Probiotics attenuate sperm damage induced by oxidative stress in rats

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***Abstract*: [Objective] To explore the preventive effect of probiotics on sperm damage induced by diet oxidative stress. [Methods] Thirty male rats were assigned to three groups randomly: group I (control) consumed a normal standard diet (5% fat, w:w), group Ⅱ (high fat diet) were fed with the standard diet supplemented with a 20% pure sunflower seed oil and group III (high fat diet + probiotics) were fed with the standard diet supplemented with 20% pure sunflower seed oil and 2% probiotics. After 3 weeks, concentration, viability and motility of sperm, and sperm cell DNA integrity were determined using a comet assay. [Results] The results showed that high fat diet could significant reduce the sperm concentration, viability, motility, and damage in sperm DNA. The damage of sperm were significantly attenuated in probiotics supplemented treatment and had no obviously difference on sperm quality compared with control group. [Conclusion] Present observation indicated the probiotics had the positive protective function on reproduction damage by improving sperm quality.**

Keywords: Probiotics; Protective; Sperm damage; free radicals

I. INTRODUCTION

Oxidative stress may occur in the body when reactive oxygen species (ROS) generation exceed scavenging ability of antioxidants[1]. Excessive ROS can damage macromolecules including lipids, proteins, polysaccharides and DNA, and lead to cell injury and detrimental effects in the form of gastrointestinal tract ulcers, decreases of immune functions, cessation of growth and reproduction and death from failure of adaptive mechanisms[2]. Reproductive consequences of ROS damage include disruption in function of spermatozoa and preimplantation embryos. It is assumed that the ROS could affect the fertility of males and many reports supported the viewpoint[3]. The ROS have both advantageous and pernicious influences on the sperm quality and then affect the process of fertilization in male animals and human. Antioxidant status may be one determinant of reproductive function in animals.

Diets high in fats, which increase fat-mediated oxidative stress and ROS levels in a variety of tissues, decrease antioxidant capacity have long been recognized. Decreasing the oxidative damage could be feasible by increasing the antioxidant level in the body. Overwhelming evidences from laboratory and animals have supported a protective role of antioxidants on reproductive function[4].

Recently, antioxidant properties of probiotics have been paid more attention. However, there are few reports concerning the protective effect of dietary probiotics on the oxidative injury of antioxidant system and reproductive performance in animals. In a previous study, we showed that probiotics had the antioxidant activity to positively modulate free radicals metabolism in rats, increasing the activities of antioxidant enzymes and decreasing content of nitric oxide and malondialdehyde[5]. It is proposed that dietary supplementation of probiotics with antimicrobial and antioxidant properties may be a promising strengthening agent for increasing pathogens resistance in animals. Reproduction is likely to fail long before life endangered by deficiency of any required nutrient, including dietary antioxidants. Up to day no study has been found reporting antioxidative probiotics on reproductive performance and oxidative status in the body, and only preliminary reports are available. The aims of the paper was to investigate the influences of oxidative stress on sperm quality and effect of probiotics supplementation on production performance and preventive function of sperm oxidative injury induced by diet stress in rats.

II. MATERIALS AND METHODS

A. Rats and probiotics

Thirty male SD rats (300 ± 30 g, SIPPR/BK Experimental Animal Ltd., China) were used and housed in a room at 22–24 °C, 60% relative humidity and 12-hour dark-light cycles. After 1 week of adaptation for a standard diet, rats were randomly assigned to one of three groups with 10 rats in each group. The control group received only a standard diet containing 5% fat. High fat diet group received a diet comprised of the standard diet supplemented with 20% pure sunflower seed oil. The final fat content of this high fat diet was 20%. Probiotics group was fed with the standard diet supplemented with 20% pure sunflower seed oil and 2% probiotics, which is produced by Chuangbo Modern Natural Agricultures Group (Shanghai, China), and is mainly based on Lactobacillus spp, Bacillus spp, beer yeast and photosynthetic bacteria co-culture and the microbial counts normally reach 5.5×10^9 cfu/g. Rats were allowed free access to their respective diets and tap water *ad libitum* for 3 weeks.

B. Samples Collection

At the end of the experimental periods, after an overnight fast, all rats were anesthetized with ethyl ether. Record the body weight and sacrificed. The testis and epididymis were dissected out and weighed to determine relative organ weight.

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C. Preparation of Sperm Suspensions

Sperm suspension was prepared according to the following procedure. Firstly, the caudal portion of the right epididymides of rats were placed in 30-mm dishes containing 2 mL physiological saline and minced into small pieces, standing for 10~15min at 37℃ incubator to let sperm swim out. Then the supernatant was diluted into 1% sperm suspension with a solution containing 5g $NaHCO_3$, 1 mL 35% formalin and 25mg/dL eosin for analysis of sperm concentration, viability, motility and nuclear DNA integrity.

D. Measurements of Sperm Count, Viability and Motility

The sperm concentration of diluted sperm suspension was counted with a haemocytometer. An aliquot of 10μL of dilution was added to counting chamber. After standing for 5min, and counted under the 400 × magnification of a light microscope (Olympus Optical Co. Ltd. Japan) The total sperm counts ($\times 10^6$/ml) were calculated.

Determination of sperm viability of diluted sperm suspension was carried out. An aliquot of 20 μL of dilution was added into 20 μL 0.05% eosin-Y and nigrosin in a slide. After standing for 2 min at room temperature, the slide was viewed at a magnification of 400× under a light microscope. Live sperms were white (unstained) and dead sperms were,stained. Counting 100 sperms per rat and calculating viability ratio (%) of sperm. Sperm motility and percentage of rapid progressive motile sperm (grade A) were categorized and assessed according to the World Health Organization standards (WHO, 1999).

E. Assessment of Sperm Nuclear DNA Integrity (Comet Assay)

The effect of probiotics on sperm nuclear DNA integrity was investigated using a comet assay (a single cell gel electrophoresis) kit (Trevigen, Gaithersburg, MD, USA). Cells stained with DNA fluorochrome SYBR Green (Trevigen) were visualized under a fluorescence microscope (Olympus Optical Co. Ltd. Japan) at 200 × magnification. 50 randomly selected areas were used for quantitative evalustion for per sample with the comet assay software project. In this study, tail length (μm), percentage of total DNA in tail (tail DNA percentage) , tail moment (TM) and olive tail moment (OTM) were used as indices of extension of DNA damage. The calculation formula of TM is to multiply tail DNA percentage by tail length. And OTM was to multiply tail DNA percentage by distance between head and tail.

Table 1 Effects of probiotics on body weight and reproductive organs weight relative to body weight of rats

Item	Control	High fat diet	High fat diet +2% probiotics
Initial body weigh (g)	381.42±15.45	382.51±16.87	379.40±15.96
Final body weight (g)	436.03±22.57	456.17±26.78	440.39±31.17
Relative testis weight (% body weight)	0.70±0.02[a]	0.68±0.02[b]	0.71±0.03[a]
Relative epididymis weight (% body weight)	0.22±0.02[a]	0.20±0.01[b]	0.22±0.01[a]

[a,b]Means in the same row with different letter superscripts are significant difference ($P < 0.05$), the same as below

F. Statistical Analyses

Data were expressed as mean ± standard deviation (SD) of 10 rats per group. A one-way analysis of variance (ANOVA) was conducted to examine significant difference among groups using SPSS 11.5 computer software (SPSS Inc., Chicago, Illinois, USA), and statistical significance was set at $P<0.05$.

III. RESULTS

A. Body Weight and Reproductive Organs Weight Coefficient

The consumption of water and feed during the period of experiment was similar among the groups (data not shown). The mean final body weight at the end of experiment was generally higher in treatment groups, compared with controls, but there didn't reach statistical significance as showed in Table 1. The testis and epididymis weight coefficient (expressed as relative to body weight) of rats fed with basal diet supplemented 20% sunflower seed oil was significantly lowered compared with control rats. Supplementation with probiotics along with the high fat diet had a similar reproductive organs weight coefficient with those of the control rats.

B. Sperm parameter analysis

To ascertain whether oxidative stress induced by high fat diet influences the reproductive performance of male rats and preventive effect of probiotics, sperm characteristic analysis was conducted. Sperm concentration, viability and motility were significantly decreased in rats fed high fat diet, damage degree of sperm was significantly attenuated in rats fed basal diet supplemented with high fat and probiotics when compared with control group. There was no significant differences in the progressive motility among groups (Table 2).

C. Sperm DNA damage

To investigate the preventive effect of probiotics on sperm DNA damage, the sperm DNA fragementation was evaluated using a comet assay. As showed in Table 3, high fat diet could provoke more DNA damage for rats, and was shown as significantly increase in tail length, tail DNA percentage, TM and OTM. Supplementation of probiotics could reduce sperm injury induced by high fat diet to some extent, shown as tail length, tail DNA percentage, TM and OTM were significantly decreased compared with model rats. Tail length, tail DNA percentage and TM in probiotics group had been decrease to level of control group ($P > 0.05$)

Table 2 Effects of probiotics on sperm parameters in rats

Parameters	Control	High fat diet	High fat diet +2% probiotics
Concentration ($\times 10^6$/mL)	26.2±3.1[a]	23.8±4.1[b]	26.8±3.4[a]
Viability (%)	89.3±3.5[a]	78.2±2.4[b]	86.2±3.7[a]
Motility (%)	87.8±2.8[a]	80.1±2.2[b]	84.5±2.4[a]
Progressive motility ($\times 10^6$/mL)	7.4±1.8	6.8±1.5	7.2±1.5

Table 3 Effect of probiotics on extent of sperm DNA damage in rats

Item	Control	High fat diet	High fat diet +2% probiotics
Tail length (μm)	3.64±1.65[b]	4.93±1.98[a]	4.12±2.53[b]
Tail DNA percent (%)	0.30±0.15[b]	2.70±0.97[a]	1.05±0.76[b]
Tail moment	0.09±0.04[b]	0.19±0.12[a]	0.05±0.06[b]
Olive tail moment	0.19±0.15[c]	0.84±0.13[a]	0.31±0.02[b]

IV. DISCUSSION

In this study, we used oxidative stress rat model induced by high fat diet[6] to explore the preventive effect of probiotics on sperm damage. Free radicals plays a significant role in pathophysiology of reproductive dysfunction in male animals and human[7]. It is well known that sperm cell membranes are rich in polyunsaturated fatty acids and are very susceptible to free radicals attack, and results in lipid peroxidation of membrane which causes increase in membrane permeability, inhibition of mitochondria respiratory function and adenosine triphosphate generation, and decrease in phosphorylation of axonemal proteins[8]. Lipid peroxidation had been accepted to be an inportant mechanism of the free radicals injured sperm. Sperm quality in rats fed high fat diet significantly declined may be caused by the decreased protective function of antioxidant enzyme in the body and the increased free radicals[6]. Excessive free radicals could decrease sperm counts and result in poor viability and motility of sperm (Table 2), even infertility. Sperm motility decreased by free radicals was presumably by a rapid loss of intracellular adenosine triphosphate, which altered axoneme structure and caused tail abnormality and decrease in sperm motility. Free radicals not only damage sperm lipid membrane, but also affect sperm cell DNA integrity. It is well known that oxidative damage of mitochondrial DNA occurs in all aerobic cells (may including sperm) because of ROS production during oxidative respiratory. Injury of cell DNA induced by ROS led to produce some peroxidation products such as malondialdehyde (MDA), 8-oxo-7,8-dihydroxyguanosine, which causes fragmentation of sperm DNA and has a mutagenic effect[9]. Higher degree of sperm cell mitochondria damage could reduce male fertility and supplementation of antioxidant could be a treatment to alleviate male infertility associated with sperm DNA damage from the clinical point of view[10].

Antioxidant supplementation can theoretically protect and prevent peroxidative damage. For example, vitamin E, as the main chain-breaking antioxidant can reverse the negative impact of antioxidant status and quality of the sperm quality induced by high polyunsaturated fatty acids supplementation on chickens diets. Probiotics are live microbial preparations that have beneficial effects on host healthy by inhibiting intestinal pathogenic microorganisms, promoting growth of beneficial microorganisms, producing bacteriocins / defensins, increasing systemic immune response. The use of probiotics for enhancing growth performance and increasing disease resistance ability and improving intestinal healthy has been well documented[11]. To our knowledge however, few studies were related to reproductive system of body. Kistanova[12] showed that probiotic (BioPro-1) can be used to improve reproductive performances of rams during breeding season. Su et al. [14] reported that the sperm motility rate, acrosome integrity rate and GSH-Px active in stock boars supplemented with selenium-enriched probiotics were better than that in control stock boars fed basal diet, and concluded that selenium-enriched probiotics supplementation could significantly enhance sperm quality in stock boars. In present study, sperm quality was significantly increased in rats fed basal diet supplemented with oil and probiotics compared with that in oxidative stress rats model, which demonstrated that incorporation of probiotics positively influenced the reproductive performance of rats in terms of higher sperm viability and motility, and lower sperm DNA fragmentation. Our study was the first reports that the effect of incorporated probiotics on protection of reproductive damage induced by oxidative stress. It is hypothesized that the preventive effect on sperm damage of probiotics might be as a result of the increase in antioxidant system and the decrease in sperm cell apoptosis in testis, which would need more avenues to approve in future.

In summary, results of the present investigation confirmed that incorporation probiotics have potential to restore quality of damage sperm induced by diet stress in rats to some extent.

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