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Lipopolysaccharide-induced mitochondrial dysfunction in boar sperm is mediated by activation of oxidative phosphorylation

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ABSTRACT

Lipopolysaccharide (LPS) has been reported to exert detrimental effects on boar sperm viability. In the present study, LPS was detected in boar semen samples at an average level of 0.62 ± 0.14 μg/mL. We treated boar sperm with 1 μg/mL LPS for 6 hours and examined alterations in sperm motility and apoptosis, together with mitochondrial functionality and mitochondrial reactive oxygen species generation. The expression and the location of toll-like receptor 4 (TLR4) and mitochondrial transcription factor A (TFAM) were determined to reveal possible mechanisms. LPS-treated sperm showed significant reduction in motility ($P < 0.05$) and viability ($P < 0.05$). LPS induced sperm mitochondrial damage *via* oxidative stress which is indicated by marked ultrastructural changes in the mitochondria including swelling, disorientation and vacuole, a decrease of mitochondrial membrane potential ($\Delta\Psi m$; $P < 0.05$), as well as an increase of malondialdehyde levels ($P < 0.01$). Moreover, the production of mitochondrial reactive oxygen species through oxidative phosphorylation (OXPHOS) was significantly ($P < 0.05$) increased, which leads to oxidative stress. The copy number of mitochondrial DNA was significantly ($P < 0.05$) higher in LPS-treated sperm. Moreover, cytochrome c oxidase subunit IV (COXIV), an important subunit in mitochondrial electron transport chain and OXPHOS, was significantly ($P < 0.05$) upregulated after LPS treatment. TFAM, the key transcription factor that activates mitochondrial DNA replication and transcription, was translocated from the head to the midpiece of sperm where mitochondria are distributed in LPS-treated sperm. Taken together, these results indicate that LPS-induced decrease of motility and viability in boar sperm is mediated by abnormal activation of OXPHOS and mitochondrial membrane lipid peroxidation. These findings may provide new insights in understanding the mechanisms underlying the bacterial infection-induced sperm damage.

1. Introduction

The presence of Gram-negative bacteria, especially *Escherichia coli*, may have detrimental effects on the viability of boar spermatozoa and, hence, negatively affects the litter size obtained from sows inseminated with such semen samples [1,2]. Soluble products of *E. coli* have been reported to induce human sperm damage [3]. Lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria, acts as the prototypical endotoxin and elicits strong immune responses [4]. LPS has been shown to reduce sperm motility and viability in human and mice [5–7]. Conversely, neutralization of LPS activity with molecules, such as polymyxin B, can rescue LPS-induced sperm motility loss and apoptosis [8].

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Previous studies indicate that excessive production of reactive oxygen species (ROS) is responsible for the decrease of human sperm viability [9]. It is well known that ROS is associated with cell membrane deterioration, DNA damage, and apoptosis [10,11]. In fact, the basal ROS formation is very low in viable sperm of fresh boar semen [12]. Intracellular ROS is primarily produced via the nicotinamide adenine dinucleotide phosphate oxidase–dependent respiratory burst, the electron transfer chain, and oxidative phosphorylation (OXPHOS) system [13]. Mammalian mitochondrial DNA (mtDNA) encodes 22 transfer RNAs, two ribosomal RNAs, and 13 proteins that are subunits of the electron transfer chain complexes [14]. Alterations in mtDNA will directly affect OXPHOS function and mitochondrial activity. Mitochondrial reactive oxygen species (mROS) have been regarded as by-products of OXPHOS [15]. Recent researches have demonstrated that mROS participate in a broad range of innate immune pathways [16]. LPS challenge led to upregulation of mtDNA transcription, OXPHOS, and ROS production in murine macrophages [17]. Another research reported that LPS downregulated the expression of mitochondrial transcription factor A (TFAM) and mtDNA, which contributes to mitochondrial dysfunction in liver cells [18]. However, whether OXPHOS and mROS production in boar sperm can be regulated by LPS is not fully elucidated.

Based on the literature summarized earlier, we speculate that the deteriorate effects of LPS may be mediated by TFAM-induced abnormal activation of mitochondrial OXPHOS and mitochondrial membrane lipid peroxidation. To test this hypothesis, we treated boar sperm with LPS and examined alterations in sperm motility and apoptosis, together with the mitochondrial functionality and mROS generation. The expression and the location of toll-like receptor 4 (TLR4) and TFAM were also determined to reveal possible mechanisms. The results may provide new insights in understanding the mechanisms underlying the bacterial infection-induced sperm damage.

2. Materials and methods

2.1. Ethics statement

The experiment was conducted following the guidelines of Animal Ethics Committee at Nanjing Agricultural University, China. The slaughter and sampling procedures complied with the “Guidelines on Ethical Treatment of Experimental Animals” (2006) No. 398 set by the Ministry of Science and Technology, China, and “the Regulation regarding the Management and Treatment of Experimental Animals” (2008) no. 45 set by the Jiangsu Provincial People’s Government.

2.2. Collection and culture of sperm

Twelve mature Duroc boars, aged 15 to 28 months, were used in this study. The sperm samples were manually collected weekly from each boar using the gloved-hand technique. Semen samples were purified and placed in a dish with prewarmed Ham’s F10 medium as previously described [19]. The semen was then incubated for 6 hours at 37 °C under 5% CO_2 atmosphere.

2.3. LPS assays

The levels of LPS in semen were measured by the limulus test using a commercial Tachypleus Amebocyte Lysate test (Chinese Horseshoe Crab Reagent Manufactory Co., Ltd., Xiamen, China) as previously described [20]. Briefly, samples were heated at 70 °C for 10 minutes and then diluted with endotoxin-free distilled water. After centrifugation, the supernatant was mixed with limulus amebocyte lysate for 10 minutes at 37 °C. The reaction was detected by Toxinn ometer ET-2000 (Synergy; BioTek, Winooski, VT, USA).

2.4. Evaluation of sperm motility

Sperm motility was evaluated by a sperm quality analyzer, which provided a quantitative estimation of sperm motility. The sperm motility index was determined in three sequential readings by introducing semen into a thin glass capillary tube (internal dimensions: depth 0.3 mm, width 3 mm, length 50 mm), which was housed in a plastic casing with a 2-mm diameter optical aperture.

2.5. Evaluation of apoptosis

Apoptosis was assayed by flow cytometry as previously described [21]. Apoptosis was assayed using an Annexin V–fluorescein isothiocyanate (FITC) apoptosis detection kit (G003; Nanjing Jiancheng Bioengineering Institute, China) and analyzed by flow cytometry (FACS Verse; BD Biosciences, USA). The sperm were stained with 1 μg/mL Annexin V–FITC and 1 μg/mL propidium iodide (PI) for 5 minutes in darkness at room temperature. The FITC and PI fluorescence signals were estimated at excitation wavelength of 488 and 549 nm, measured at emission wavelengths of 530 and 585 nm, respectively, and 10,000 events were acquired on flow cytometer.

2.6. Immunofluorescence staining

Boar sperm were fixed in 4% neutral paraformaldehyde for 30 minutes, blocked with 10% FCS, and then incubated with rabbit anti-TLR4 antibody (sc-10741; Santa Cruz, USA) or rabbit anti-TFAM antibody (ab176558; Abcam, USA) overnight at 4 °C. The tetramethylrhodamine-labeled goat anti-rabbit IgG (1:1000; KPL Inc., Gaithersburg, MD, USA) was used as the second antibody. The 4′,6-diamidino-2-phenylindole (DAPI; Sigma–Aldrich, USA) was used as a marker for cell nuclei. Negative control sections were incubated with normal serum instead of primary antibodies. Mounted slides were visualized using a fluorescence microscope (DMI6000 B; Leica, Germany).

2.7. Protein extraction and Western blot analysis

Sperm samples were homogenized in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP40, 0.25% Na-deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and