Original Article

Effect of Huaji Jianpi Decoction on the semen quality of high-fat diet-induced obese mice

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Background: At present, the incidence of obesity is increasing. Several studies have shown that obesity can reduce male fertility by affecting spermatogenesis and semen quality. Traditional Chinese medicine (TCM) has fewer side effects and stable efficacy in the treatment of related diseases. This study aimed to investigate the effect of Huaji Jianpi Decoction on the semen quality of high-fat diet-induced obese mice.

Methods: The obese male mice model was constructed by using high-fat diet and the Huaji Jianpi Decoction was processed into an aqueous extract. Mice were allocated in the normal group (n=30) and five different treatment groups (n=50). Huaji Jianpi Decoction was applied in low-, medium- and high-dose [17.52 g/(kg·d), 35.04 g/(kg·d) and 70.07 g/(kg·d), respectively]. The body weight, body fat, testis wet weight, testis coefficient, and routine sperm parameters were detected and analyzed. Meanwhile, transmission electron microscope (TEM) was used to observe testis ultrastructure. reverse-transcription quantitative PCR (RT-qPCR) was used to measure the expression of tumour necrosis factor α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1).

Results: Compared with normal mice (ND), the testis wet weight and testis coefficient of mice in the blank group were significantly decreased, while the number of mitochondria was observed to be decreased on testis ultrastructure examination, and apoptotic cells and germ cells in the spermatogenic tubules were shed. After Huaji Jianpi Decoction administration, the body fat and blood lipid levels of obese mice were decreased, the testis wet weight and testis coefficient were increased, and semen parameters were increased. Different doses of Huaji Jianpi Decoction could improve testicular weight, sperm density, sperm motility, forward motility, and total sperm motility. Huaji Jianpi Decoction could also downregulate TNF-α and MCP-1 expression and inhibit germ cell apoptosis to improve semen quality.

Conclusions: Huaji Jianpi Decoction can improve the semen quality of high-fat diet-induced obese mice by reducing weight and lipid levels.

Keywords: Huaji Jianpi Decoction; obese mice; semen quality; tumour necrosis factor α (TNF-α); monocyte chemoattractant protein-1 (MCP-1)

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Introduction

The obesity rate has risen in recent years, and more than 10% of adults worldwide meet the obesity criteria (1). Obesity has become a global health problem. According to statistics, 1 in 25 obese adult men suffer from male infertility. Moreover, the incidence of infertility in obese and overweight men is ever increasing. Obesity leading to reduced male fertility has become a hot issue in current research. Recently, scholars have proposed that obesity reduces male fertility by affecting spermatogenesis and semen quality (2,3), mainly through changes in testicular weight, spermatogenic tubule diameter, and number of spermatogenic cells, as well influencing semen parameters (such as the decrease of sperm count, density, and viability) (4). Currently, the treatment of male hypogonadism is mostly through western medicine, but long-term use exhibits serious side effects. Therefore, traditional Chinese medicine (TCM), with low side effects and stable efficacy, is considered to be a more effective alternative treatment option. Nevertheless, the process of TCM intervention involves various components, pathways, and targets, and the interaction network of corresponding functional protein targets is complex (5). Huaji Jianpi Decoction is a kind of TCM made with 14 herb ingredients which can be used to invigorating spleen for eliminating dampness according to ancient Chinese prescriptions.

At present, no unified criteria for evaluating TCM treatment of obesity-induced reproductive impairment has been established. In this study, by applying the high-fat diet-induced obese mice model, whether Huaji Jianpi Decoction could lower lipids to improve the semen quality of obese male mice was investigated, and the effect of Huaji Jianpi Decoction on the treatment of obesity-induced low fertility was explored, with an aim to provide basic information for the treatment of obesity and obesity-induced low male fertility by TCM. We present the following article in accordance with the ARRIVE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-115/rc).

Methods

Ethical statement

All animal research was approved by the Ethics Committee of Hebei University (approval ID: IACUC-2021XS038). The animal research was performed in compliance with the Hebei University Laboratory Animal Welfare and Ethics guidelines of the Animal Welfare and Ethical Committee of Hebei University.

Huaji Jianpi Decoction preparation

The Huaji Jianpi Decoction included stir-fried Atractylodes (30 g), stir-fried Citrus aurantium (15 g), stir-fried Atractylodes Atractylodes (30 g), Poria cocos (15 g), French Pinellia (10 g), tangerine peel (10 g), Codonopsis (15 g), Jiao Hawthorn (15 g), Jiao Shenqu (15 g), Jiao Malt (15 g), stir-fried Coix seed (30 g), fried Alisma (15 g), lotus leaf (10 g), and roasted licorice (6 g). First, all medicinal materials were mixed using a Waring blender, soaked in double-distilled water (50 times the medicinal materials) for 2 h, and boiled for 1 h. After cooling, the decoction was filtered through 2 layers of cotton gauze. The filtrates obtained from 3 cycles of the procedure were combined. After filtration, the solution was concentrated into a residue in a vacuum evaporator and distilled into liquid. The yield of the aqueous extract was 3.85 g/mL and the volume was 60 mL (based on the original amounts of the herbal ingredients). After preparation, the reagents were put into glass bottles and stored at 4 ℃.

Animal models

A total of 100 specific pathogen-free (SPF)-grade healthy 3-week C57BL/6J male mice weighed about 15 g (The Charles River Laboratories, Beijing, China) were adaptively bred for 1 week and randomly allocated into the normal group (30 mice on a normal diet) and the model group (70 mice on a high-fat diet). Food intake and water consumption were recorded daily. After 10 weeks of high-fat diet exposure, the obese mice were screened by weighing mice and calculating Lee's index. Obesity was defined as a 20% increase in weight compared with the normal group. Moreover, micro-CT was applied to further confirm the total volume of the adipose tissue. The high-fat feed ingredient was D12492 (Research Diets, Inc.; New Brunswick, USA), including 20 kcal% protein, 20 kcal% carbohydrate, and 60 kcal% fat. The ordinary feed was purchased from Weitonglihua (Beijing Weitong Lihua Laboratory Animal Technology Co., LTD.; No. 1184, Baishan Village, Baishan Town, Changping District, Beijing, China).

Drug intervention

A total of 50 obese model mice were selected and randomly allocated into the model blank group, orlistat group [0.091 g/(kg·d) dose], low-dose TCM group [17.52 g/(kg·d) dose], medium-dose TCM group [35.04 g/(kg·d) dose], and

high-dose TCM group [70.07 g/(kg·d) dose]. The dose of Huaji Jianpi Decoction was determined by converting the dose of human in to mice according to the body surface area. Mice in the drug experimental groups were given corresponding drugs, and mice in the normal group and the model blank group were given corresponding volumes of distilled water by oral gavage at 08:00 every day for 6 weeks. The dose was adjusted according to the weekly weight changes of mice. During the administration period, mice in the drug experimental group were still fed with a high-fat diet. Drugs were administered by gavage after 20 min in a water bath at 37 ℃. After administration, the drugs were stored at 4 ℃.

Testis coefficient evaluation in mice

After 6-week intragastric administration, the mice were sacrificed by the cervical dislocation method 2 h after the last administration. Subsequently, the mice were fixed, and their abdomens were sprayed using 75% alcohol. After disinfection, the abdominal cavity of the mice was quickly opened. Both testicles were taken, around which the fatty tissue was cleaned up. An electronic balance was used for weighing, and the wet testis weight of the mice was measured to calculate the testis coefficient. The testis coefficient was defined using the following formula: testis coefficient = (testis weight/mice weight) × 100%. The tissue was stored in a freezer at –80 ℃ for subsequent use.

Semen detection in mice

After intragastric administration for 6 weeks, the mouse epididymal tail tissue was obtained. The removed epididymal tail was washed twice in PBS solution and placed in 0.9% NaCl solution. The semen was squeezed out using tweezers under a stereo microscope (Olympus, Japan), followed by 30 min incubation in an incubator. After full dissociation, the sperm was placed on a counting plate (pre-placed on a 37 ℃ constant temperature plate in advance). The sperm suspension incubated in the 37 ℃ cell incubator (Thermo Scientific, USA) was taken out and mixed thoroughly. Next, 10 μL of semen was dropped on the sperm counting plate. The glass slide was covered and placed on the thermostatic plate. More than 200 sperm were observed and counted under the microscope. The sperm quality analysis system was used to detect the sperm indicators.

Transmission electron microscope (TEM) observation

The mouse testis tissues were placed on ice cubes and quickly cut into 1 mm^3 pieces. Subsequently, mouse testis tissues were fixed in 2.5% glutaraldehyde solution for 2 h, washed 3 times with phosphate buffer saline (PBS) (0.1 mol/L), and added with 1% osmium acid for 1 h fixation. After 3 washes with PBS (0.1 mol/L), the tissues were dehydrated using gradient alcohol (30%, 50%, 70%, 85%, 95%, and 100%) and acetone, and embedded in Epon 812. The semi-thin sections (0.5–2 nm) were stained with toluidine blue to observe mouse testis morphological changes under an optical microscope. After ultrathin sectioning, copper netting, and lead citrate and uranyl acetate staining, testis ultrastructure was observed using a TEM (Japan Electronics Corporation, Japan).

Reverse-transcription quantitative PCR (RT-qPCR)

Total RNA from mouse testis tissues was extracted using TRIpure Reagent Total RNA Extraction Reagent (Beijing Adler Biotechnology Co., Ltd., China) as per the manufacturer's instructions. RT-qPCR was performed for expression level analysis using the 5× HiScript II Select qRT SuperMix II and AceQ qPCR SYBR Green Master Mix (Vazyme, China) with the 7300 Real-Time PCR System (Applied Biosystems, USA). The data were normalized to β-actin expression and then further normalized to the negative control unless otherwise indicated. Custom primers for C/EBP-α, PPAR-γ, tumour necrosis factor α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) were synthesized as follows: β-actin forward primer 5'-ACTCATCGTACTCCTGCTTGCTGA-3' and reverse primer 5'-AGGGAAATCGTGGGTGACATCAAA-3'; C/EBP-α forward primer 5'-CCTTCAACGACGAGTTCCTG-3' and reverse primer 5'-TGGCCTTCTCCTGCTGTC-3'; PPAR-γ forward primer 5'-CTGGCCTCCCTGATGAATAA-3' and reverse primer 5'-GGCGGTCTCCACTGAGAATA-3'; TNF-α forward primer 5'-CAGATTGACCTCAGCGCTGAGTTG-3' and reverse primer 5'-ACCCTCACACTCAGATCATCTTCT-3'; MCP-1 forward primer 5'-GGGATCATCTTGCTGGTGAA-3' and reverse primer 5'-AGGTCCCTGTCATGCTTCTG-3'. The three-step method was used. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

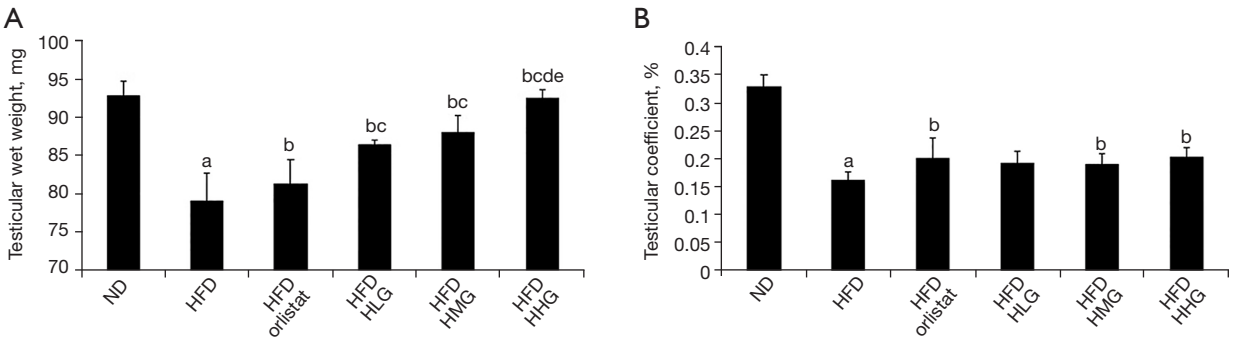

Figure 1 Testicular wet weight and testicular coefficient were detected in this study. (A) Huaji Jianpi Decoction effect on the testicular wet weight. (B) Huaji Jianpi Decoction effect on testicular coefficient. a, P<0.05 *vs.* the normal group; b, P<0.05 *vs.* the model blank group; c, P<0.05 *vs.* the orlistat group; d, P<0.05 *vs.* the low dose group; e, P<0.05 *vs.* the medium dose group. ND, normal group; HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group.

Statistical analysis

All data were statistically analyzed using SPSS 19.0 software. The measurement data were represented as mean ± standard deviation ($\bar{x}\pm s$). Comparisons between groups were analyzed using one-way analysis of variance, and comparisons among multiple groups were analyzed using the LSD method. The difference was statistically significant when P<0.05.

Results

Huaji Jianpi Decoction could improve the semen quality of obese mice

As indicated by the results of the animal model, obese mice showed a much lower testicular wet weight and testicular coefficient than normal mice (ND) (P<0.05). Sperm density, sperm motility, sperm forward motion force (PR), and total sperm motility (PR + NP) of mice in the model blank group were all lower than those in the normal group (P<0.05).

The testicular wet weight and testicular coefficient were strongly associated with semen quality. Compared with mice in the control group (HFD), the testicular wet weight and testicular coefficient of mice with drug administration were increased to varying degrees (P<0.05). No difference in testicular wet weight was found between the low-dose (HFD + HLG) group and the medium-dose (HFD + HMG) group (*Figure 1A*). However, the testicular wet weight in the remaining groups showed differences, with the high-dose group (HFD + HHG) showing more obviously increased testicular wet weight (P<0.05). There was no difference in testicular coefficient between the orlistat group (HFD + orlistat) and the Huaji Jianpi Decoction group (P>0.05) (*Figure 1B*).

Based on the results in this study, the sperm density of mice with drug administration was increased to varying degrees relative to those in the HFD group (P<0.05) (*Figure 2A*). The sperm density of mice in the HFD + HHG, HFD + HMG, and HFD + HLG groups was higher than that in the HFD + orlistat group (P<0.05), while compare with other groups, only the difference was observed between the Huaji Jianpi Decoction groups, HFD + HMG, and HFD + HHG groups. Mice in the HFD + HHG group showed more obvious sperm density improvement (P<0.05) (*Figure 2B*). The sperm motility of the HFD + orlistat group showed no difference to that of the HFD group (P>0.05), but the Huaji Jianpi Decoction groups exhibited much higher sperm motility than that of the HFD group (P<0.05). No difference among the Huaji Jianpi Decoction groups was found (P>0.05). Compared with the ND group, the PR of the drug administration groups was improved to varying degrees. In addition to the HFD + orlistat group and the HFD + HMG group (P>0.05), the drug groups showed different PR. The HFD + HHG group exhibited more obvious effects (P<0.05) (*Figure 2C*). The PR + NP of the HFD + orlistat, HFD + HMG, and HFD + HHG groups was notably higher than that in the HFD group (P<0.05). The PR + NP of the HFD + orlistat group was different from that of the HFD + HMG and HFD + HHG groups (P<0.05), and the HFD + HHG group showed a more significant increase in PR + NP (P<0.05) (*Figure 2D*).

In summary, Huaji Jianpi Decoction administration caused increased testicular wet weight, testicular coefficient,

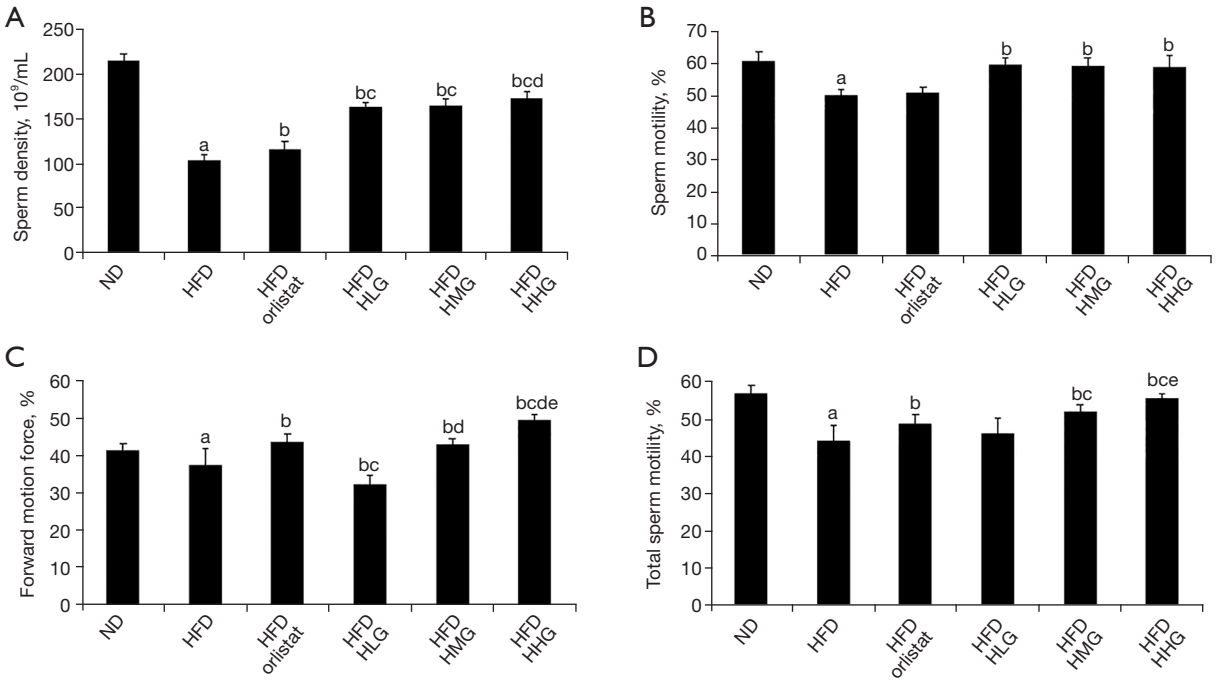

Figure 2 Semen parameters after Huaji Jianpi Decoction administration were detected in this study. (A) Huaji Jianpi Decoction effect on sperm density. (B) Huaji Jianpi Decoction effect on sperm motility. (C) Huaji Jianpi Decoction effect on forward motion force. (D) Huaji Jianpi Decoction effect on total sperm motility. a, P<0.05 *vs.* the ND group; b, P<0.05 *vs.* the HFD group; c, P<0.05 *vs.* the HFD + orlistat group; d, P<0.05 *vs.* the HFD + HLG group; e, P<0.05 *vs.* the HFD + HMG group. ND, normal group; HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group.

and semen parameters. Huaji Jianpi Decoction could also improve testicular weight, sperm density, sperm motility, forward motility, and total sperm motility.

Huaji Jianpi Decoction could reduce the Morphological structure of spermatogenic cells and seminiferous tubules in obese mice

As shown in *Figure 3*, the male mice of each group showed the complete basic structure of the seminiferous tubules with no obvious tissue damage. The substrate membrane of the seminiferous tubule (whose tube cavity has 5–8 layers of cells) was relatively complete. All levels of sperm cells closely connected to supporting cells were clear in all groups except for the HFD group. The HFD group exhibited decreased cell layers in the seminiferous tubule, accompanied by ambiguous and disordered structure of sperm cells at all levels, and supporting cells are lost to apoptosis (*Figure 3B*).

As shown in *Figure 4*, the seminiferous tubules surrounded by Myoid cell substrate membrane and connected to Sertoli cells and spermatogenic cells were thin and regular in the ND (*Figure 4A*), HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups (*Figure 4C-4F*). Compared with the ND group, the HFD (*Figure 4B*) group showed more obvious microstructural changes (severely damaged, thickened, and irregular substrate film). Meanwhile, a wide range of cell gaps was observed in the luminal epithelial cells, which caused cellular separation and destruction. Large areas where cytoplasm was not present were found in luminal epithelial cells, showing swollen mitochondria and cytoplasm with empty bubbles.

Compared with the ND group (*Figure 5A*), a wide space around the nucleus and dramatically decreased mitochondrial numbers were observed in the HFD group (*Figure 5B*). According to our results, mitochondria were observed in the cytoplasm of spermatogonia with an obvious nucleolus surrounded by chromosome clumping (as shown by the black arrow) in the ND, HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups (*Figure 5C-5F*). No other obvious differences were observed in addition to these differences.

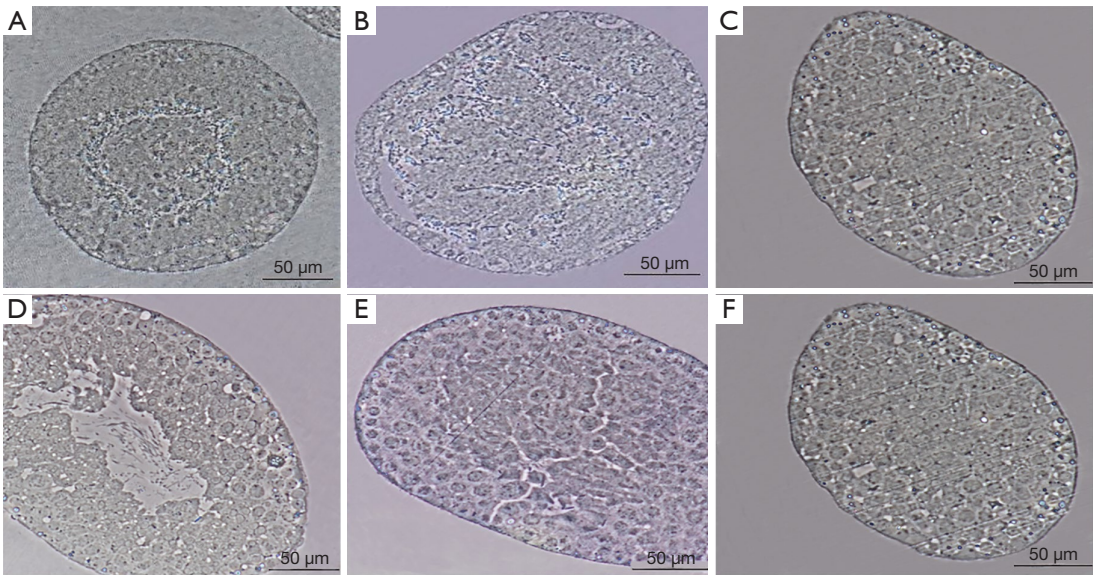

Figure 3 The morphology of the seminiferous tubules of mice in each group was observed. Semithin section of testicular tissue under the transmission electron microscope (toluidine blue staining method, ×400) was observed. (A) The substrate membrane of seminiferous tubules was intact; the epithelium of spermatogenic cells was thick; the boundaries of spermatogenic cells at all levels are obvious. (B) The epithelium of spermatogenic cells thinning was observed; the spermatogenic cells loosely peel off. (C-F) The basic structure of the seminiferous tubules was complete, showing no obvious tissue damage and clear spermatogenic cells at all levels.

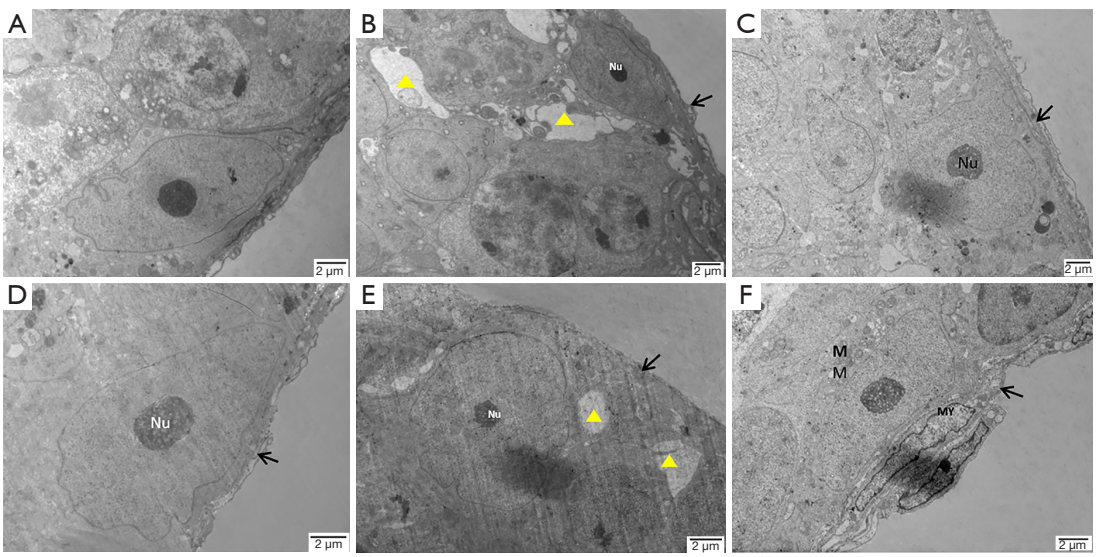

Figure 4 Ultrastructure of substrate membrane and supporting cells of seminiferous tubule. Ultrastructure of substrate membrane and supporting cells of seminiferous tubule under the transmission electron microscope (×400) was observed (A) The seminiferous tubules were surrounded by the thin and regular substrate membrane and myoid cells. A large number of mitochondria in cytoplasm and irregular prominent nucleolus could be observed. (B) There were wide cell gaps, thickened substrate film (As shown by the black arrow), and cytoplasm with empty bubbles. There was no significant change in the nucleolus of supporting cells. (C-F) The ultrastructure of substrate membrane and supporting cells of seminiferous tubule in the HFD + orlistat, HFD + HLG, HFD + HMG and HFD + HHG groups. HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group. The yellow triangles stand for lipid vacuoles, Nu (nucleolus; irregular cell nucleolus) and M (mitochondria) in the legend.

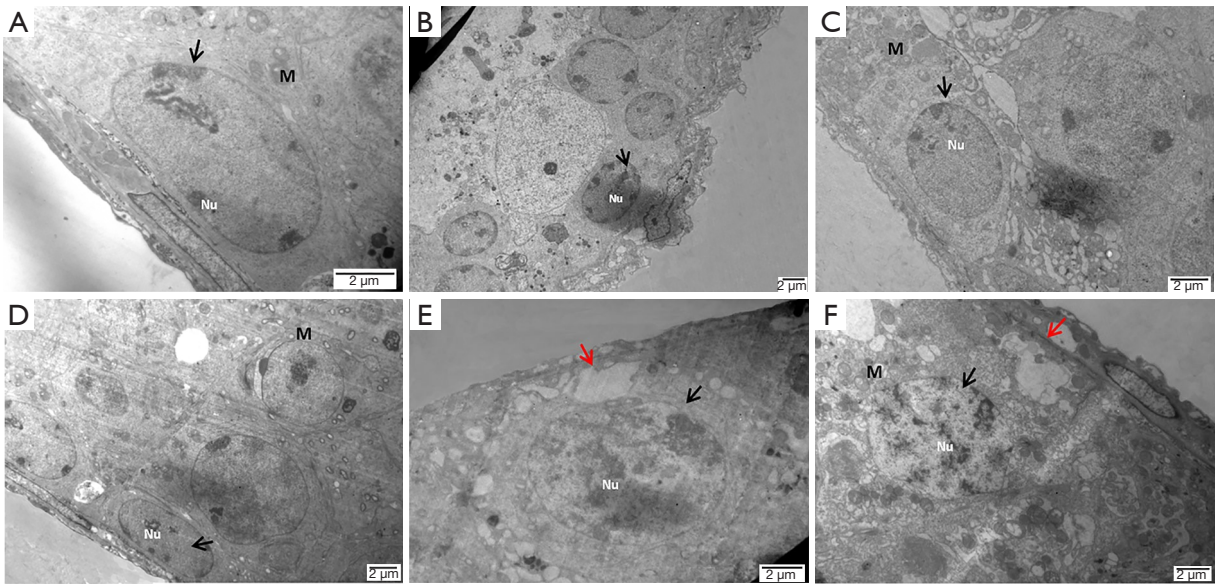

Figure 5 Ultrastructure of spermatogonia in the seminiferous tubule. Ultrastructure of spermatogonia in the seminiferous tubule under the transmission electron microscope (×400) was observed. (A) Mitochondria were observed in the spermatogonia with obvious nucleolus surrounded by chromosome clump (as shown by the black arrow). (B) The spermatogonia with whose more complete nucleus have less mitochondria. The substrate film with an irregular shape has wide cell gaps (as shown by the red arrow). (C-F) The substrate film was relatively smooth and neat, but there were still empty bubbles (as shown by the red arrow). The black arrows stand for thickened basal tubules, Nu (nucleolus; irregular cell nucleolus) and M (mitochondria) in the legend.

The ultrastructure of rounded sperm cells showed similar results to that of spermatogonia. Compared with the ND group (*Figure 6A*), in the HFD group (*Figure 6B*), early sperm cells exhibited malformation and an area without cytoplasm due to the lack of an acrosome cap. Early sperm cells whose proximal part was covered by an acrosome cap presented as a circle in the ND HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups (*Figure 6C-6F*).

According to the results of the ultrastructure of rounded sperm cells, compared with the HFD group, the proximal part of the round nucleus of early sperm cells was covered by a vesicle, and small empty bubbles were observed in the cytoplasm of other groups (*Figure* 7).

The head of long-form sperm cells showed an abnormal acrosome in the HFD group (as shown by the asterisk), while the sperm nucleus was normal. Compared with that in the ND group, no difference in the structure of long-form sperm cells was found in the HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups (*Figure 8*).

Huaji Jianpi Decoction downregulated the expression of TNF-α and MCP-1

The expression of TNF-α and MCP-1 was improved in the HFD group ($P<0.05$) compared with the ND group. No difference in MCP-1 expression was observed between the HFD and HFD + HLG groups ($P>0.05$). The expression of TNF-α and MCP-1 was decreased to varying degrees in the HFD + orlistat, HFD + HMG, and HFD+HHG groups ($P<0.05$). No difference was found in TNF-α mRNA expression between the HFD + orlistat and HFD + HLG groups, and the HFD + HMG and HFD + HHG groups ($P>0.05$). Nevertheless, there was a difference in the expression of TNF-α mRNA between the drug administration groups, with the HFD + HHG group showing a more obvious reduction ($P<0.05$). There was no difference in the expression of MCP-1 mRNA in the HFD + orlistat and HFD + HHG groups ($P>0.05$). Most obviously, the expression of TNF-α and MCP-1 mRNA was reduced in the HFD + HHG group (*Figure 9*).

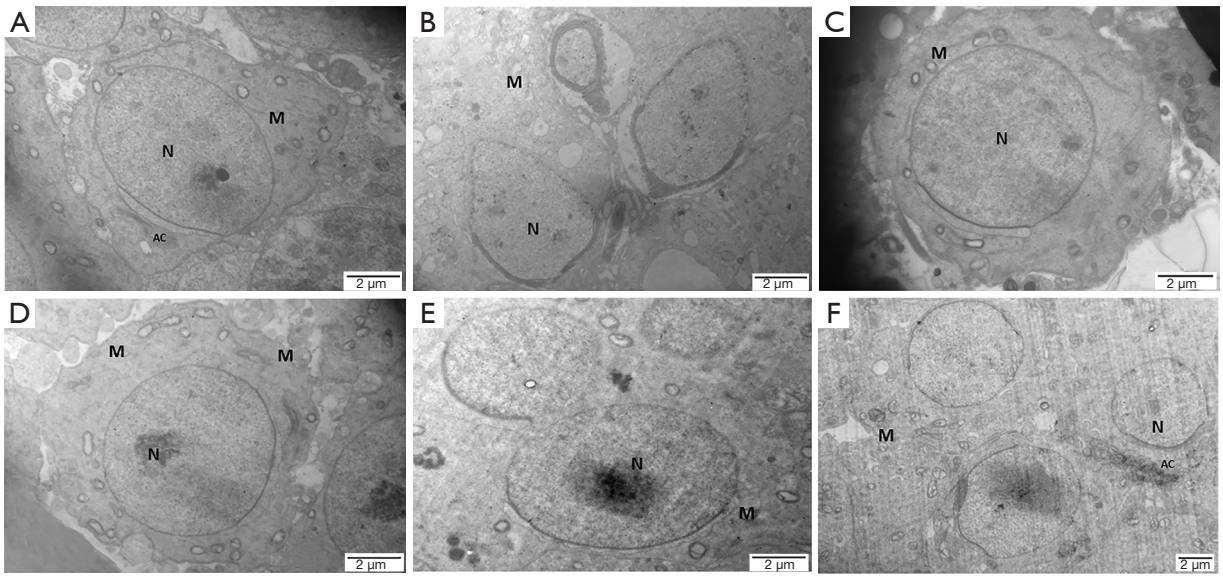

Figure 6 Ultrastructure of rounded sperm cells of seminiferous tubules. Ultrastructure of rounded sperm cells of seminiferous tubules under the transmission electron microscope (×400) was observed (A-F). The ultrastructure of rounded sperm cells of the ND, HFD, HFD + orlistat, HFD + HLG, HFD + HMG and HFD + HHG groups. The AC letters in the figure meant the acrosomal cap, M meant mitochondria, and N meant nucleolus. ND, normal group; HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group. AC (acrosomal cap), N (nucleus, round nucleus were observed), and M (mitochondria).

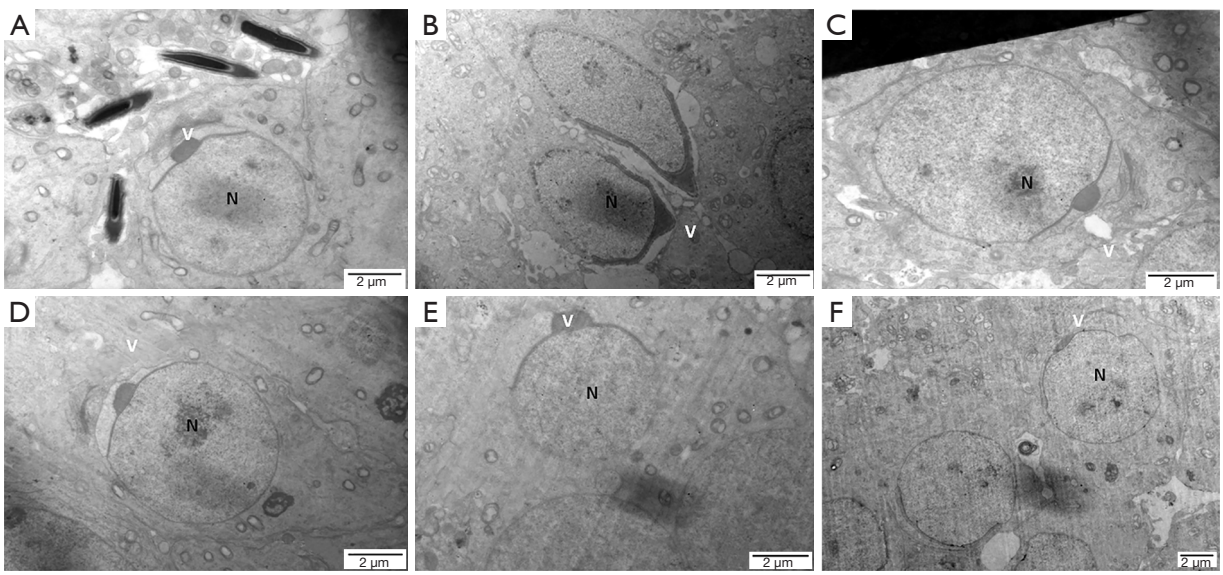

Figure 7 Ultrastructure of rounded sperm cells of seminiferous tubules. Ultrastructure of rounded sperm cells of seminiferous tubules under the transmission electron microscope (×400) was observed (A-F). The ultrastructure of rounded sperm cells of the ND, HFD, HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups. ND, normal group; HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group. N (nucleus, round nucleus were observed) and V (vesicle, the proximal part of the nucleus was covered by its vesicle).

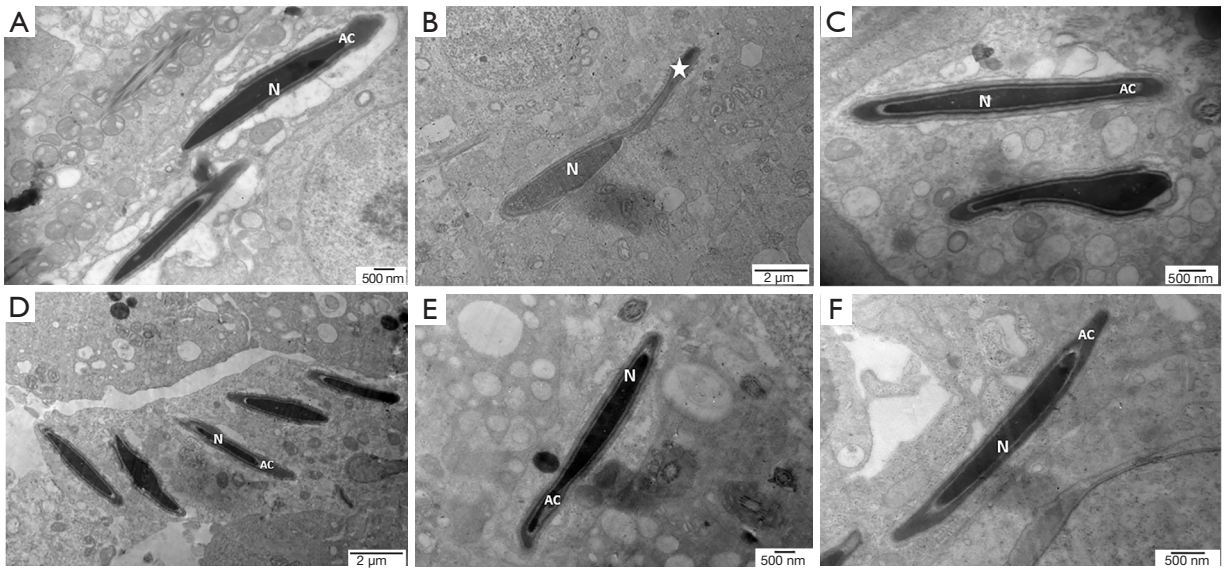

Figure 8 Ultrastructure of long-form sperm cells of seminiferous tubules. Ultrastructure of long-form sperm cells of seminiferous tubules under the transmission electron microscope (×400) was observed (A-F). The ultrastructure of long-form sperm cells of the ND, HFD, HFD + orlistat, HFD + HLG, HFD + HMG, and HFD + HHG groups. (A) The head of late sperm cell was made up of nucleus surrounded by the acrosomal cap. (B) The late sperm cells had irregular contractile nucleus. (C-F) There was no significant difference between the ND and other groups. ND, normal group; HFD, control group; orlistat, orlistat group; HLG, low-dose group; HMG, medium-dose group; HHG, high-dose group. AC (acrosomal cap), N (nucleus, round nucleus were observed), and M (mitochondria). The stars stand for acrosome abnormalities in the head of long sperm in the model group.

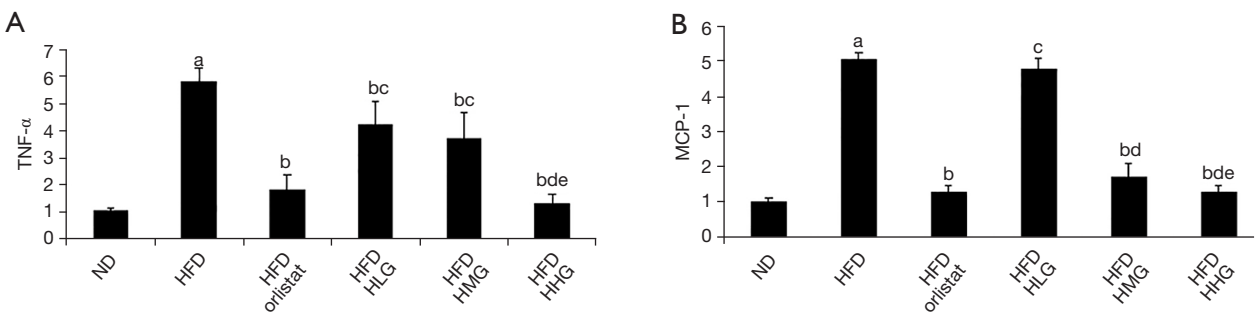

Figure 9 The effect of Huaji Jianpi Decoction on inflammatory factors (TNF-α and MCP-1). (A,B) The expression of TNF-α and MCP-1 was detected. a, P<0.05 *vs.* the ND group; b, P<0.05 *vs.* the HFD group; c, P<0.05 *vs.* the HFD + orlistat group; d, P<0.05 *vs.* the HFD + HLG group; e, P<0.05 *vs.* the HFD + HMG group. ND, normal group; HFD, control group; orlistat, orlistat group; HLG low-dose group; HMG, medium-dose group; HHG, high-dose group; TNF-α, tumour necrosis factor α; MCP-1, monocyte chemoattractant protein-1.

Discussion

This study provided evidence that Huaji Jianpi Decoction could decrease the body fat and blood lipid levels of obese mice, increase the testicular wet weight and testis coefficient, and enhance semen parameters. Different doses of Huaji Jianpi Decoction could improve testicular weight, sperm density, sperm motility, forward motility, and total sperm motility to varying degrees, downregulate TNF-α and MCP-1 expression, inhibit germ cell apoptosis, and improve semen quality. Recent study has demonstrated that obesity is a chronic metabolic disease, which leads to multiple complications due to excessive fat accumulation or abnormal distribution in the body as a result of the body's

energy intake exceeding consumption (6). A previous study proposed that obesity exerts negative effects on sperm motility and semen parameters, and is a high-risk factor for male infertility (7). It has been shown that the fertility of obese male mice was improved following lipid lowering and weight loss by vertical banding gastric decongestion (8). After Huaji Jianpi Decoction administration into obese male mice for 6 weeks, the body fat content and blood lipid levels were reduced, the testicular wet weight and semen content were increased, and semen parameters were improved. The above results suggest that Huaji Jianpi Decoction can improve semen quality in obese mice through weight loss and lipid reduction.

Growing studies have revealed that increased obesity rates coincide with decreased sperm quality and increased male infertility (9,10), suggesting that obesity is associated with male fertility (11). After 4 weeks of the high-fat diet, mouse sperm concentration and sperm motility were reduced. Previous study also demonstrated that sperm mitochondria were impaired, ATP was reduced, and reactive oxygen species (ROS) were elevated (12). Moreover, total sperm count was markedly lower in obese males than in males with normal weight (13). Obese individuals have lower sperm mitochondrial activity, as well as higher ROS and DNA fragmentation levels (14). Our results showed that sperm density, viability, sperm forward motility, and total sperm viability were significantly reduced in obese mice relative to those in ND. These results could be attributed to the mitochondrial aerobic respiratory response and the uncoupling of ATP, suggesting that obesity not only increases the risk of sperm DNA breakage, but is also associated with reduced mitochondrial activity. Furthermore, apoptosis is crucial for the development of male germ cells, and excessive apoptosis is present in the sperm of both infertile patients and infertile mice (15). High-fat foods induce apoptosis in murine germ cells, which then promotes infertility (16). According to the results in this study, the testicular wet weight and testis coefficient were significantly reduced in the model blank group. Reduced mitochondria in the testicular ultrastructure and the apoptosis of supporting cells and germ cells in the lumen of the germinal ducts were observed, which is possibly associated with obesity-induced apoptosis of spermatogonia, spermatocytes, and Sertoli cells (17). Spermatogenesis, as a complex process, relies on coordinated cell proliferation and apoptosis, and excessive apoptosis can lead to sperm abnormalities (18). Therefore, inhibiting excessive apoptosis and re-establishing the balance between cell proliferation and apoptosis may be effective means for treating abnormal spermatozoa. It was hypothesized that Huaji Jianpi Decoction could improve the reproductive function of mice by protecting the mitochondrial morphology of testes and inhibiting the excessive apoptosis of spermatogenic cells.

Our results showed that fat volume and distribution in normal and obese mice were significantly higher than in ND. Fatty acids can synthesize and secrete a variety of adipokines that can regulate reproductive function through the neuroendocrine system or by acting directly on the testis. However, obesity can lead to adipocyte dysfunction and cause a systemic inflammatory response (19), which can be characterized by abnormal secretion of pro-inflammatory cytokines (TNF-α and MCP-1) and the activation of inflammatory pathways (20). These pathways can act on the testis indirectly or directly and affect the functioning of the reproductive system. TNF-α induces Fas upregulation through NF-κB activation in mouse support cells. Fas upregulation initiates apoptotic pathways and triggers apoptosis in testis cells, thereby causing damage to the blood-testis barrier (21). In particular, Fas activates the release of Cytc and increases the content of intercellular membrane protein Cytc, which further activates apoptosis-related factors (such as caspase-3) and increases the content of Cytc (22).

To investigate the effects of Huaji Jianpi Decoction on inflammatory factors and apoptosis, we used RT-qPCR to analyze TNF-α and MCP-1 mRNA expression. It was found that the expression levels of TNF-α and MCP-1 in the testicular tissues of mice in the model blank group were significantly higher than those of mice in the normal group. Obesity may stimulate the recruitment and activation of testicular macrophages, thereby causing an inflammatory response and promoting apoptosis, ultimately leading to decreased semen quality and conception. The relative expression of inflammatory factors was reduced to varying degrees by Huaji Jianpi Decoction. It was hypothesized that Huaji Jianpi Decoction may improve male semen quality by inhibiting the inflammatory response *in vivo* and reducing excessive apoptosis of germ cells.

In conclusion, we observed that Huaji Jianpi Decoction could inhibited TNF-α and MCP-1 mRNA expression caused by obesity and suppressed obesity-induced hypogonadism in mice via reducing TNF-α and MCP-1 mRNA expression. Our results suggest that Huaji Jianpi Decoction can improve the reproductive function of obese mice by improving the level of lipid metabolism. Our study may provide a new theoretical basis for the future clinical

treatment of reproductive system diseases in obese males.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-22-115/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-115/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All animal research was approved by the Ethical Committee of Hebei University (approval ID: IACUC-2021XS038). The animal research was performed in compliance with the Hebei University Laboratory Animal Welfare and Ethics guidelines of the Animal Welfare and Ethical Committee of Hebei University.

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