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高脂饮食诱导肠道菌群紊乱对小鼠雌性生殖的影响

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摘要 高脂饮食引发的过度肥胖是导致女性生殖能力降低的主要原因。研究发现过度肥胖导致肠道菌群紊乱。但肥胖诱导肠道菌群紊乱是否会影响雌性生殖力, 目前尚不清楚。通过建立四种小鼠模型, 包括正常饮食组(ND组)、高脂饮食组(HFD组)、粪便菌群移植的正常饮食组(ND-FMT组)和粪便菌群移植的高脂饮食组(HFD-FMT组), 以探究高脂饮食诱导肠道菌群紊乱对雌性生殖力的影响。结果显示, HFD组小鼠卵母细胞成熟率、胚胎发育率、产仔数和卵母细胞质量显著低于ND组。通过检测体质量、血糖以及16S rDNA测序等方法验证, 粪便菌群移植后肠道菌群紊乱小鼠模型构建成功, 其中HFD-FMT组比ND-FMT组中拟杆菌门相对丰度明显降低, 厚壁菌门明显升高。最重要的是, HFD-FMT组小鼠卵母细胞成熟率、胚胎发育率和卵母细胞质量显著低于ND-FMT组。综上所述, 高脂饮食引起的肠道菌群失衡可导致雌性生殖力降低。

关键词 高脂饮食; 肠道菌群; 粪便菌群移植; 卵母细胞

Effects of High-Fat Diet-Induced Disturbance of Intestinal Flora on Female Reproduction in Mice

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Abstract Excessive obesity resulting from a high-fat dietary regimen is a predominant factor contributing to the decline in female reproductive capacity. Research findings indicate that obesity of this nature results in disruption of the gut microbiota. Nevertheless, the question of whether obesity-induced alterations in gut microbiota influence female fertility remains unresolved. To explore this issue, four murine models were developed: a normal diet group (ND), a high-fat diet group (HFD), a normal diet plus fecal microbiota transplantation group (ND-FMT), and a high-fat diet with fecal microbiota transplantation group (HFD-FMT). The objective was to examine the effects of high-fat diet-induced gut microbiota dysbiosis on female fertility. The results revealed that the HFD group exhibited significantly lower rates of oocyte maturation, embryo development rate, litter size, and oocyte quality compared with the ND group. Evidence from body mass measurements, blood glucose detection, and 16S rDNA sequencing confirmed the successful establishment of a gut microbiota dysbiosis model in mice following fecal microbiota transplantation, with the HFD-FMT group showing a marked reduction in the relative abundance of Bacteroidetes and a concomitant increase in that of Firmicutes when compared with the ND-FMT group. Most critically, the maturation rate of oocytes, embryo development rate, and oocyte quality in the HFD-FMT group were substan-

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tially inferior to those in the ND-FMT group. Collectively, these data suggest that the imbalance of gut microbiota caused by a high-fat diet can adversely affect female reproductive capacity.

Keywords high-fat diet; gut microbiota; fecal microbiota transplantation; oocyte

In recent years, there has been significant interest in the relationship between a high-fat diet, obesity, and female reproduction [1-2]. Obesity caused by a high-fat diet is a major contributor to decreased oocyte quality [3], and as BMI increases, the risk of infertility also rises due to factors such as reduced gonadotropin levels (which cause reduced ovulation and anovulation) [4]. This is mainly reflected in lower gonadotropin levels in obese women [5], reduced fertility [6], higher miscarriage rates [7], and lower successful *in vitro* fertilization rates [2,8]. Increasing evidence suggests that obesity is also associated with alterations in gut microbiota [9], including changes in microbiota diversity [10] and dominant flora [11]. The gut microbiota regulates multiple metabolic processes through interactions with metabolic, signaling, and immune-inflammatory pathways [12]. Intestinal microorganisms decompose food residues in the intestine and regulate intestinal epithelial cells, immune cells, and intestinal mucosal barriers, thus impacting energy intake [13-15], digestion and absorption [16], early immune system development [17-19], and regulation of immune function [20-23]. Furthermore, several studies have shown that the metabolites of intestinal microbes—and not just the microbes themselves—play a role in regulating host immune and metabolic functions [24-26]. Obesity-induced disturbances in intestinal flora can also lead to ovarian dysfunction, including impaired oocyte development, disruption of the estrous cycle and ovulation abnormalities, and may even affect the entire process of embryonic development [3,27]. Furthermore, obesity-related inflammation adversely affects oocyte quality by impairing meiosis and cytoplasmic maturation, ultimately contributing to reproductive disorders. In addition, the gut microbiota of obese mothers during pregnancy affects the microbiota colonization and metabolism of the offspring [28]. Given that alterations in gut microbiota lead to obesity [9] and that a strong relationship exists between obesity and infertility [29], it is possible that obesity-induced imbalances in gut microbiota may lead to abnormalities in reproductive function.

FMT (fecal microbiota transplantation) involves transplanting flora from animal feces into the gastrointestinal tract of other animals to alter and remodel their gut microbiota composition [30]. Research has shown that disrupting the gut microbiota of obese model mice with a high-fat diet leads to endotoxemia and epididymal inflammation in male mice, which in turn affects sperm quality [31]. However, ZHANG et al [32] found that FMT could improve sperm quality and male fertility. Further research is needed to investigate the relationship between altered gut microbiota, obesity, and infertility. Exploring the mechanism by which gut microbiota affects female fertility can provide a foundation for using gut microbiota to adjust and improve infertility.

In this study, to investigate whether disruption of gut microbiota caused by a high-fat diet affects female fertility, we established four murine groups: mice fed a ND (normal diet); mice fed a HFD (high-fat diet); mice on a ND-FMT (normal diet undergoing FMT); and mice on a HFD-FMT (high-fat diet undergoing FMT). The gut microbiota composition of the mice was analyzed by 16S rDNA sequencing. The oocyte maturation rate, embryo development rate, and litter size of the four groups were evaluated by quantitative counting. Finally, the oocyte quality of the four groups was assessed. It was found that high-fat diet-induced imbalance of gut microbiota resulted in reduced oocyte maturation and early embryo development.

1 Material and methods

1.1 Experimental animals

Female ICR mice (5-6 weeks old, average weight 25 g) were purchased from the Labora-

tory Animal Center of Xi'an Jiaotong University (SCXK[SHAAN]2017-003). The mice were kept in an environment with a constant temperature of 21 °C-25 °C, an ambient humidity of 50%-60%, a 12-hour light/dark cycle, and free access to food and water. All experimental operations were conducted in accordance with the Regulations on the Management of Laboratory Animals of the Second Affiliated Hospital of Air Force Medical University, Xi'an, China. Every effort was made to minimize the animals' pain. In this study, each group consisted of 30 mice. Within each group, at least 6 mice were allocated for fertility assessment, at least 3 were used for IVM (*in vitro* oocyte maturation) and IVF (*in vitro* fertilization), and the remaining mice were subjected to subsequent processing, sampling, and analysis.

1.2 Mouse models and intestinal FMT

We randomly divided 6-week-old mice into four groups: mice fed a ND (normal diet); mice fed a HFD (high-fat diet); mice on a ND-FMT (normal diet undergoing FMT); and mice on a HFD-FMT (high-fat diet undergoing FMT). Prior to the start of the experiment, all mice underwent an acclimatization period under the same conditions to ensure that they adapted to the experimental environment and feeding conditions. After the acclimatization period, mice in the ND and HFD groups were fed a normal diet and a high-fat diet (Jiangsu Xietong Medicine Bioengineering Co., Ltd., XTHF60, Jiangsu, China) respectively, for one week. Subsequently, we removed these ND and HFD mice from their original, uncleaned cages and carefully placed them into new cages. The uncleaned cages were then used to house the ND-FMT group and the HFD-FMT group for one week so that they could undergo FMT. During this week, the ND-FMT and HFD-FMT mice were fed a normal diet. At the end of the one-week period, we switched the mice in the ND and HFD groups back to their original cages. Thereafter, ND and ND-FMT mice switched cages once weekly, as did the HFD and HFD-FMT groups. The entire trial period is 15 weeks. This approach ensured that mice in the ND-FMT and HFD-FMT groups continuously acquired intestinal flora from the ND and HFD groups, respectively, to mimic the effects of intestinal flora transplantation. Through this alternating cage-switching method, we were able to cross-control the dietary and environmental conditions between groups to minimize experimental errors and ensure the reliability and accuracy of the experiments. This experimental design helped us to study the effects of intestinal flora disruption on oocyte quality in mice. Body weights of all mice were measured and recorded every other week.

1.3 Glucose tolerance testing

One day before the experiment, each group of mice was placed into a clean cage labeled according to the group within it (ND, HFD, ND-FMT, HFD-FMT). During this period, mice were subjected to fasting for 12 hours. After fasting, mice were weighed and their fasting blood glucose levels were measured. Subsequently, an OGTT (oral glucose tolerance test) was performed, in which each mouse was administered glucose (2 g/kg) orally. Blood samples were collected from the tail vein at 0, 15, 30, 45, 60, 90 and 120 minutes after glucose loading and glucose concentration was measured using a glucose analyzer.

1.4 Oocyte collection

To collect GV (germinal vesicle) stage oocytes, mice were injected intraperitoneally with 10 IU pregnant mare serum gonadotropin (PMSG; Shu Sheng Hormone, China) and euthanized 46-48 h after injection. The skin and peritoneum were incised to expose the peritoneal cavity, the ovaries were removed and placed in pre-equilibrated M2 culture medium (Sigma-Aldrich, USA), and the follicles were punctured to collect GV stage oocytes. The GV-stage oocytes were aspirated with a glass needle and washed three times in M2 culture medium before being placed in pre-equilibrated M2 culture medium, covered with paraffin oil (Sigma-Aldrich, USA), and incubated in an atmosphere of 5% CO_2 at 37 °C. The oocyte maturation rate of each group of mice was measured after 14-16 h.

Stage MII oocytes were collected and injected with 10 IU of human chorionic gonadotropin (hCG, Shu Sheng Hormone, China) 48 h after PMSG injection, and the mice were euthanized 14-16 h after hCG

injection. Next, COCs (cumulus-oocyte complexes) were collected from the ampullary segment of mouse oviducts and transferred to M2 medium containing 0.1% hyaluronidase to remove cumulus cells and allow collection of oocytes that had attained the MII (mature metaphase II) stage.

1.5 *In vitro* fertilization

The ampullary segments of mice oviducts were collected as described above. COCs were collected under a microscope and then incubated in a droplet containing IVF medium (Vitrolife, Sweden). At the same time, sperm were obtained from the epididymis tails of adult male mice and transferred to the droplets containing IVF medium and oocytes for fertilization after enabling treatment for 6 h. Subsequently, the fertilized eggs were collected and transferred to hyaluronidase droplets for treatment. The digested oocytes were then washed three times in a KSOM (Sigma) culture medium, placed into the KSOM culture medium with a layer of paraffin oil, and then incubated in an atmosphere of 5% CO_2 at 37 °C. The incubation was continued in an incubator. Cleavage and blastocyst rates were calculated at 24 h and 96 h, respectively, for each group of mice.

1.6 Mating and littering

Different groups of female mice were mated with males of appropriate age. Mice were provided with sufficient food and water during the period, and the number of offspring was recorded after mice gave birth.

1.7 Immunofluorescence staining

Oocytes at the MII stage were fixed and treated with 4% paraformaldehyde for 30 min at room temperature. After fixation, the oocytes were permeabilized on a shaker at room temperature for 20 min. The oocytes were blocked with blocking buffer for 2 h at room temperature, after which they were washed, placed in a wet box and incubated overnight with anti-Tubulin primary antibody (1:1 000, Proteintech, China) at 4 °C. Subsequently, the oocytes were washed several times and transferred to a culture medium containing secondary antibody and incubated for 2 h at room temperature in a darkened environment. After incubation, oocytes were washed again and transferred to slides with drops of DAPI for nuclear staining for 5 minutes. Next, a drop of anti-fluorescent bursting agent was added to seal the slides. Finally, the coverslips were sealed with petroleum jelly and slides were observed under a fluorescence microscope (Olympus BX53F) and photographed for documentation.

1.8 Mitochondrial assay

Fresh stage MII oocytes were washed three times in PBS-PVA, placed in 200 nmol/L Mito Tracker® Red CMX Ros staining solution (Beyotime, China), and placed in an incubator at 5% CO_2 and 37 °C for 30 minutes in a darkened environment. After three washes in PBS-PVA, the slide was supported with petroleum jelly to form a square area, into which the oocytes were placed. They were then observed and photographed under a fluorescence microscope (Olympus BX53F).

1.9 16S rDNA sequencing of fecal flora

Fresh fecal samples were collected from the four groups of mice (ND, HFD, ND-FMT, and HFD-FMT), quickly snap-frozen in liquid nitrogen for 15 min, and subsequently stored at –80 °C. Samples were packed in dry ice to ensure their integrity during transit. Genomic DNA from the fecal samples was extracted using the CTAB method, and DNA purity and concentration were examined using agarose gel electrophoresis. Subsequently, DNA was diluted to a concentration of 1 ng/μL with sterile water in a centrifuge tube. Primers were designed to amplify the daughter generation and PCR products were detected using 2% agarose gel electrophoresis. Aliquots were mixed well according to the concentration of the PCR product before undergoing 2% agarose gel electrophoresis again; the target bands were recovered using a gel recovery kit. Next, the NEBNext® UltraTM II DNA Library Prep kit was used to construct sequencing libraries. Online sequencing was performed after the libraries passed quality control. Sequencing data were analyzed using FastQC (version 0.11.9) for quality control, reads were quantified using Subread (version 2.0.1), and

differentially abundant bacterial taxa were identified using the R package DESeq2 (version 1.34.0).

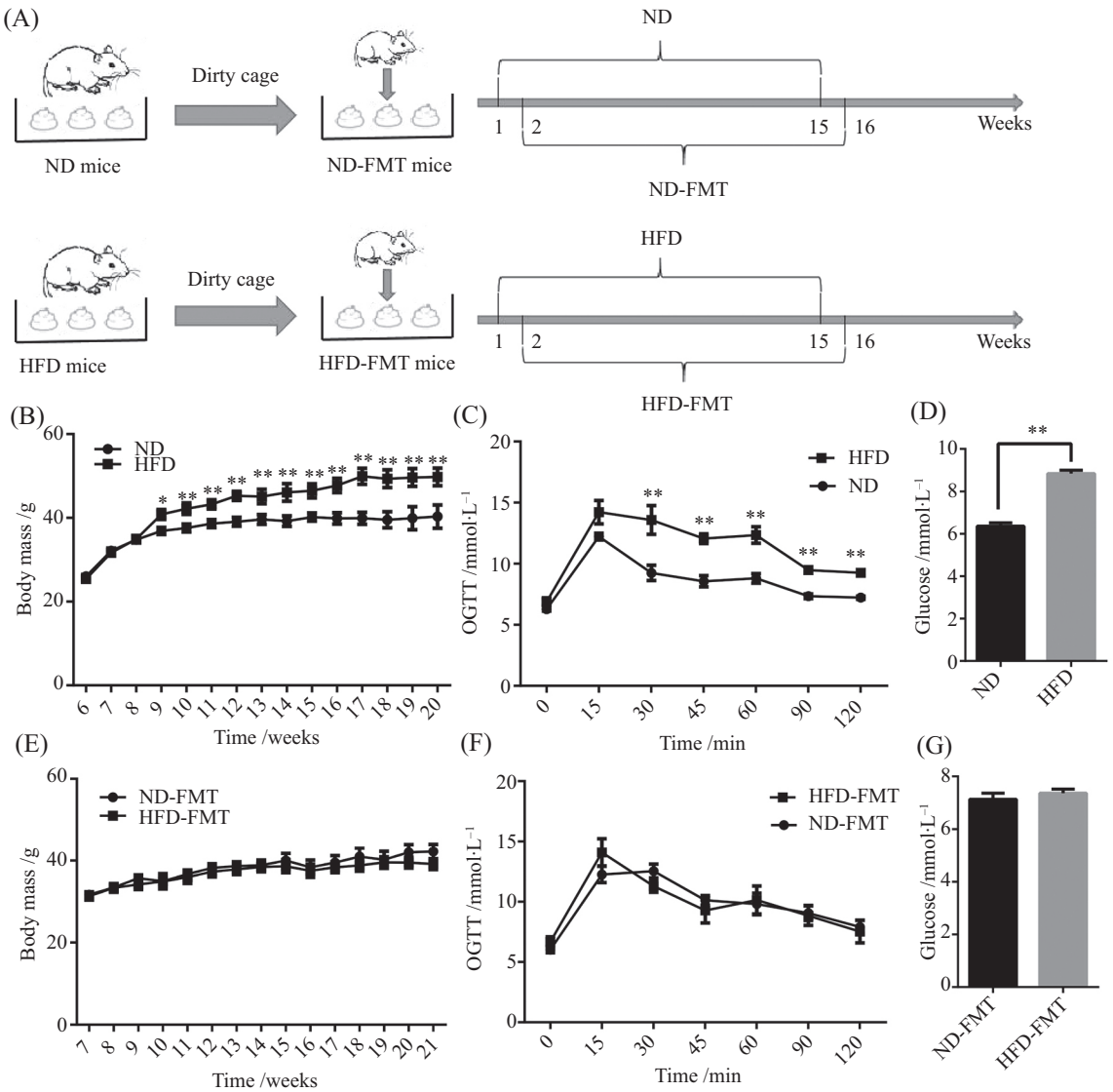
1.10 Statistical analysis

The fluorescence intensity of the immunofluorescence-stained images was quantified using Image-Pro Plus software (v6.0; Media Cybernetics). Independent sample *t* tests were used to analyze the data using GraphPad Prism 8 software (GraphPad Software Corporation). Data are presented as mean ± standard error. $P<0.05$ was considered significant. Each experiment was repeated three times.

2 Results

2.1 HFD and FMT mouse models

As shown in Fig.1A and Fig.1B, starting from week 9, mice in the HFD group had significantly higher



A: ND-FMT和HFD-FMT小鼠模型的构建; B: ND和HFD组体质量变化; C: ND和HFD组血糖浓度; D: ND和HFD组空腹血糖浓度; E: ND-FMT和HFD-FMT组体质量统计; F: ND-FMT和HFD-FMT组血糖浓度; G: ND-FMT和HFD-FMT组空腹血糖浓度。**$P<0.01$。$n=6$。

A: construction of the ND-FMT and HFD-FMT mouse models; B: body mass changes in the ND and HFD groups; C: blood glucose concentration in the ND and HFD groups; D: fasting glucose concentration in the ND and HFD groups; E: body mass statistics in the ND-FMT and HFD-FMT groups; F: blood glucose concentration in the ND-FMT and HFD-FMT groups; G: fasting glucose concentration in the ND-FMT and HFD-FMT groups. **$P<0.01$. $n=6$.

图1 粪便菌群移植(FMT)小鼠模型的构建

Fig.1 Construction of the FMT (fecal microbiota transplantation) mouse model

body mass than mice in the ND group. Further, OGTT tests showed that blood glucose concentrations significantly increased along with body mass in HFD mice (Fig.1C and Fig.1D), demonstrating that these animals had developed glucose intolerance. These results indicated that the obese mouse model was successfully established.

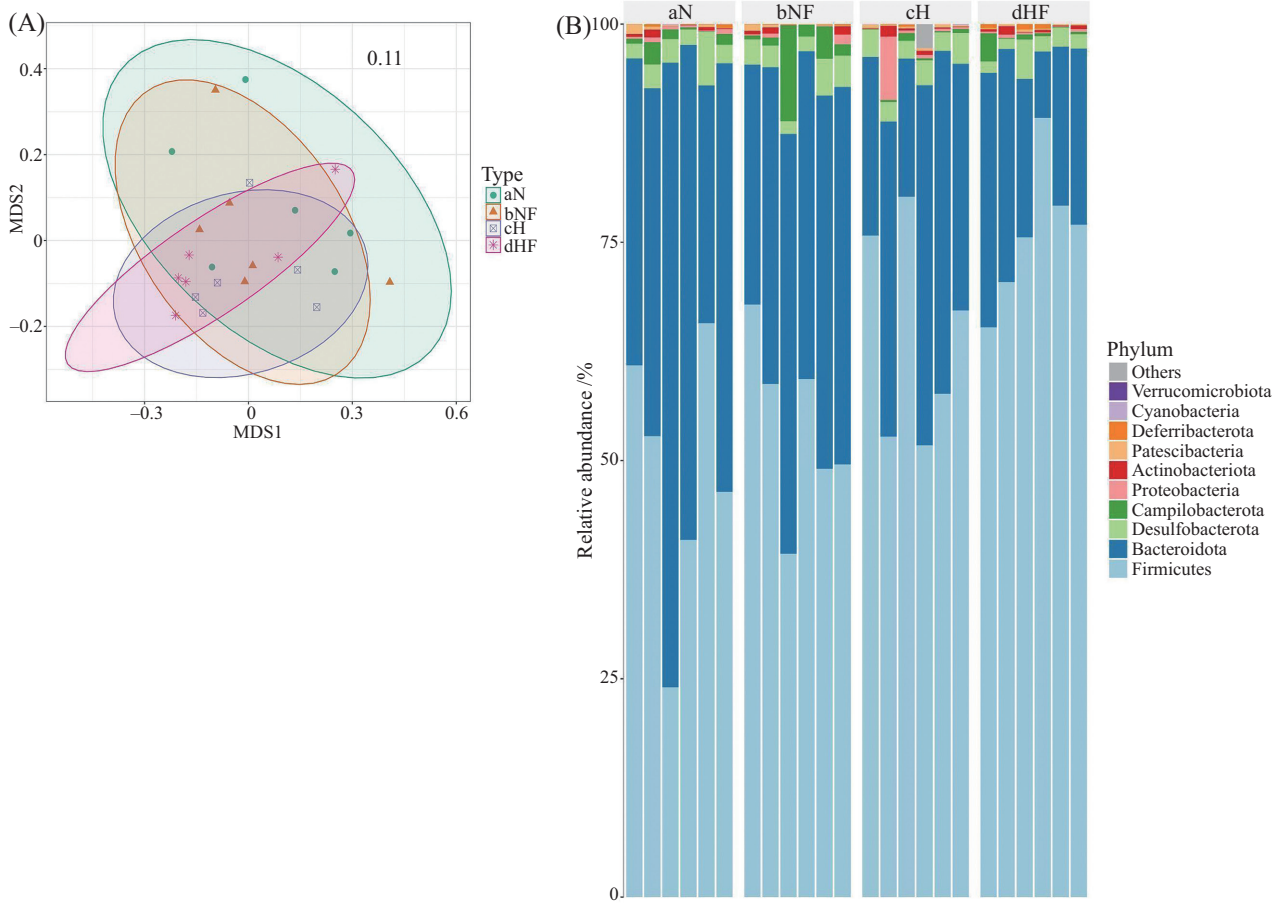
There were no significant differences in body mass, blood glucose concentration or glucose tolerance between mice in the ND-FMT and HFD-FMT groups (Fig.1E-Fig.1G), confirming that FMT mice did not exhibit significant obesity or glucose metabolism disorders.

2.2 Successful colonization of gut microbiota in FMT mice

Results of 16S rDNA sequencing analysis of fecal samples showed that the relative abundance of Bacteroidetes was significantly decreased, while that of Firmicutes was significantly increased, in the intestinal tract of HFD mice compared with ND mice (Fig.2A). This indicates that a high-fat diet led to changes in the composition of gut microbiota. Following FMT, the Bacteroidetes phylum also significantly decreased, and the Firmicutes phylum significantly increased, in the intestinal tract of HFD-FMT mice when compared with ND-FMT mice (Fig.2B). These results confirmed the successful colonization of gut microbiota in mice undergoing FMT.

2.3 The impact of a high-fat diet on the reproductive capacity of female mice

To investigate the effects of a high-fat diet on mouse oocytes, we counted the number of MII-stage oocytes collected from ND and HFD mice and calculated the rate of first polar body extrusion. The rate of

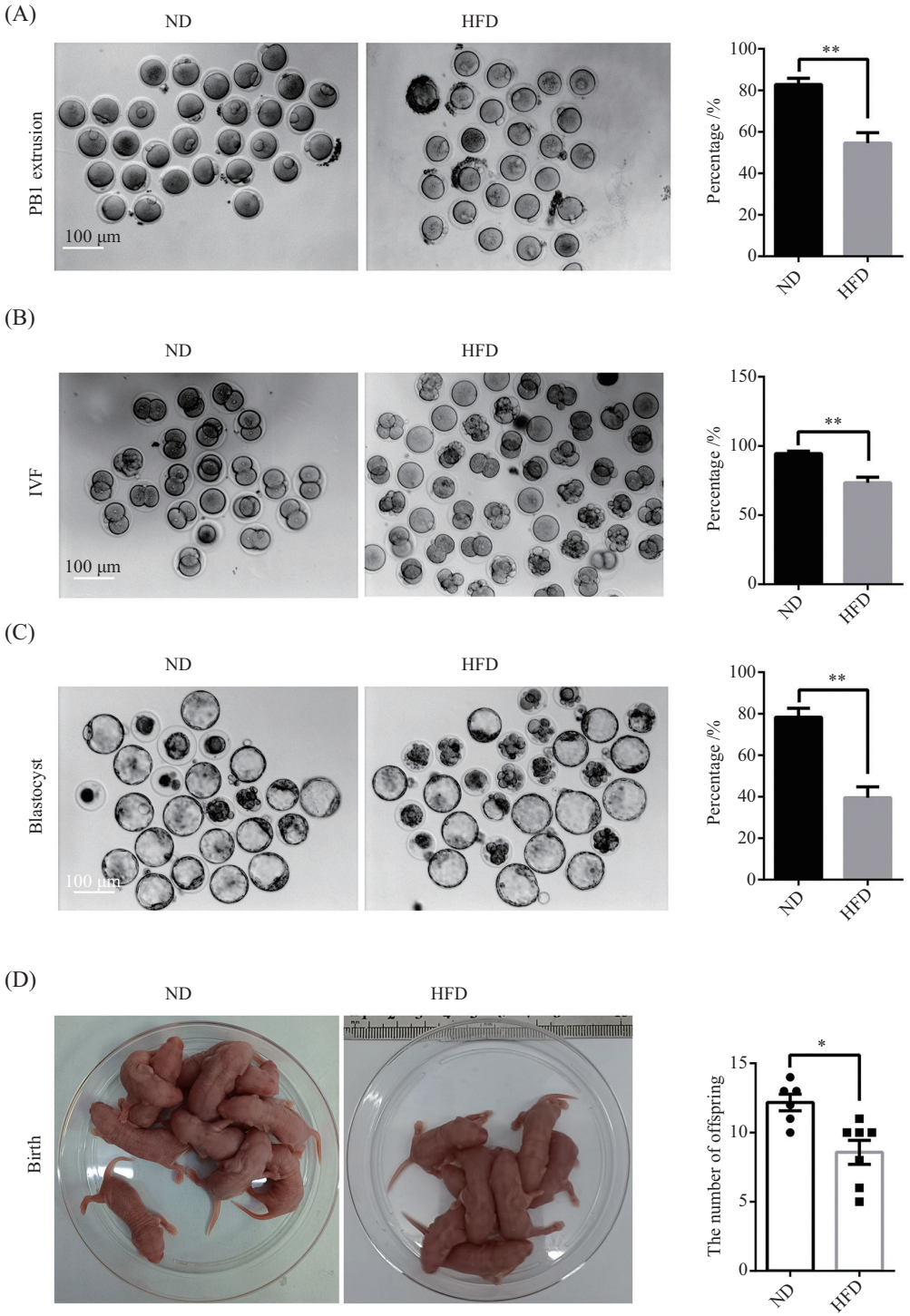


A: MDS图分析四组小鼠(ND、ND-FMT、HFD、HFD-FMT)粪便中菌群组成差异; B: 四组小鼠粪便中不同菌群的相对丰度。

A: MDS plots analyzing differences in the composition of bacterial flora in the feces of the four groups of mice (ND, ND-FMT, HFD, HFD-FMT); B: relative abundance of different flora in the feces of the four groups of mice.

图2 16S rDNA测序分析

Fig.2 16S rDNA sequencing



A: ND组和HFD组小鼠卵母细胞成熟率; B: ND组和HFD组体外受精胚胎卵裂率; C: ND组和HFD组囊胚发育率; D: ND与HFD组小鼠产仔数。*P<0.05, **P<0.01。

A: oocyte maturation rate in ND and HFD mice; B: cleavage rate of fertilized eggs in ND and HFD mice; C: blastocyst rate in ND and HFD mice; D: litter size in the ND and HFD groups. *P<0.05, **P<0.01.

图3 ND与HFD小鼠生育力比较

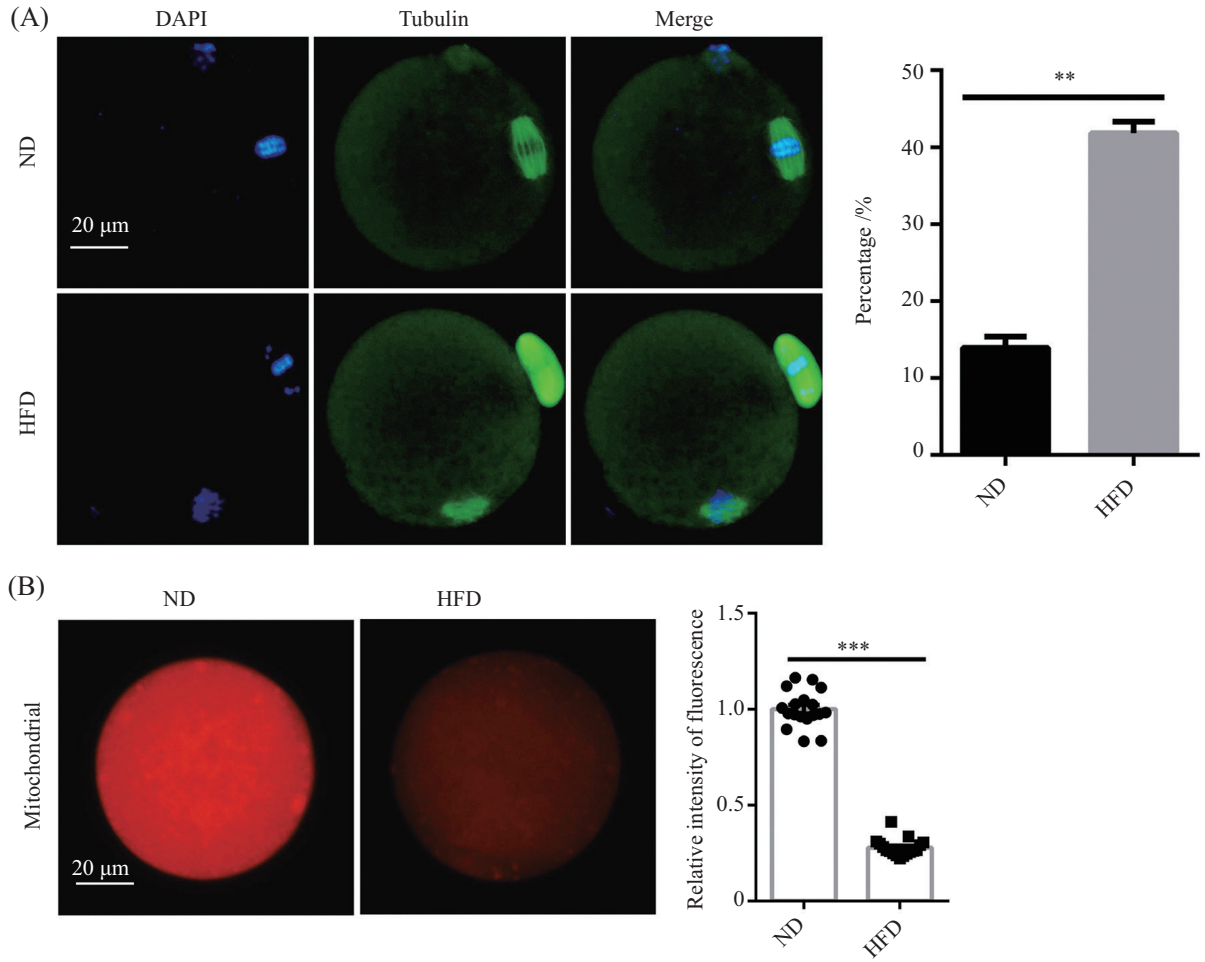
Fig.3 Comparison of fertility between ND and HFD mice

first polar body extrusion in oocytes was significantly lower in the HFD group (n=125) than in the ND group (82.77%±3.05% *vs* 54.62%±4.96%, P<0.01; Fig.3A; n=116). Subsequently, *in vitro* fertilization experiments found that the oocyte cleavage rate (94.50%±1.74%, n=135 *vs* 73.38%±3.93%, n=116; P<0.01) and blas-

tocyst rates (78.34%±4.34%, n=86 *vs* 39.52%±5.19%, n=91; P<0.01) in the HFD group were significantly lower than in the ND group (Fig.3B and Fig.3C). To further understand the impact of a high-fat diet on mouse reproductive function, we performed a fertility assay following prolonged feeding. As shown in Fig.3D, Mating experiments also showed that the average litter size was significantly smaller in the HFD group (12.17±0.60, n=6) than in the ND group (8.57±0.86, n=7; P<0.05). Together, these results suggested that high-fat diet had a negative impact on the quality and developmental competence of mouse oocytes, as well as adverse effects on mouse reproductive function.

2.4 The impact of a high-fat diet on oocyte quality

To assess the impact of a high-fat diet on oocyte quality, we conducted immunofluorescence staining experiments. Oocytes from HFD mice (n=62) also showed significantly higher proportions of abnormal spindle morphology and chromosome misalignment than those from ND mice (P<0.01; Fig.4A; n=43). Furthermore, we used Mito Tracker® Green for specific immunofluorescence staining to label mitochondria in oocytes. Results of immunofluorescence staining experiments showed a significantly lower mitochondrial fluorescence level in oocytes from the HFD group (n=12) than in oocytes from the ND group (P<0.001; Fig.4B; n=18). These results indicated that a high-fat diet damaged oocyte quality.



A: ND和HFD小鼠卵母细胞纺锤体形态和染色体排列代表性图表; B: ND和HFD小鼠MII期间卵母细胞的线粒体活性染色图和荧光强度分析。**P<0.01, ***P<0.001。

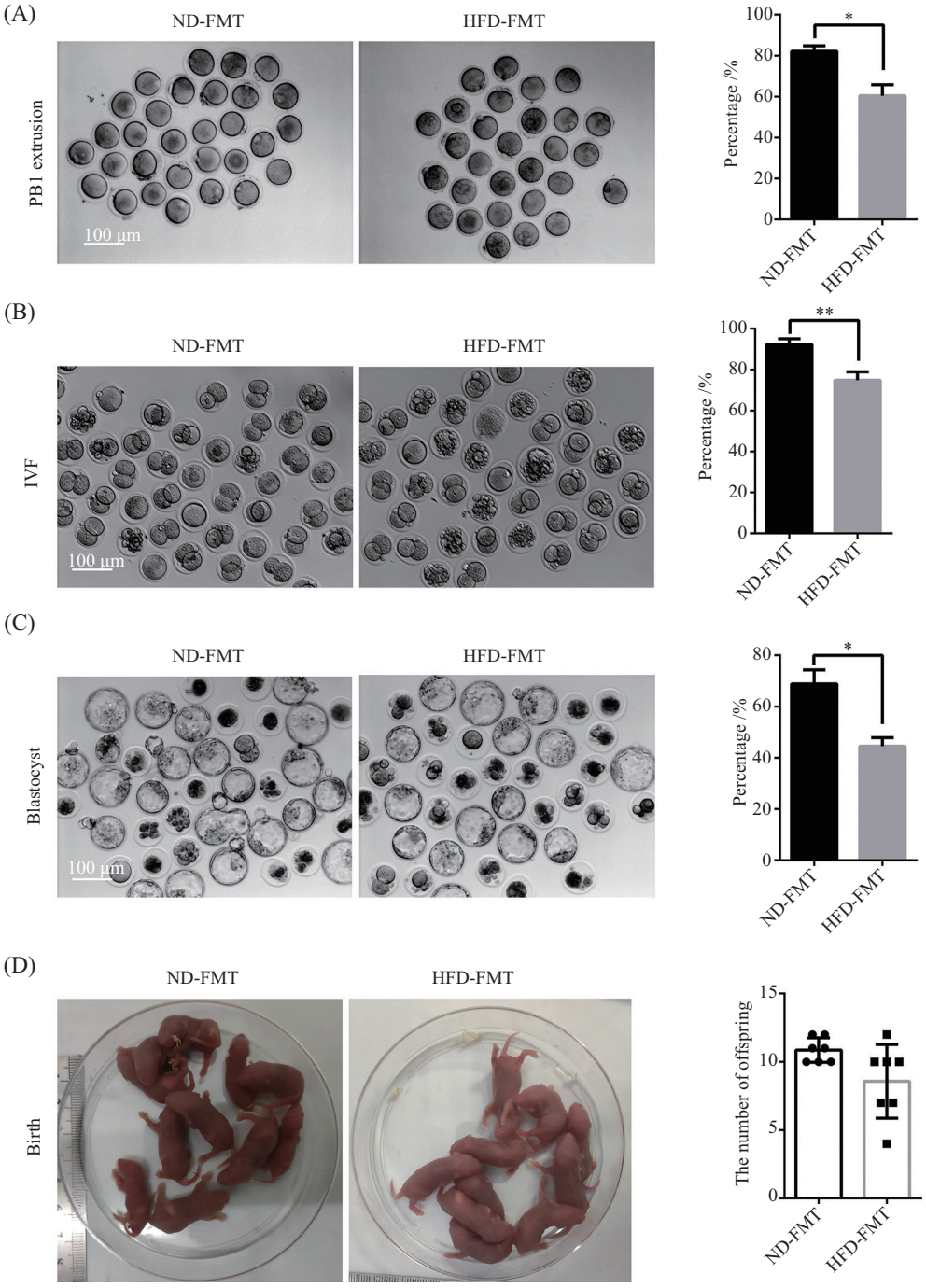
A: representative diagrams of spindle morphology and chromosome arrangement in the oocytes of ND and HFD mice; B: representative mitochondrial activity staining plots and fluorescence intensity of oocytes during MII in ND and HFD mice. **P<0.01, ***P<0.001.

图4 ND和HFD组卵母细胞质量分析

Fig.4 Oocyte quality assay in the ND and HFD groups

2.5 High-fat diet-induced gut microbiota dysbiosis leads to impaired oocyte maturation and compromised early embryonic developmental competence

After FMT, the rate of first polar body extrusion in oocytes from the HFD-FMT group was significantly lower than in the ND-FMT group (82.17%±2.66%, n=103 *vs* 60.39%±5.40%, n=92; P<0.05; Fig.5A). Further, *in vitro* fertilization experiments found



A: ND-FMT组与HFD-FMT组小鼠卵母细胞成熟率; B: ND-FMT组与HFD-FMT组小鼠组体外受精胚胎卵裂率; C: ND-FMT组与HFD-FMT组小鼠囊胚率; D: ND-FMT组与HFD-FMT组小鼠产仔数。*P<0.05, **P<0.01。

A: oocyte maturation rate in ND-FMT and HFD-FMT mice; B: IVF embryo cleavage rate in ND-FMT and HFD-FMT mice; C: blastocyst rate in ND-FMT and HFD-FMT mice; D: representative pictures of mouse offspring in the ND-FMT and HFD-FMT groups. Litter size in the ND-FMT and HFD-FMT groups. *P<0.05, **P<0.01.

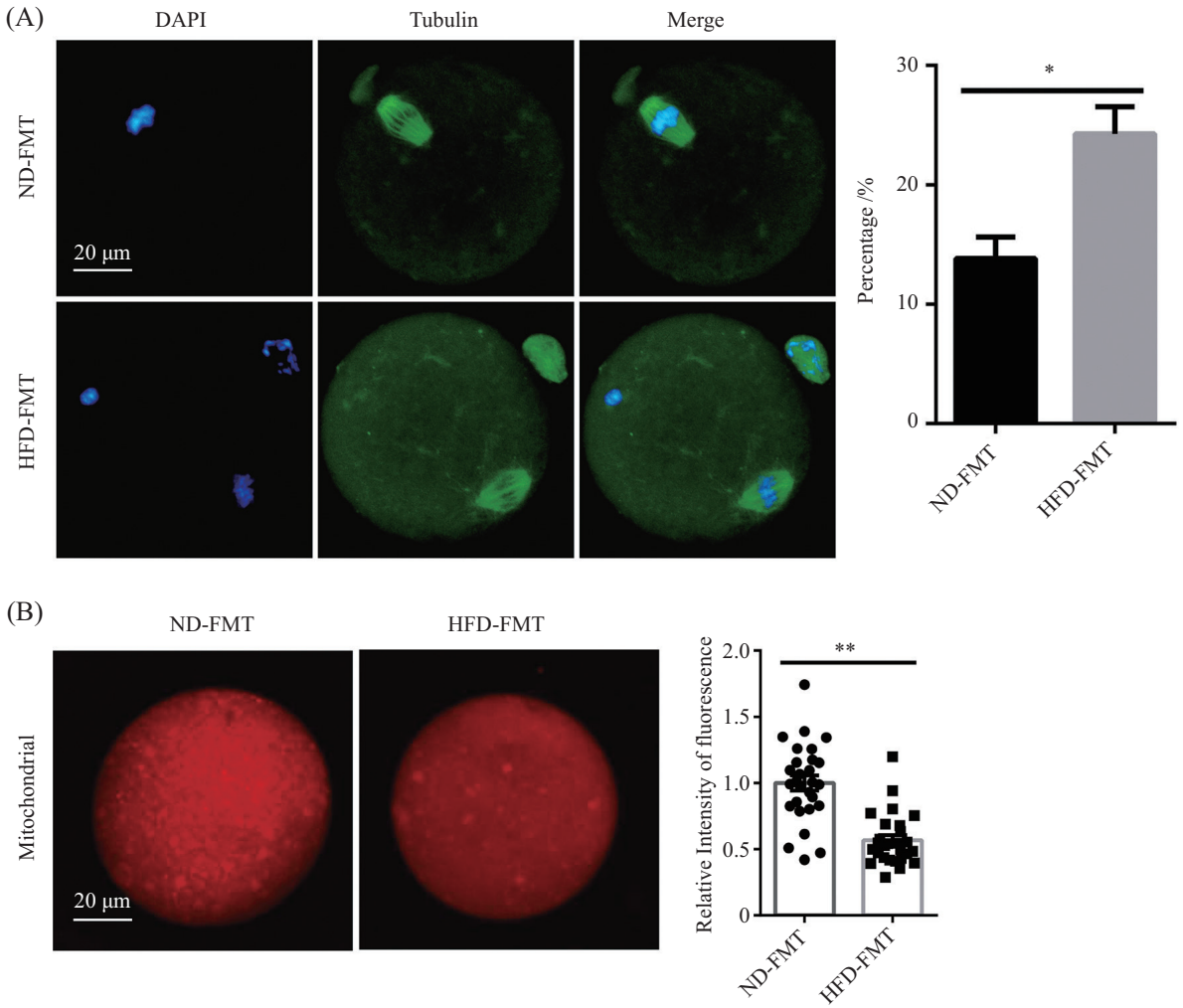
图5 ND-FMT组与HFD-FMT组小鼠生育力比较

Fig.5 Comparison of fertility between ND-FMT and HFD-FMT groups

that oocyte cleavage (92.32%±2.73%, n=103 *vs* 74.83%±4.07%, n=96; P<0.05) and blastocyst rates (68.81%±5.50%, n=97 *vs* 44.55%±3.28%, n=91; P<0.05) were significantly lower in the HFD-FMT group versus the ND-FMT group (Fig.5B and Fig.5C). These results suggested that the gut microbiota dysbiosis induced by a high-fat diet led to impaired oocyte maturation and compromised early embryonic developmental competence. However, in mating and littering experiments, there was no significant difference in average litter size between the HFD-FMT (8.57±1.02, n=7) and ND-FMT groups (10.86±0.34, n=7; Fig.5D).

2.6 An imbalance of gut microbiota leads to decreased oocyte quality

Oocytes from HFD-FMT mice (n=49) showed a significantly higher proportion of abnormal spindle morphology and chromosome misalignment than ND-FMT mice (P<0.05, Fig.6A, n=53). Additionally, Mitochondrial fluorescence staining showed that the HFD-FMT group (n=22) exhibited significantly lower mitochondrial fluorescence levels than the ND-FMT group (P<0.01, Fig.6B, n=29). These results indicated that the gut microbiota imbalance induced by a high-fat diet negatively impacted oocyte quality.



A: ND-FMT和HFD-FMT小鼠卵母细胞纺锤体形态和染色体排列代表图; B: ND-FMT和HFD-FMT小鼠卵母细胞线粒体活性染色代表图。*P<0.05, **P<0.01。

A: representative diagrams of spindle morphology and chromosome arrangement in the oocytes of ND-FMT and HFD-FMT mice; B: representative diagrams of mitochondrial activity staining in the oocytes of ND-FMT and HFD-FMT mice. *P<0.05, **P<0.01.

图6 ND-FMT和HFD-FMT组卵母细胞质量测定

Fig.6 Oocyte quality assay in the ND-FMT and HFD-FMT groups

3 Discussion

Obesity causes various diseases that can seriously impact health, and it also causes reproductive challenges in women of childbearing age. Previous studies have shown that oocytes from obese patients tend to result in lower embryo development rates, attachment rates, pregnancy rates, and live birth rates [33]. To investigate whether the disturbance of intestinal flora caused by high fat affects female fertility, in the current study, we found reduced oocyte maturation rates, lower *in vitro* fertilization cleavage and blastocyst rates, and lower birth rates in HFD mice compared with ND mice, suggesting that a high-fat diet reduces oocyte quality. Since spindle structure is closely related to oocyte maturation [34], we also examined spindle structure and found that abnormal spindles were increased in the oocytes of HFD (versus ND) mice. As mitochondria provide energy to support oocyte meiosis [35], we also examined mitochondrial activity and found it to be lower in the oocytes of HFD mice than in their ND counterparts.

In addition to reducing oocyte quality, obesity also disrupts gut microbial homeostasis [11]. To further explore the potential association between decreased oocyte quality and disordered gut microbiota in high-fat diet-induced obese mice, we used FMT to examine the effects of colony transplantation of gut microbiota from ND or HFD mice into females recipients on a normal diet. We found that the Bacteroidetes phylum was significantly reduced and the Firmicutes phylum was significantly increased in the HFD-FMT group compared with the ND-FMT group, which proved that the mouse model of intestinal flora disorder after fecal microbiota transplantation was successfully constructed. Oocyte maturation rates, cleavage rates and blastocyst rates were also significantly lower in HFD-FMT mice versus ND-FMT mice. Further, analysis of spindle morphology and mitochondrial activity yielded similar results in HFD-FMT mice as in HFD mice: namely, increased spindle abnormalities and reduced mitochondrial activity. Puzzlingly, there was no significant difference in the birth rate of the offspring between HFD-FMT and ND-FMT mice. We speculated that it might be due to the difference in embryo development *in vivo* and *in vitro*. These findings confirmed that obesity-induced disturbances in intestinal flora could directly contribute to a reduction in oocyte quality.

Conflict of interest

There are no conflicts of interest to declare.

Author contributions

WANG Jingjing: study design; MA Yefei, LANG Ranran, ZHAO Limin, and LIU Jun: experimental performance; MA Yefei: data analysis and writing-original draft.

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