Animal Reproduction Science 164 (2016) 47–56

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Effects of pH during liquid storage of goat semen on sperm viability and fertilizing potential

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ARTICLE INFO

Article history:
Received 29 August 2015
Received in revised form 16 October 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Liquid semen storage
Extender pH
Sperm motility
Fertilizing potential
Goat

ABSTRACT

A specific problem in goat semen preservation is the detrimental effect of seminal plasma on sperm viability in extenders containing yolk or milk. Thus, the use of chemically defined extenders will have obvious advantages. Although previous studies indicate that the initial pH of an extender is crucial to sustain high sperm motility, changes in extender pH during long-term semen storage have not been observed. Monitoring extender pH at different times of semen storage and modeling its variation according to nonlinear models is thus important for protocol optimization for long-term liquid semen preservation. The present results showed that during long-term liquid storage of goat semen, both sperm motility and semen pH decreased gradually, and a strong correlation was observed between the two. Whereas increasing the initial extender pH from 6.04 to 6.25 or storage with stabilized pH improved, storage with artificially lowered pH impaired sperm motility. Extender renewal improved sperm motility by maintaining a stable pH. Sperm coating with chicken (Gallus gallus) egg yolk improved motility by increasing tolerance to pH decline. A new extender (n-mZAP) with a higher buffering capacity was formulated, and n-mZAP maintained higher sperm motility, membrane integrity and acrosome intactness than the currently used mZAP extender did. Goat semen liquid-stored for 12 d in n-mZAP produced pregnancy and kidding rates similar to those obtained with freshly collected semen following artificial insemination. In conclusion, maintenance of a stable pH during liquid semen storage dramatically improved sperm viability and fertilizing potential.

1. Introduction

Liquid-stored semen can be an alternative to frozen-thawed semen for artificial insemination (AI), because semen cryopreservation is an expensive process. However, although much research has already been conducted to prolong the in vitro viability and fertilizing potential of stored liquid semen, limited improvements have been achieved in different species (Weitze, 1990; De Pauw et al., 2003; Leboeuf et al., 2003). A specific problem in the preservation of goat semen has been the detrimental effect of seminal plasma on the viability of the spermatozoa in extenders containing egg yolk or milk (Leboeuf et al., 2000). The use of milk- or yolk-containing extenders in this species therefore requires the removal of the seminal plasma by washing before semen dilution. Because washing is a complex and time-consuming process, and it causes damage (Harrison and White, 1972) and some loss (Corteel,

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http://dx.doi.org/10.1016/j.anireprosci.2015.11.011
0378-4320/

1981) of spermatozoa, the use of chemically defined extenders will have obvious advantages in liquid storage of goat semen.

However, reports on the liquid storage of goat semen in chemically defined extenders which contain neither milk nor yolk are few (Leboeuf et al., 2003; Paulenz et al., 2005); so are reports on cryopreservation of goat spermatozoa in such chemically defined media (Kundu et al., 2000, 2002; Khalifa and El-Saidy, 2006). One study (Xu et al., 2009) has shown that the self-made mZAP extender, formulated based on the Zorlesco extender (Gottardi et al., 1980) and the Androhep® extender (Weitze, 1990) used for liquid storage of porcine semen, performed the best among the extenders tested. A forward sperm motility of over 30% was maintained for 9 d and successful pregnancies were induced after AI with semen stored for 7 d when goat semen was diluted and stored in the mZAP extender. Another study (Zhao et al., 2009) examined effects of semen cooling velocity, sperm coating and extender renewal on sperm viability during goat semen storage. With the optimized protocol, a sperm motility of 48% was maintained for 13 d, and an in vitro-fertilizing potential similar to that of fresh semen was maintained for 11 d.

The pH of freshly ejaculated boar semen is between 7.2 and 7.5 (Johnson et al., 2000). When glucose was present in the extender, all visible compartments of the boar spermatozoa as well as the extender were acidified to pH 6.2 within 20 h (Kamp et al., 2003). Although it is recognized that a lower pH can reduce sperm motility and metabolic activity, which is good for keeping spermatozoa viable during manipulation or preservation (Johnson et al., 2000), metabolic acidosis may prevent prolonged storage if no proper buffer is present in the extenders. Thus, our previous study demonstrated that whereas 6.04 was the most suitable pH for liquid storage of goat semen, sperm viability decreased significantly when semen was stored at either pH 6.61 or pH 5.54 (Xu et al., 2009). While these results and those from other studies (Maxwell and Salamon, 1993; Johnston et al., 2000) indicate that the initial pH of an extender is crucial to sustain high sperm motility during semen manipulation or storage, changes in semen pH during long-term storage have not been reported. Monitoring extender pH at different times of semen storage and modeling its variation according to nonlinear models is thus of great importance for protocol optimization for long-term liquid semen preservation.

The objective of this study was to observe the effect of pH during liquid semen storage in chemically defined extenders on goat sperm viability and fertilizing potential. Experiment 1 monitored and correlated semen pH and sperm motility changes during the storage of coated or non-coated spermatozoa with or without extender renewal. In experiment 2, effects of artificially adjusting semen pH on sperm motility were observed. The adjustments made included stabilizing or lowering pH during storage and increasing the initial extender pH. In experiment 3, a new extender that possesses a higher buffering capacity was developed and its effect on sperm function and fertilizing potential (AI outcomes) was evaluated.

2. Materials and methods

2.1. Ethics statement

The experimental procedures were approved by the Animal Care and Use Committee of the Shandong Agricultural University P. R. China (Permit number: SDAUA-2001-001).

All chemicals and media used were purchased from Sigma Chemical Co (St. Louis, MO, USA), unless specified otherwise.

2.2. Animals and semen collection

The study was conducted at the Animal Station of the Shandong Agricultural University, Shandong Province (122° to 114°E; 34° to 38°N) of China. Male goats were kept in sheltered pens separated from females, and fed hay and concentrate, with water available ad libitum. The Lubei White male goats ($n = 5$) used in this study were of meat breed. Male goats aged between 2 and 4 yr were trained to ejaculate into an artificial vagina at a doe mount. Semen collection was scheduled to be at 3-d intervals. The ejaculate was collected into a pre-warmed empty tube.

2.3. Extenders

The mZAP extender, which contained 63.9 mM glucose, 80.6 mM fructose, 39.8 mM sodium citrate, 5.1 mM ethylenediaminetetraacetic acid (EDTA), 14.9 mM $NaHCO_3$, 37.8 mM Hepes, 0.25 g/100 mL polyvinyl alcohol (PVA), 5000 IU/100 mL penicillin and 0.1 g/100 mL streptomycin, was prepared as reported previously (Xu et al., 2009). The new mZAP (n-mZAP) extender contained the same constituents as the mZAP did except that $NaHCO_3$ and Hepes in the mZAP extender was replaced with 2-morpholinoethanesulfonic acid (MES) as buffers. The pH of the extenders was adjusted to 6.04 with 1N HCl unless specified otherwise.

2.4. Sperm coating and semen dilution and packaging

In experiments not involving sperm coating, ejaculates of original volume were 1:10 diluted with pre-warmed (35 °C) extender. In the sperm-coating experiment, ejaculates were 1:1 diluted with the extender supplemented with 20% chicken (Gallus gallus) egg yolk. Within 5 min, the coated ejaculates were centrifuged at 30 °C for 10 min at 200 × *g*. Following removal of the supernatant, the coated ejaculates were 1:10 diluted with pre-warmed extender. Both the non-coated and coated ejaculates that had been 1:10 diluted were packaged in 1.5-mL microfuge tubes (0.5 mL semen per tube) and the tubes were then placed in a water bath immediately after packaging and maintained at 35 °C before cooling and storage.

2.5. Semen cooling and storage

After packaging, the semen was cooled to and stored at 5 °C in an incubator. Cooling down to 5 °C was achieved by incubating at 5 °C the storage tubes with extended semen