Theriogenology 86 (2016) 839–849

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Effects of glucose metabolism pathways on sperm motility and oxidative status during long-term liquid storage of goat semen

Jian-Hua Qiu [a,1], You-Wei Li [b,1], Hong-Li Xie [a], Qing Li [a], Hai-Bo Dong [a], Ming-Ju Sun [b], Wei-Qiang Gao [a], Jing-He Tan [a,b,*]

[a] College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai-an City, P. R. China
[b] College of Life Science, Northeast Agricultural University, Harbin, P. R. China

ARTICLE INFO

*Article history:*
Received 10 December 2015
Received in revised form 3 March 2016
Accepted 3 March 2016

*Keywords:*
Glucose metabolism
Spermatozoa
Semen
Liquid semen storage
Goat

ABSTRACT

Although great efforts were made to prolong the fertility of liquid-stored semen, limited improvements have been achieved in different species. Although it is expected that energy supply and the redox potential will play an essential role in sperm function, there are few reports on the impact of specific energy substrates on spermatozoa during liquid semen storage. Furthermore, although it is accepted that glucose metabolism through glycolysis provides energy, roles of pentose phosphate pathway (PPP) and tricarboxylic acid cycle remain to be unequivocally found in spermatozoa. We have studied the pathways by which spermatozoa metabolize glucose during long-term liquid storage of goat semen. The results indicated that among the substrates tested, glucose and pyruvate were better than lactate in maintaining goat sperm motility. Although both glycolysis and PPP were essential, PPP was more important than glycolysis to maintain sperm motility. Pentose phosphate pathway reduced oxidative stress and provided glycolysis with more intermediate products such as fructose-6-phosphate. Pyruvate entered goat spermatozoa through monocarboxylate transporters and was oxidized by the tricarboxylic acid cycle and electron transfer to sustain sperm motility. Long-term liquid semen storage can be used as a good model to study sperm glucose metabolism. The data are important for an optimal control of sperm survival during semen handling and preservation not only in the goat but also in other species.



## 1. Introduction

The increase in the utilization of artificial insemination has brought about the development of techniques for midterm or long-term liquid semen storage. Liquid-stored semen can be an alternative to frozen-thawed semen because liquid semen storage is less costly than semen freezing [1]. In addition, goat seminal plasma has detrimental effects on spermatozoa when semen is preserved in extenders containing yolk or milk [2]. Thus, the use of chemically defined extenders will have obvious advantages in liquid storage of goat semen. However, although much research has been conducted to prolong sperm viability and fertility of liquid-stored semen, limited improvements have been made in different species [3–5].

It is reasonable to think that energy supply and the redox potential would be essential for sperm function during liquid semen storage. Some studies have suggested that semen liquid storage may cause oxidative stress that damage sperm DNA and plasma membrane [6–8]. However, there are few reports on the impact of specific energy substrates on spermatozoa during liquid semen storage [9].

* Corresponding author. Tel.: +86 538 8249616; fax: +86 538 8241419.
*E-mail address:* tanjh@sdau.edu.cn (J.-H. Tan).
[1] These authors contributed equally to this work.

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.03.005

Studies using other models suggest that mammalian spermatozoa obtain energy from monosaccharides through glycolysis alone or glycolysis combined with the mitochondrial tricarboxylic acid (TCA) cycle [1], and that the TCA cycle and the pentose phosphate pathway (PPP) of spermatozoa may produce molecules with great reducing capacity [10]. However, although it is well accepted that glucose metabolism through glycolysis provides energy for spermatozoa [11], the existence and role of PPP have not yet been unequivocally found in spermatozoa. For example, although a functional PPP has been reported in mouse [12,13], human [14–16], and rabbit [17] spermatozoa at different stages of the sperm life, no PPP activity was observed in bull [18,19], ram [18,20], and dog [18] spermatozoa, which showed a remarkable glycolysis at the time of analysis. Furthermore, although some researchers have reported that energy from the TCA cycle is absolutely necessary to maintain sperm motility in bull [21–25], others have concluded that the TCA cycle energy is not essential for sperm motility in mice [26].

According to Rodriguez-Gil [1], sperm equilibrium between glycolysis and the TCA cycle is controlled by the external factors (such as medium composition, $O_2$ pressure, and extracellular pH), the metabolic phenotype of the species, and the functional status of spermatozoa at the time of analysis. However, sperm metabolism of glucose during liquid semen storage has not been reported to our knowledge although a clear knowledge about this will be important for optimal control of sperm survival during semen handling and preservation. In the present study, we investigated the pathways by which goat spermatozoa metabolize glucose during liquid semen storage. The molecular mechanism was elucidated by regulating activities of the related enzymes and observing the effect on sperm motility and levels of oxidative stress during long-term liquid storage of goat semen.

Glucose-6-phosphate dehydrogenase (G6PDH) is a rate-limiting enzyme of PPP, which converts glucose-6-phosphate into 6-phosphoglucono-δ-lactone. Glyceraldehyde 3-phosphate dehydrogenase catalyzes the conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate, the sixth step in glycolysis. The monocarboxylate transporters (MCTs) are a family of proton-linked plasma membrane transporters that carry molecules having one carboxylate group (monocarboxylates), such as lactate and pyruvate, across biological membranes [27]. The electron transport chain in the mitochondrion is the site of oxidative phosphorylation where the NADH and succinate generated in the TCA cycle are oxidized to produce ATP [28]. In this study, to observe the roles of PPP and glycolysis in sperm glucose metabolism, G6PDH and glyceraldehyde 3-phosphate dehydrogenase were inhibited with 6-aminonicotinamide (6-AN) and iodoacetate, respectively and to determine how pyruvate is used by spermatozoa, the electron transport chain and MCTs were inhibited with rotenone and α-cyano-4-hydroxy cinnamate (4-CIN), respectively.

## 2. Materials and methods

The experimental procedures were approved by the Animal Care and Use Committee of the Shandong Agricultural University P. R. China (Permit number: SDAUA-2001-0510). Unless otherwise specified, all chemicals and reagents used in the present study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### 2.1. Semen collection

The research was carried out at the Animal Station of the Shandong Agricultural University, Shandong Province (122°–114°E; 38°–34°N), People's Republic of China. The male goats were kept in sheltered pens separated from females and fed with hay and concentrate with water available ad libitum. Male goats (n = 6) of Lubei White breed (a native meat breed) were used at the age from 1.5 to 4 years. A doe mount was used to train the male goats to ejaculate into an artificial vagina. Semen was collected at 3-day intervals into a prewarmed empty tube.

### 2.2. Extenders

Our preliminary experiments indicated that sperm motility was better maintained in PBS without $Ca^{2+}$ and $Mg^{2+}$ (data not shown). Thus, $Ca^{2+}$- and $Mg^{2+}$-free PBS (hereinafter referred to as PBS) was used as basic extender in this study. The PBS was composed of 136 mM NaCl, 8.1 mM $Na_2HPO_4$, 1.47 mM $KH_2PO_4$, 2.68 mM KCl, 1 g/L polyvinyl alcohol, 0.06 g/L penicillin, and 0.05 g/L streptomycin. Depending on experiments, PBS was supplemented with different energy substrates and/or metabolism regulators. The osmotic pressure of the extender was adjusted by decreasing the amount of NaCl accordingly when the concentration of the added energy substrate was high enough to change the osmotic pressure. For example, the concentration of NaCl was decreased from 136 mM to 126 mM when 10 mM pyruvate or lactate was added to the extender. The pH of the extender was adjusted to 7.4 with 1N HCl or 1N NaOH when necessary.

The metabolism regulators used in this study were as follows: 6-AN was used to inhibit the G6PD in PPP; iodoacetate was used to inhibit the glyceraldehyde-3-phosphate dehydrogenase in glycolysis; 4-CIN was used to block the MCT; and rotenone was used as an inhibitor of the electron transport chain. To prepare stock solutions, 6-AN (10 mM), iodoacetate (5 mM), 4-CIN (100 mM) were dissolved in PBS, rotenone (1 mM) were dissolved in DMSO. All the stock solutions were stored in aliquots at −20 °C and diluted to desired concentrations with proper extenders immediately before use.

### 2.3. Semen centrifugation and dilution

Immediately after collection, each ejaculate was mixed with equal volume of PBS that had been prewarmed to 30 °C to 35 °C. An aliquot of semen was taken from each ejaculate and examined for sperm motility. Only ejaculates with a sperm motility of over 90% were used for experiments. The ejaculate was then centrifuged (200 × *g*) for 10 minutes at 30 °C. After the supernatant was removed, the bottom sperm pellet was resuspended with the same volume of PBS as that of the removed supernatant to restore the original volume and sperm concentration of ejaculates. Then, the ejaculate was 1:10 diluted with corresponding extenders and packed