Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Age-associated expression of vitamin D receptor and vitamin D-metabolizing enzymes in the male reproductive tract and sperm of Hu sheep

Xiaolei Yao[a,b], M.A. EI-Samahy[a,b], Hua Yang[a,b], Xu Feng[a,b], Fengzhe Li[a,b], Fanxing Meng[b], Haitao Nie[a,b], Feng Wang[a,b,*]

[a] Jiangsu Engineering Technology Research Center of Mutton Sheep and Goat Industry, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu, 210095, China

[b] College of Animal Science and Technology, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu, 210095, China

ARTICLE INFO

Keywords:
Vitamin D receptor
Testis
Epididymis
Spermatozoa
Hu sheep

ABSTRACT

The cellular response to 1,25-dihydroxyvitamin D_3 (Vit D_3; biologically active form of Vitamin D) is complex and depends not only on Vitamin D receptor (VDR) expression but also on cellular uptake of circulating Vit D_3 and the presence and activity of Vitamin D-metabolizing enzyme. This study evaluated the expression of VDR and Vitamin D-metabolizing enzymes in the ram reproductive tract at different developmental stages and in spermatozoa. Nearly all cell types in the testes and epithelial cells of the caput, corpus, and cauda expressed VDR, CYP27B1, and CYP24A1 proteins. The mRNA and protein expression of CYP2R1, CYP27A1, and CYP27B1 in the testes and cauda increased significantly with increasing age ($P < 0.05$). However, epididymal VDR mRNA and protein expression showed no significant difference ($P < 0.05$) between adult (9- and 24-month-old) and prepubertal (3-month-old) rams. Furthermore, VDR and CYP24A1 were mainly concentrated in the mid-piece of ejaculated or cauda epididymis spermatozoa or both. Additionally, VDR and CYP27B1 mRNA and protein expression levels were significantly higher in ejaculated spermatozoa than in cauda epididymal spermatozoa ($P < 0.05$). Moreover, VDR and CYP24A1 expression was significantly higher in high-motility than in low-motility spermatozoa ($P < 0.05$). The diverse expression patterns of VDR and Vitamin D-metabolizing enzymes in the ram reproductive tract at different developmental stages and spermatozoa suggest it plays a potential role in spermatogenesis.

1. Introduction

The formation of mature spermatozoa involves numerous cell-developmental stages that begin in the male reproductive organ and end with capacitation in the female reproductive tract. Immature spermatozoa produced in the testes acquire motility and fertilizing capacity in the epididymis, where they are stored until ejaculation (Xie et al., 2016). Before ejaculation, the spermatozoa mix with seminal plasma secreted by the epididymal tail and the accessory sex glands, leading to dilution of the spermatozoa to facilitate sperm transportation. Further, the seminal plasma contains biochemical components that protect the sperm and functionally affect the sperm surface (Ramos Angrimani et al., 2017).

* Corresponding author at: Jiangsu Engineering Technology Research Center of Mutton Sheep and Goat Industry, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu, 210095, China.
E-mail address: caeet@njau.edu.cn (F. Wang).

https://doi.org/10.1016/j.anireprosci.2018.01.003
Received 25 September 2017; Received in revised form 6 January 2018; Accepted 9 January 2018

1,25-dihydroxyvitamin D_3 (Vit D_3; biologically active form of Vitamin D) is a steroid hormone that plays critical roles in diverse biological functions in various organs. An organ is regarded as a Vit D_3-target organ if it expresses the vitamin D receptor (VDR). Vit D_3 must be activated by two enzymatic steps before binding VDR with high affinity (Prosser and Jones, 2004). It normally starts in the skin following ultraviolet B (UVB) radiation converts 7-dehydrocholesterol to cholecalciferol. It is subsequently metabolized through two hydroxylation steps 25-hydroxylases (CYP2R1 and CYP27A1), before 25-hydroxycholecalciferol ($25(OH)D_3$) relocates to the circulation, where the renal 1α-hydroxylase (CYP27B1) converts $25(OH)D_3$ to the active Vit D_3. Vit D_3 binds and activates VDR in target cell, until 24-hydroxylase (CYP24A1) inactivates it (Krause et al., 2017).

The expression of VDR and Vitamin D-metabolizing enzymes (CYP2R1, CYP27A1, CYP27B1, and CYP24A1) has been widely demonstrated in numerous reproductive tissues, such as testes, epididymis, ovaries, and uterus, as well as in spermatozoa (Jensen et al., 2010; Mahmoudi et al., 2013; Bergada et al., 2014; Jensen, 2014; Brozyna et al., 2015; Rodriguez et al., 2016), which suggests that target-cell responsiveness is affected not only by circulating $25(OH)D_3$ levels, but also the cellular expression of Vitamin D-metabolizing enzymes. Particularly, CYP27B1 and CYP2R1 are more strongly expressed in the testes than in other organs. Whereas in normal mice, the downregulation of CYP27B1 and upregulation of CYP24A1 can mediate the Vit D_3 level, high CYP27B1 and low CYP24A1 expression were detected in the testes of VDR-null mice, which confirmed the testicular feedback system of Vit D_3 by functional VDR (Boisen et al., 2017). VDR-null mice showed an impaired fertility later in life because of low sperm quality and reduced sperm motility that could be partly restored by calcium (Ca) supplementation. However, VDR may not be vital for testicular development in mice as two out of three VDR-null strains and CYP27B1knockout mice had clearly normal testicular histology (Jensen, 2014; Boisen et al., 2017). Besides its roles in Ca level regulation and lipid metabolism in spermatozoa, Vit D_3 influences human sperm quality through epigenetic signatures, which is reflected in spermatozoa development and motility (Schagdarsurengin and Steger, 2016; Toman et al., 2016). These findings indicate that Vit D_3 plays an important role in reproductive physiology.

To enhance our knowledge of Vit D_3 function in male reproduction, we conducted the first comprehensive analysis of the expression of VDR and Vitamin D-metabolizing enzymes in the ram reproductive tract at different developmental stages. Moreover, for the first time, we determined whether the expression of these molecules differs between the cauda epididymis and ejaculated spermatozoa, and between spermatozoa exhibiting distinct motilities.

2. Materials and methods

All animal experimental procedures were performed in accordance with the National Institutes of Health (NIH) Guidelines and the guide for the Chinese Association for Laboratory Animal Science. All antibodies were obtained from commercial sources (Table 1).

2.1. Experiments and sample collection

The experiments were conducted at the Jiangyan Hai Lun sheep Farm (Jiangsu, China) from April to May 2016. Experimental animals were housed under similar conditions of free access to food and water in natural lighting and were fed normal diet.

Experiment 1: In total, 15 Hu sheep were divided into three groups according to age (3 months: 16.07 ± 0.20 kg; 9 months: 40.07 ± 2.75 kg; and 24 months: 76.65 ± 4.38 kg; n = 5/group). The epididymis (caput, corpus, and cauda) and testes were collected from each animal for further study. Tissue samples were divided into two parts: one part was fixed in 4% formaldehyde for immunohistochemistry while the other was minced with surgical scissors and stored at −80 °C for further analysis.

Experiment 2: The epididymis was collected from 24 sexually mature Hu sheep and processed within 3 h after surgery, and spermatozoa were flushed with RNase-free phosphate-buffered saline (PBS) as described by (Chang et al., 2016). Additionally, fresh ejaculates were manually harvested from 6 sexually mature Hu sheep after 3–5 days of sexual abstinence, and the semen was centrifuged (1400 × *g*, 10 min) to obtain spermatozoa. The collected cauda epididymis and fresh ejaculate spermatozoa were immediately frozen in liquid nitrogen and stored at −80 °C until total protein and mRNA extraction.

Experiment 3: Spermatozoa exhibiting distinct motilities were obtained from fresh ejaculates using our previously described methods (Yao et al., 2017). Briefly, fresh ejaculates were harvested from 12 sexually mature rams. Sperm motility was detected by computer-assisted semen analysis using a SpermVision instrument (Minitüb, Tiefenbach, Germany), which was connected with an Olympus CX31 microscope, monitoring 20 fields per slide of sperm suspension (~2000 spermatozoa/sample, 37 °C). Spermatozoa motility was ≥80% in five rams, 50%–80% in four rams, and ≤50% in three rams. Finally, the ejaculates with sperm motility of ≥80% and ≤50% were selected as high- and low-motility models, respectively. After motility determination, all samples were immediately frozen at −80 °C until use.

Table 1
Primary antibodies used for immunohistochemistry (IHC), western blot (WB), and immunofluorescence (IF) analyses.

Antibody	Catalog No.	Company	Specificity	Dilution, IHC/IF	Dilution, WB
VDR	14526-1-AP	ProteinTech., Chicago, IL, USA	Human, mouse, rat	1:200	1:500
CYP24A1	21582-1-AP	ProteinTech.	Human, mouse, rat	1:100	1:500
CYP27B1	bs-14146R	Bioss Inc., Woburn, MA, USA	Sheep and others	1:200	1:500
β-actin	bs-0061R	Bioss Inc., Woburn, MA, USA	Sheep and others	–	1:2000