Theriogenology 87 (2017) 276–283

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Relationships between mitochondrial DNA content, mitochondrial activity, and boar sperm motility

Huiduo Guo [a], Yabin Gong [a], Bin He [a,*], Ruqian Zhao [a,b]

[a] Key Laboratory of Animal Physiology and Biochemistry, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, China
[b] Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, Nanjing Agricultural University, Nanjing, P. R. China

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 3 September 2016
Accepted 3 September 2016

Keywords:
Sperm motility
Mitochondrial DNA
Mitochondrial activity
Boar

ABSTRACT

Energy produced by mitochondria via oxidative phosphorylation (OXPHOS) is essential for mammalian sperm motility. Mammalian mitochondrial DNA (mtDNA)–encoded proteins are subunits of the OXPHOS system. Paradoxically, there are less mitochondrial and mtDNA contents in motile sperm than less motile sperm. Here, mature boar sperm was used as a model to investigate the relationships between mtDNA content, mitochondrial activity, and sperm motility. Motile and less motile sperm were separated by centrifugation on a discontinuous percoll density gradient. The contents and expression of mtDNA as well as mitochondrial activity and biosynthesis were determined to reveal possible mechanisms. Motile sperm showed less mitochondrial ($P < 0.01$) and mtDNA ($P < 0.05$) contents as compared to less motile sperm. Higher mitochondrial activity in motile sperm indicated by mitochondrial ultrastructure, higher mitochondrial transmembrane potential ($P < 0.01$), as well as higher mitochondrial respiratory chain complex I activity ($P < 0.05$). Moreover, more mitochondrial reactive oxygen species ($P < 0.01$) suggested higher mitochondrial biosynthesis in motile sperm. Although less mtDNA contents in motile sperm, accompanied by the higher expression of transcription factors, the level of mtDNA-encoded protein (cytochrome c oxidase subunit 1) which play pivotal role in OXPHOS was higher in motile sperm. The results are helpful to interpret why mtDNA-less sperm have higher mitochondrial activity and better motility.

1. Introduction

Nearly, all eukaryotes examined to date inherit mitochondrial DNA (mtDNA) from only the female, termed maternal inheritance [1]. As sperm mtDNA may be damaged by reactive oxygen species (ROS) involved in fertilization, thus causing some mitochondrial disorders in offsprings, elimination of sperm mtDNA is an advantageously evolutionary event [2]. Several models, including specific nuclease-dependent system, ubiquitin-proteasome system and autophagy have been proposed to explain the degradation of sperm mtDNA during spermatogenesis or after fertilization [3–6]. Recently, several studies indicate that motile sperm which can be found in the oviduct have less mtDNA molecules than less motile sperm in mice [7,8].

Mammalian sperm move relied on large amounts of adenosine triphosphate (ATP), which is used by axonemal dynein to drive sperm motility [9]. Mitochondria produce ATP via oxidative phosphorylation (OXPHOS), which takes place within the electron transfer chain (ETC) [10]. Paradoxically, there were less mitochondrial contents in motile sperm than less motile sperm. It has been reported that defective OXPHOS does not inhibit sperm motility in mice [11]. In boar sperm, glycolysis was the main pathway of glucose utilization [12]. Until now, whether mitochondrial OXPHOS works as a pathway for energy production in boar sperm is not clear.

* Corresponding author. Tel./fax: +86-25-84396413.
E-mail address: heb@njau.edu.cn (B. He).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.005

Mammalian mtDNA encodes 22 transfer RNAs, two ribosomal RNAs, and 13 proteins which are subunits of the ETC complexes [13]. Alterations in mtDNA will directly impact OXPHOS function and mitochondrial activity. The replication and transcription of mtDNA is controlled by factors both in intramitochondria and intermitochondria [14]. In human sperm, the contents of nuclear-encoded transcription factors which can be translocated to the mitochondria are associated with sperm quality [15]. Peroxisome proliferator–activated receptor gamma coactivator-1 alpha (PGC-1α) controls many aspects of oxidative metabolism, including mitochondrial biogenesis and respiration through the coactivation of many nuclear receptors, and factors outside the nuclear receptor family [16]. In particular, PGC-1α regulates transcription of mitochondrial transcript factor A (*TFAM*), a gene encodes a high mobility group protein that activates mtDNA transcription in mammals [17]. However, what is the relationship between less mtDNA and higher mitochondrial activity in motile sperm is still unclear.

In the present study, we use mature boar sperm as model to gain insight into the relationships between mtDNA content, mitochondrial activity, and sperm motility. We separated motile and less motile sperm by discontinuous density-gradient centrifugation and compared the differences in mitochondrial content, mtDNA content, and mitochondrial activity [7,8]. To determine the relationship between mtDNA content and the mitochondrial activity, mitochondrial biosynthesis and the contents of mtDNA-encoded protein were compared. The results will help to delineate the mechanism why mtDNA-less sperm have better motility.

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. Reagents

The MitoFluor Red 589 dye (M-22424), a mitochondrion-selective probe, was purchased from Molecular Probes, Inc. (USA). The MitoSOX Red (M36008), a specific fluorescent probe for superoxide produced by mitochondria was purchased from Invitrogen Co., Ltd., (USA). The JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′tetraethylbenzymidazolyl carbocyanine iodide) mitochondrial membrane potential (ΔΨm) kits were purchased from Nanjing KeyGen Development Co., Ltd. (China). The 2′,3′-dideoxycytidine (D5782), rotenone (R8875), and carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP, C2920) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The 3-methyladenine (3-MA, 189490) was purchased from Merck Millipore Co., Ltd., (USA).

2.3. Collection and culture of sperm

The mature Duroc boars, aged 15 to 28 months, were used in this study. The sperm-rich fraction was collected weekly from each boar using the gloved-hand technique. The semen was then incubated for 6 hours at 37 °C under 5% CO_2 atmosphere.

2.4. Sperm separation

Motile sperm has a higher density than less motile sperm [18]. Motile and less motile sperm were separated by Percoll gradient centrifugation as previously described [8]. Primarily, place all components of the upper (45%) and lower phase (90%) and semen samples in an incubator at 37 °C for 20 minutes. Transfer 2 mL of the lower phase into a sterile conical-bottom, disposable centrifuge tube. Layer 2 mL of the upper phase on top of the lower phase used a transfer pipet. Slowly dispense the upper phase lifting the pipet up the side of the tube as the level of the upper phase rises. The sample is centrifuged at 650 × *g* for 30 minutes. At the end of centrifugation, each sperm is located at the gradient level that matches its density. The leukocytes were distributed in upper phase and were removed. After carefully removing Percoll solutions, sperm sediment was resuspended in 0.9% physiological saline solution and centrifuged at 430 × *g* for 10 minutes once. Sperm motility of separated sperm was evaluated by a sperm quality analyzer.

2.5. Determination of mtDNA copy number

Total genomic DNA was isolated and the mtDNA copy number was determined using real-time polymerase chain reaction (PCR) as previously described with some modifications [19]. Primers specific for mtDNA were used for quantification (F: 5′-TCCTACTGGCCGTAGCATTCCT-3′, R: 5′-TTGAGGATGTGGCTGGTCGTAG-3′). Peptidylprolyl isomerase A (*PPIA*, also known as cyclophilin A) was chosen as a reference gene (F: 5′-GACTGAGTGGTTGGATGG-3′, R: 5′-TGATCTTCTTGCTGGTCTT-3′). All primers were synthesized by Generay Biotech Co., Ltd. (Shanghai, China). Real-time quantitative PCR was performed with a Mx3000P real-time PCR detection system (Stratagene, USA). The amplification specificity of each gene was checked by melting curve analysis. Relative mtDNA copy number was calculated with $2^{-\Delta\Delta Ct}$ method.

2.6. Transmission electron microscopy observation of mitochondria

The motile sperm and less motile sperm were fixed with 2% glutaraldehyde, postfixed with 1% osmium tetroxide, and embedded in resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. Sperm ultrastructure was determined with a transmission electron microscope (Hitachi H-7650, Hitachi Technologies, Tokyo, Japan).