti-c-kit antibody (Abcam ab24870, Cambridge, MA, USA) was added and incubated at 4°C for 30 min. Dead cells were excluded from analysis by forward-scatter gating. Samples were analyzed using FACSCalibur flow cytometer. A minimum of 20,000 events was acquired for each sample. C-kit expressing cells were spermatogonia.

Sex hormones and metabolic features

The hormone levels, including LH (luteinizing hormone, Elabscience, E-EL-R0026c), FSH (follicular stimulating hormone, Elabscience, E-EL-R0391c), T (testosterone, Elabscience, E-EL-0072c), and E₂ (estradiol, Elabscience, E-EL-0065c), were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Sandwich ELISA protocols were used, and calibration was done with standard LH, FSH, T and E₂. Intra- and inter- assay coefficients of variation (CV) for hormone assays were FSH: 3.8 and 7.2%, LH: 3.9 and 7.1%, T: 4.2 and 7.2%, and E₂: 6.8 and 8.9%, respectively.

Serum levels of TG (triglyceride), TC (cholesterol), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol) were measured by BeckmanCx5PRO automatic biochemical analyzer (Beckman Coulter, Inc. Brea, California, USA).

Quantitative real-time PCR analysis

Quantitative real-time PCR assay was used to assess the transcriptional expression of *mTOR*. Total RNAs were extracted from spermatogonia cells or testicular tissues, and reverse-transcribed with a kit from TaKaRa (Otsu, Japan) using a SYBR Green dye kit (TaKaRa) as described previously [17]. β-actin was used as the endogenous housekeeping gene to normalize the mRNA levels. The specific primers were: *mTOR*, 5'- CACATCACTCCCTTCACCA-3' (forward) and 5'- GCAACAACGGCTTTCCAC-3'; β-actin, 5'-CCTGAGGCTCTTTTCCAGCC-3' (forward) and 5'- TAGAGGTCTTTTACGGATGTC AACGT-3' (reverse). The results were expressed as the mean of 2-ΔΔCt (calculated according to the manufacturer's instructions)±standard deviation.

Histological examination

Testicular tissues were fixed in 10% formalin, embedded in paraffin, cut into 5-μm sections, and then deparaffinized and rehydrated. Subsequently slides were stained with hematoxylin and eosin. Images were acquired on BX40 microscope (Olympus, Tokyo, Japan).

Immunohistochemistry

For immunohistochemistry (IHC) analysis, the sections from the formalin fixed, paraffin-embedded tissues were deparaffinized in xylene and rehydrated in descending concentrations of ethanol. Subsequently slides were boiled for 10min in 0.01M citrate buffer to retrieve antigen and incubated with 0.3% H₂O₂ in methanol to block endogenous peroxidase. And the sections were incubated with the anti-mTOR (1:100, Protein Tech Group Inc. Chicago, IL, USA, cat. no. 20657-1-AP) followed by incubation with secondary antibody tagged with the peroxidase enzyme and were visualized with 0.05% DAB until the desired brown reaction product was obtained. Images were acquired on an Olympus microscope (BX40, Tokyo, Japan).

Western blot analysis

Western blot analysis was used to evaluate levels of mTOR, p70S6 and Phospho-p70S6. Briefly, the spermatogonia or testicular tissues were collected and lysed on ice in radio immunoprecipitation assay reagent (RIPA, P1003B, Beyotime Biotech, Shanghai, China). Samples containing equal amount of proteins were separated in 10% SDS-PAGE and blotted onto PVDF membranes. The membranes were blocked with 5% bovine serum albumin and incubated with anti-mTOR (Protein Tech Group Inc. Chicago, IL, USA, cat. no. 20657-1-AP), p70S6 (Protein Tech Group Inc. Chicago, IL, USA, cat. no. 14485-1-AP), Phospho-p70S6 (Cell Signaling Technology, Boston, MA, USA, #9234), or anti-β-actin antibody (Protein Tech Group Inc. Chicago, IL, USA, cat. no. 60008-1-Ig). All primary antibody dilutions were 1:1000. The preparations were then exposed to goat anti-rabbit antibody (1:2000 dilution, Protein Tech Group Inc. Chicago, IL, USA, cat. no. SA00001-2) conjugated with horseradish peroxidase. The proteins of interest were detected using Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analysis

All values in the text and figures are presented as the mean±standard deviation (SD), and statistically analyzed using one-way ANOVA followed by the Student-Newman-Keuls test. All the statistical analyses were performed using GraphPad Prism software (GraphPad Software, CA, USA). The differences were considered statistically significant when P<0.05.

Results

Changes in gross anatomy of the testes

The average final body weight and weight gain were significantly higher in the HFD group than in the control group. A tendency for decreased weight gain and body weight was observed in rats fed HFD supplemented with fluvastatin (Table 1); however, the opposite phenomenon was observed for testicle weight. The testes of the control group were oval

Table 1. Effects of fluvastatin on the body weight, weight gain, testicular weight, testicular coefficient, testicular cell, and sperm parameters after 8 weeks. All data are expressed as mean±S.E.M. ^aindicates a statistical difference when compared with the control group (P<0.05), ^bindicates a statistical difference when compared with HDF group (P<0.05)

	Control group (n=8)	HFD group (n=8)	Fluvastatin group (n=8)
Final body weight (g)	326.74±25.19	392.40±28.05 ^a	348.50±38.59 ^b
Weight gain (g)	128.55±14.27	187.09±22.84 ^a	144.50±25.31 ^b
Testicular weight (g)	2.63±0.37	1.88±0.31 ^a	2.43±0.27 ^b
Testicular coefficient (g/kg)	8.05±1.05	5.71±0.94 ^a	6.97±0.53 ^b
Spermatogenesis			
Spermatogonia /hpf	24.86±5.72	14.38±3.61 ^a	23.33±5.27 ^b
Leyding cells /hpf	7.99±0.39	4.05±0.13 ^a	6.01±0.82 ^b
Sertoli cells /hpf	9.15±0.76	5.16±0.59 ^a	8.06±1.05 ^b
Sperm parameters			
Concentration (×10 ⁶ /ml)	56.68±4.95	47.62±4.16 ^a	53.82±8.11 ^b
Vitality (%)	96.43±1.28	94.01±0.75 ^a	97.31±1.47 ^b
Motility (%)	67.92±6.22	54.38±5.24 ^a	66.05±5.08 ^b
Normal morphology (%)	85.36±2.07	80.19±1.73 ^a	86.02±2.26 ^b

Fig. 1. Morphological changes in the testes in male rats. Haematoxylin and eosin staining in three groups. The seminiferous tubules displayed normally in control and fluvastatin groups. The seminiferous tubules displayed atrophy and vacuolation in HFD group.

Table 2. Sex hormone and Lipid levels in the three groups. All data are expressed as mean±S.E.M. a indicates a statistical difference when compared with the control group ($P<0.05$), b indicates a statistical difference when compared with HFD group ($P<0.05$)

	Control group (n=8)	HFD group (n=8)	Fluvastatin group (n=8)
Lipid levels			
TG (mmol/l)	0.39±0.08	1.18±0.12 ^a	0.51±0.15 ^b
TC (mmol/l)	1.47±0.10	3.56±0.28 ^a	1.68±0.19 ^b
HDL-C (mmol/l)	0.96±0.05	1.69±0.14 ^a	1.04±0.10 ^b
LDL-C (mmol/l)	0.79±0.03	3.52±0.19 ^a	0.94±0.08 ^b
Sex hormone levels			
FSH (ng/ml)	6.04±0.83	6.99±1.20	6.40±1.88
LH (ng/ml)	2.11±0.69	1.74±0.55	1.71±0.57
T (ng/ml)	3.71±1.09	2.37±0.42 ^a	3.44±0.62 ^b
E ₂ (pg/ml)	9.17±1.23	15.83±2.72 ^a	10.29±1.11 ^b

and larger, with a smooth surface and plump appearance. They were opaque white and elastic with clear vascular texture. In contrast, the testes of the HFD group were atrophied, with visible differences observed between the HFD ± fluvastatin groups. Compared with the control group, the testicular coefficient of the HFD group was lower (5.71 ± 0.94 vs 8.05 ± 1.05 , $P<0.05$), and fluvastatin treatment provided significant protection against the testicular atrophy observed in the HFD group (5.71 ± 0.94 vs 6.97 ± 0.53 , $P<0.05$, Table 1).

HFD significantly decreases sperm production

Compared to the control and fluvastatin groups, the concentration, viability, and motility of sperms were significantly reduced in the HFD group and had abnormal morphology ($P<0.05$). Apart from sperm concentration, there were no significant differences in those indices between fluvastatin and control groups (Table 1). Haematoxylin and eosin staining also revealed that the size of the seminiferous tubules decreased and appeared vacuolated in the HFD group (Fig. 1). Additionally the size of seminiferous tubules in the fluvastatin group was increased compared with the HFD group and vacuolation of the seminiferous tubules was rescued to some extent (Fig. 1). Compared with control group, the average number of spermatogonia, sertoli cells, and Leydig cells was significantly reduced in the HFD group ($P<0.05$), while fluvastatin treatment protected testicular tissue from the damage caused by the HFD (Table 1).

Changes in sex hormones and lipid levels

SD rats fed HFD had presented significantly increased serum levels of TG, TC, HDL-C and LDL-C accompanied by body weight gain. After fluvastatin intervention, all these indexes of metabolic abnormalities decreased, and no significant differences were observed between the fluvastatin and control groups (Table 2). Furthermore, abnormalities in serum sex hormone levels were observed, with obviously decreased T and increased E₂ levels in the

Fig. 2. The expression of mammalian target of rapamycin (mTOR) and its targeted protein p70s6k in three groups. (A) Quantitative RT-PCT analysis data showed that mRNA levels of mTOR in testicular tissue treated with HFD were significantly decreased compared to control and fluvastatin groups. (B) mTOR immunohistochemistry in the testis in three groups. (C) a quantitative analysis of mTOR immunohistochemistry integrated option density (IOD) by Image-Pro Plus (IPP). Values are mean $\pm$ SD. (D) The protein expression of mTOR and p70s6k in rat testes as determined by Western blot. (E) the ratio of phospho-p70s6k/p70s6k was significantly lower in HFD group than control and fluvastatin groups. * P <0.05 compared with control group.

HFD group (P <0.05). However, there were no significant differences in the levels of FSH and LH among the three groups (Table 2).

Changes in mTOR and p-p70s6 expression

Analyses using quantitative RT-PCR (Fig. 2A), and Immunohistochemistry (Fig. 2B and C) showed that mTOR was primarily expressed in spermatogonia and was weakly expressed in HFD group. No significant differences were observed between the HFD+fluvastatin and control groups. Although the expression of mTOR target p70s6k did not obviously change, the phosphorylated form p-p70s6 significantly decreased in the HFD group and there were no significant differences between HFD+fluvastatin and control group (Fig. 2D and E). These results demonstrate that the reduction of spermatogenesis induced by obesity was related to the inhibition of mTOR expression, and fluvastatin was able to improve spermatogenesis by increase the expression of mTOR.

mTOR regulates p70s6 activation in spermatogonia after rapamycin

Haematoxylin and eosin staining also revealed that the seminiferous tubules size decreased and seemed vacuolated after rapamycin treatment (Fig. 3A). Moreover, after rapamycin treatment, the expression levels of p-p70s6k were significantly decreased in the testis, whereas p70s6k expression did not change (Fig. 3B and C). To further confirm that

Fig. 3. Morphological changes in the testes and the activation of p70s6 activation in spermatogonia after rapamycin treatment. (A) Haematoxylin and eosin staining in Rapamycin and control groups. The seminiferous tubules displayed normally in control. The seminiferous tubules displayed atrophy and vacuolation in rapamycin group. (B) Subsequently, the protein expression patterns of mTOR and its target by western blot were examined. (C) After rapamycin treatment, the ratio of p-p70s6k was significantly decreased in the testis, whereas p70s6k expression did not change. * $P < 0.05$ compared with control group. (D) Optical microscopy showed that spermatogonial cells were round or oval and flow cytometry analysis using c-kit surface expression. (E) Flow cytometry results showed that spermatogonial cells were blocked in G1 phase after rapamycin treatment compared with blank and DMSO groups. (F) A quantitative presentation of the data shown in E. (G) The protein expression of mTOR and p70s6k in spermatogonia determined by Western blot. (H) p-p70s6k expression level was significantly down-regulated in the rapamycin group, however significantly up-regulated in okadaic acid group ($P < 0.05$). * $P < 0.05$ compared with control group.

mTOR regulates spermatogonial proliferation by controlling downstream target activation, spermatogonia were cultured *in vitro* and treated with 50nM rapamycin for 24h with 0.5 μ M okadaic acid. Optical microscopy showed that spermatogonial cells were round or oval (Fig. 3D). The expression of the spermatogonia marker c-kit reached 88% (Fig. 3D).

Flow cytometry results showed that the percentage of spermatogonial cells in G1 phase significantly ($P < 0.05$) increased after rapamycin treatment compared with the control group, suggesting that cell proliferation was blocked (Fig. 3E and 3F). As shown in Fig. 3G and 3H, p-p70s6k expression level was significantly downregulated in the rapamycin group, however significantly upregulated in okadaic acid group ($P < 0.05$). Therefore, mTOR is likely involved in the spermatogonial cell proliferation process through the regulation of p70s6k.

Discussion

Obesity, which is one of the most widespread metabolic disorders in contemporary society, has become a challenge for the developed and developing societies of the world [18-20]. It is closely related to many diseases such as cardiovascular diseases, insulin resistance, hypertension and endocrine disorders. Furthermore, in recent years, growing evidence suggests that men who are obese due to consumption of HFD have lower quantity and quality sperm, which result in low fertility rate, in comparison with normal weight men [21-23]. Weight loss in obese patients can improve or prevent many of the obesity-related risk factors for reduced spermatogenesis and can improve semen quality [24].

Statins are the most effective cholesterol-reducing agents, which act by inhibiting intracellular hydroxyl-methyl glutaryl coenzyme A (HMG-CoA) reductase, the key enzyme for cholesterol biosynthesis [25-28]. Recently, statins have been found to exert biological effects on osteoporosis, inflammation and other diseases in addition to their hypocholesterolemic activities [29-31]. Furthermore, several studies have shown that treatment with statins can reduce reproductive damage induced by hypercholesterolemia, however, the mechanisms by which obesity impairs spermatogenic function and how statins help recover from obesity-induced damage in the testes, sperm parameters, sex hormones, and metabolism is still unclear [32]. Here we provide data supporting a protective role for fluvastatin in reproductive damage in HFD-induced obese male rat model and the possible role of mTOR signaling in these effects.

In the present study, we investigated the gross morphology and HE staining of testicular tissues and the results showed that HFD not only results in rat obesity but also leads to atrophy of the testes. This was supported by the reduction in the testicular weight and coefficient in the HFD group compared with the control group, which was consistent with the Yan et al.'s findings [33]. Furthermore, the decreased concentration, viability, motility, and morphology of sperm in HFD rats indicated poor sperm quality. Moreover, the number of spermatogonia, Leydig cells, and Sertoli cells in the fluvastatin group was significantly higher than that in the HFD group. This suggests that fluvastatin may have a protective effect against HFD induced testicular damage. In spermatogenesis, FSH, LH, and testosterone are the pivotal endocrine factors controlling testicular functions. Our study showed that with the increased body mass, the serum levels of hormones such as E2 increased in the HFD group, while T decreased, indicating that obesity impairs male reproductive function by disrupting the homeostasis of these hormones. This is supported by studies in obese men where T levels decrease and E2 levels increase most probably due to increased aromatase activity [34]. Furthermore, after fluvastatin treatment, the hormone concentrations were restored to close to normal levels. The data suggests that fluvastatin may have certain maintenance effect on hormone levels disrupted by HFD.

Mammalian target of rapamycin (mTOR) is a serinethreonine protein kinase that belongs to the phosphatidylinositol kinase-related kinase family, regulated by proline [35-37]. One study has found that mTOR plays crucial role in the normal spermatogenesis process and can preferentially regulate p70s6k rather than 4e-bp1 to promote cell proliferation [12, 38, 39]. Simvastatin has been shown to induce proliferation, migration and reduce apoptosis of endothelial cells via mTOR signaling pathways [40]. We hypothesized that fluvastatin may act through similar mechanisms on spermatogonia in our model. In our study, we found that mTOR and p70s6k, was primarily expressed in spermatogonia in control animals and was weakly

expressed in the HFD group. Fluvastatin intervention partially improved the expression of mTOR and p70s6k in HFD exposed animals. These results indicate that the reduction of spermatogenesis induced by obesity was related to the inhibition of mTOR expression, and fluvastatin can improve spermatogenesis through improving the expression of mTOR. In addition, we cultured spermatogonia *in vitro*, and the HFD groups had a significant number of cells blocked in the G1 phase compared to the control group. When spermatogonia were treated with mTOR inhibitor, rapamycin, p-p70s6k expression was downregulated, however treatment with the global phosphorylator okadaic acid was able to reverse this effect. These results confirmed that mTOR is likely involved in the spermatogonial cell proliferation process through the regulation of p70s6k supporting a previous study [12].

In summary, the present study indicates that obesity induced by HFD can cause detrimental effects on spermatogenesis, semen quality, endogenous hormone levels, and apoptosis of testicular cells in rats. Earlier studies have suggested that treatment with statins can reverse this phenomenon [28]. Fluvastatin intervention improves spermatogenesis in obese male rats, possibly due to its effect on signaling of mTOR. Further studies are needed to validate the mechanisms of fluvastatin and mTOR signaling pathway on testicular spermatogenic function. Meanwhile, the influence of other mechanisms of HFD and fluvastatin, such as hormones changes, caloric restriction, reactive oxygen species (ROS) and other metabolic changes, on male reproductive function should also be investigated. These issues will be addressed systematically in subsequent studies.

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Disclosure Statement

There are no conflicts of interest.

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