Effect of sugars on characteristics of Boer goat semen after cryopreservation


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A B S T R A C T

In order to improve Boer goat semen quality during cryopreservation process, the influence of sugar supplementation on semen characteristics of sperm were investigated. Three experiments were carried out to investigate the effect of (a) addition of two monosaccharides (fructose and glucose) and two disaccharides sugars (trehalose and sucrose) (b) sugar combination (fructose and trehalose, sucrose and trehalose, glucose and trehalose), and control (glucose without trehalose) (c) different concentrations of trehalose on cryopreservation using Tris based extender. The total motility, forward motility, viability, normal spermatozoa, acrosome integrity and membrane integrity were assessed subjectively. Differences were not detected among monosaccharides, but glucose increased \( P < 0.05 \) sperm forward motility in post-thaw goat semen compared to trehalose or sucrose supplementation. Semen quality did not differ \( (P > 0.05) \) among disaccharide sugar supplementation. Combination of glucose and trehalose significantly improved the characteristics of Boer spermatozoa after cryopreservation \( (P < 0.05) \). Supplementation of trehalose (198.24 mM) into the glucose extender significantly increased total motility, forward motility, live spermatozoa, acrosome integrity and membrane integrity following cryopreservation \( (P < 0.05) \). In conclusion, glucose had the better ability to support Boer sperm motility and movement patterns. Combination of monosaccharide (glucose) and disaccharide (trehalose) improved semen quality following cryopreservation. Trehalose supplementation at the concentration of 198.24 mM to the glucose extender conferred the greater improvement of semen quality for Boer semen cryopreservation.

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1. Introduction

Boer goats are one of the most popular breeds of meat goat in the world due to their fast growth rate and excellent carcass qualities. Moreover, Boer goats have a high resistance to diseases and adapt well to hot, dry semi-desert climates. For the improvement of genetic selection and economic advantages in Boer goat production, the use of artificial insemination (AI) is necessary. In order to optimize extender medium for the achievement of best post-thaw semen characteristics which would infer greater fertility, it is important to study the influence of different sugars on Boer goat spermatozoa.
Sugar maintains the osmotic pressure of the diluents by inducing cell dehydration and less ice crystal formation into the spermatozoa (Leibo and Songasen, 2002; Purdy, 2006). Moreover, sugar has the ability to form a glass (vitrification) by depressing the membrane phase transition temperature of dry lipids. It also interacts with phospholipid membranes at low hydration and thus causes stabilization of the membranes (Aisen et al., 2002; Hincha et al., 2006). Furthermore, sugar is utilized by spermatozoa as an energy source through glycolysis and mitochondrial oxidative phosphorylation to support sperm motility and movement (Salisbury and Vandemark, 1961; Salisbury et al., 1978; Ponglowhapan et al., 2004).

Many researchers have studied the effect of sugar supplementation in semen extender on the quality of cryopreserved spermatozoa. Glucose was suggested to be more suitable sugar than fructose, lactose or raffinose in Tris based media in ram (Salamon and Visser, 1972). Abdelhakeam et al. (1991) reported that addition of ten different sugars did not significantly improve the survival of unfrozen ram spermatozoa, but maltose yielded the best post-thaw motility in TEST extender. There was no effect of sugar type (fructose, glucose, lactose, sucrose, or trehalose) on post-thaw motility of ram spermatozoa (Molinia et al., 1994). In contrast, trehalose, xyllose and fructose significantly increased total active sperm rates compared to other sugars (glucose, sucrose, galactose, lactose, maltose and raffinose) in frozen thaw samples of dog spermatozoa (Yildiz et al., 2000). Neither types nor concentration of the two sugars (glucose and fructose) influenced the quality of spermatozoa in canine semen (Ponglowhapan et al., 2004). Monosaccharide, especially fructose enhanced quality of spermatozoa in canine semen (Ponglowhapan et al., 2009) semen, but failed to improve fertility in frozen bull (Foote et al., 1993), rabbit (Dalmata and Graham, 1997) or brown hare (Kozdrowski, 2009) semen. Aboagla and Terada (2003) reported that the substitution of a Tris–citric acid diluents composition with trehalose only significantly improved sperm motility, acrosome integrity and membrane fluidity of Japanese native miniature goat. However, there is the paucity of information on the influence of sugar combination and trehalose on characteristics of Boer goat spermatozoa. These studies were conducted to determine the effect of two monosaccharide and two disaccharide sugars in Boer goat semen cryopreservation. A second aim was to explore the effect of trehalose in different doses and in combination with other sugars on sperm characteristics in post-thaw Boer goat semen.

2. Materials and methods

2.1. Animals and semen collection

A total of seven Boer bucks (body weights from 110 to 125 kg) were used as sperm donors. Semen was collected by using an artificial vagina twice a week for 6 weeks. The semen samples were assessed for volume, colour, consistency, mass activity, sperm concentration, sperm morphology, and percentage of motile spermatozoa (Evans and Maxwell, 1987). Only ejaculates between one and two millilitres volume with a concentration of greater than 2.5 × 10⁹ sperm/ml having >75% progressively motile sperm and >85% of the sperm with normal morphology were selected for cryopreservation. A total of 52 ejaculates were individually frozen. Fourteen ejaculates were used in experiment 1 (two replications from each of seven Boer goats). Eighteen ejaculates were used in experiment 2 (three replications from each of six Boer goats) and twenty ejaculates were used in experiment 3 (four replications from each of five Boer goats).

2.2. Extender preparation

All chemicals were reagent grade and were purchased from Sigma–Aldrich, St. Louis, MO. The cooling extender consisted of 250 mM Tris, 88.5 mM citric acid, and 18% (v/v) egg yolk, and antibiotics (500 IU penicillin, 5 mg streptomycin, 10 mg neomycin per ml). The freezing extenders comprised of 250 mM Tris, 88.5 mM citric acid, and 18% (v/v) egg yolk, and glycerol 8% (v/v) (Foote, 1970; Liu et al., 1998). Three experiments were conducted to examine the effect of sugars on semen characteristics.

2.2.1. Experiment 1

Four cooling and freezing extenders were prepared by addition of two monosaccharide sugars (glucose, fructose) at the concentration of 69.38 mM and two disaccharide sugars (sucrose and trehalose) at the concentration of 33.04 mM, respectively.

2.2.2. Experiment 2

Three cooling extenders were prepared by adding 69.38 mM glucose, 69.38 mM fructose, and 33.04 mM sucrose. The freezing extender was made by addition of trehalose at the concentration of 33.04 mM.
in a cooling chamber at 5–10 min. Subsequently, the extended semen were placed in cooling extenders and incubated at room temperature for by two step dilution methods. Semen was diluted with supernatant was removed and semen dilution was performed into the liquid nitrogen for storage. After 2 days, thawing was done immediately after 30 s. Final concentration was adjusted to the cooling extender. The different concentrations of trehalose (33.04, 49.59, 66.08, 198.24, 264.32 mM) were added to the freezing extender.

2.3. Frozen semen processing

After initial evaluation, semen was diluted with normal saline as washing solution and then centrifuged at 1500 x g for 3 min to remove seminal plasma. The supernatant was removed and semen dilution was performed by two step dilution methods. Semen was diluted with cooling extenders and incubated at room temperature for 5–10 min. Subsequently, the extended semen were placed in a cooling chamber at 5 °C and maintained for 2.5 h. Each of the extended semen was diluted in the freezing extender and kept for 30 min. Final concentration was adjusted to 150 x 10^6 spermatozoa for a straw. The sperm suspension was loaded into 0.25 ml plastic straws immediately. The straws were horizontally placed on an aluminum rack and frozen in liquid nitrogen vapour, 5 cm above the surface of liquid nitrogen (~120 °C) for 7 min, and then immersed into the liquid nitrogen for storage. After 2 days, thawing was carried out in a water bath (37 °C) for 30 s.

2.4. Semen evaluation

Immediately after thawing, total motility, forward motility, percentage of live spermatozoa, normal spermatozoa, acrosome integrity, and membrane integrity were measured. The percentage of motility of spermatozoa in each specimen was subjectively evaluated under a phase contrast microscope at 200x magnification by placing a 10 μl drop of diluted semen on a slide and covered with a glass cover slip (22 mm x 22 mm) from three selected representative fields. The mean of the three successive estimations was recorded as final motility score. Sperm motility was assessed by modified category of the WHO laboratory manual (WHO, 2002). Live and dead spermatozoa and morphologically normal spermatozoa percentages were assessed using nigrosin–eosin stain (Evans and Maxwell, 1987). The percentage of acrosome integrity (i.e., with normal apical ridges) was determined by evaluation of sperm smears stained with nigrosin–eosin under phase contrast microscope at 1000x magnification

2.3.1. Experiment 2

Glucose at the concentration of 69.38 mM was added to the cooling extender. The different concentrations of trehalose (33.04, 49.59, 66.08, 198.24, 264.32 mM) were added to the freezing extender.

2.3.2. Experiment 3

After initial evaluation, semen was diluted with normal saline as washing solution and then centrifuged at 1500 x g for 3 min to remove seminal plasma. The supernatant was removed and semen dilution was performed by two step dilution methods. Semen was diluted with cooling extenders and incubated at room temperature for 5–10 min. Subsequently, the extended semen were placed in a cooling chamber at 5 °C and maintained for 2.5 h. Each of the extended semen was diluted in the freezing extender and kept for 30 min. Final concentration was adjusted to 150 x 10^6 spermatozoa for a straw. The sperm suspension was loaded into 0.25 ml plastic straws immediately. The straws were horizontally placed on an aluminum rack and frozen in liquid nitrogen vapour, 5 cm above the surface of liquid nitrogen (~120 °C) for 7 min, and then immersed into the liquid nitrogen for storage. After 2 days, thawing was carried out in a water bath (37 °C) for 30 s.

2.5. Statistical analysis

Results were expressed as the mean ± S.E.M. Mean were analyzed using a one-way analysis of variance, followed by the Tukey’s post hoc test to determine significant differences in all the parameters between groups using the SPSS software system (Version 12.0, SPSS, Chicago, IL). Differences with values of P < 0.05 were considered to be statistically significant.

3. Results

In experiment 1, semen quality parameters pre-freezing and post-thawing are presented in Table 1. There were no differences on all semen quality parameters during the cooling step (P > 0.05). Glucose increased (P < 0.05) sperm forward motility in post-thaw goat semen compared to trehalose or sucrose supplementation. There were no differences between fructose and glucose in forward motility (P > 0.05). Percent of normal spermatozoa was not affected. Differences were not noted among the two disaccharide sugars.

In experiment 2, effects of sugar combination on semen characteristics of Boer goat pre-freezing and post-thawing are shown in Table 2. Differences in measured semen quality parameters were not different (P > 0.05) prior to freezing. The combination of glucose with trehalose or sucrose with trehalose increased (P < 0.05) total and forward motility, as well as membrane integrity in post-thaw semen compared to sucrose with trehalose or glucose

Table 1

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Pre-freezing</th>
<th>Progressive motility%</th>
<th>Intact acrosome%</th>
<th>Live spermatozoa%</th>
<th>Normal spermatozoa%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>73.8 ± 1.9</td>
<td>81.4 ± 1.7</td>
<td>77.2 ± 1.8</td>
<td>95.4 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>73.6 ± 1.3</td>
<td>80.9 ± 1.7</td>
<td>77.4 ± 1.8</td>
<td>96.4 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>72.8 ± 1.3</td>
<td>82.0 ± 1.7</td>
<td>76.4 ± 1.9</td>
<td>96.1 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>71.8 ± 1.4</td>
<td>82.4 ± 1.5</td>
<td>76.9 ± 1.9</td>
<td>94.8 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Post-thawing</th>
<th>Total motility%</th>
<th>Forward motility%</th>
<th>Acrosome integrity%</th>
<th>Membrane integrity%</th>
<th>Live spermatozoa%</th>
<th>Normal spermatozoa%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>60.5 ± 1.2</td>
<td>51.2 ± 2.1b</td>
<td>68.9 ± 1.4</td>
<td>63.6 ± 1.3</td>
<td>62.3 ± 1.2</td>
<td>88.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>57.9 ± 1.9</td>
<td>46.7 ± 2.3ab</td>
<td>66.9 ± 1.7</td>
<td>61.1 ± 1.7</td>
<td>59.5 ± 1.7</td>
<td>88.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>56.7 ± 1.8</td>
<td>41.3 ± 2.0a</td>
<td>64.8 ± 1.2</td>
<td>59.7 ± 1.8</td>
<td>57.9 ± 1.4</td>
<td>89.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>56.1 ± 1.4</td>
<td>43.7 ± 2.1a</td>
<td>65.4 ± 1.7</td>
<td>59.2 ± 1.5</td>
<td>57.8 ± 1.6</td>
<td>88.4 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM within each column, mean with different alphabetical superscripts are significantly different (ANOVA–post hoc test at P < 0.05).
alone. Although differences were not detected in the percent of normal spermatozoa, the best sperm quality was obtained with the combination of trehalose with glucose (Table 2).

In experiment 3, differences in semen quality were not detected prior to freezing (Table 3). Addition of 198.24 mM trehalose (T4) provided significantly better effect on semen quality than the others (T5, T3, T2, T1) (P<0.05). Perhaps the middle doses improved quality but the highest concentration decreased quality after freezing and thawing (P<0.05). Normal spermatozoa percentage was not affected (P>0.05) by addition of different concentrations of trehalose.

4. Discussion

The effects of sugar supplementation to the extender on the cryopreserved spermatozoa vary according to types of sugar because of their different functionality of chemical and molecular weight. Low molecular weight molecules can pass through the plasma membrane of spermatozoa and provide energy to function in metabolism and normal physiological manner. High molecular weight sugars are not capable of diffusion across a plasma membrane and create an osmotic pressure leading to eventually induce cell dehydration. Therefore it caused lower incidence of intracellular ice formation and gave the greater survival of spermatozoa (Nagase et al., 1964; Purdy, 2006).

Glucose addition to the extender conferred better improvement in forward motility percentage than the other disaccharides addition after cryopreservation. This finding confirms the reports by Corteel (1974) and Ponglowsahan et al. (2004) who describe that glucose was essential for energy utilization by spermatozoa and to support sperm motility and movement patterns. Glucose was suggested to be an excellent sugar for metabolism of goat spermatozoa (Corteel, 1974; Purdy, 2006). Although differences among monosaccharides or disaccharides were not detected, motility following cryopreservation was improved in extenders supplemented with monosaccharides (glucose or fructose). It seems that monosaccharides are more efficient than disaccharides at the concentration used.}

<table>
<thead>
<tr>
<th>Pre-freezing</th>
<th>Progressive motility%</th>
<th>Intact acrosome%</th>
<th>Live spermatozoa%</th>
<th>Normal spermatozoa%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>77.3 ± 2.2</td>
<td>87.3 ± 3.6</td>
<td>83.0 ± 1.5</td>
<td>92.4 ± 2.2</td>
</tr>
<tr>
<td>St</td>
<td>76.9 ± 2.1</td>
<td>89.2 ± 3.8</td>
<td>83.8 ± 2.1</td>
<td>93.1 ± 1.9</td>
</tr>
<tr>
<td>Ft</td>
<td>76.2 ± 1.6</td>
<td>87.2 ± 3.7</td>
<td>82.8 ± 1.7</td>
<td>93.4 ± 2.3</td>
</tr>
<tr>
<td>Gt</td>
<td>75.5 ± 1.9</td>
<td>88.8 ± 3.9</td>
<td>82.7 ± 1.6</td>
<td>93.0 ± 1.9</td>
</tr>
</tbody>
</table>

Post-thawing Total motility% Forward motility% Acrosome integrity% Membrane integrity% Live spermatozoa% Normal spermatozoa%

<table>
<thead>
<tr>
<th>Pre-freezing</th>
<th>Progressive motility%</th>
<th>Intact acrosome%</th>
<th>Live spermatozoa%</th>
<th>Normal spermatozoa%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>50.1 ± 2.1*</td>
<td>29.1 ± 2.4*</td>
<td>58.5 ± 3.2*</td>
<td>53.7 ± 1.9*</td>
</tr>
<tr>
<td>St</td>
<td>47.2 ± 2.6*</td>
<td>32.4 ± 6.1*</td>
<td>63.2 ± 2.0*</td>
<td>51.4 ± 1.5*</td>
</tr>
<tr>
<td>Ft</td>
<td>53.9 ± 1.7*</td>
<td>43.2 ± 1.5*</td>
<td>60.3 ± 2.4*</td>
<td>62.8 ± 1.6*</td>
</tr>
<tr>
<td>Gt</td>
<td>67.4 ± 1.4*</td>
<td>54.2 ± 1.5*</td>
<td>75.1 ± 3.3*</td>
<td>69.2 ± 1.4*</td>
</tr>
</tbody>
</table>

Mean ± SEM within each column, mean with different alphabetical superscripts are significantly different (ANOVA–post hoc test at P<0.05). G = glucose (control), St = combination of sucrose and trehalose, Ft = combination of fructose and trehalose, and Gt = combination of glucose and trehalose.
halocon clockwise those obtained from Aboagla and Terada (2003), who found that 375 mM of trehalose gave best results in Japanese miniature Shiba goat. In fact, they used a base extender with only trehalose, even in absence of glucose.

Beneficial effect of sugar supplementation to post-thaw viability of spermatozoa varies among species due to differences in physical and chemical composition of the sperm (Purdy, 2006). Observed differences in semen characteristics may be due to many factors such as species or individual variation (Holt, 2000; Aisen et al., 2002; Thurston et al., 2002; Garde et al., 2008), storage temperature (Lapwood and Martin, 1966), type of buffer (Abdelhakeam et al., 1991), rate of freezing (Hay et al., 1988), or composition of extender including the presence of cryoprotectants (Bakas and Disalvo, 1991; Chen et al., 1993; Gutiérrez-Pérez et al., 2009; Kozdrowski, 2009).

In conclusion, our results indicate that the practical and beneficial effect in cryopreservation of Boer goat spermatozoa can be obtained using an extender containing sugar combination (glucose 69.38 mM and trehalose 198.24 mM). However, these results are based on in vitro evaluations, further fertility trials are required.

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References


