e-ISSN 2581-0405
Asian Journal of Applied Research for Community Development and Empowerment
Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

The Role of Glucose and Fructose for the Energy Recovery of Washed Boer Buck Spermatozoa

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ARTICLE INFO

Article History:
Received: 12 April 2025
Final Revision: 22 May 2025
Accepted: 23 May 2025
Online Publication: 29 May 2025

KEYWORDS

Fructose, glucose, goat, quality, semen, sperm washing

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A B S T R A C T

Semen contains various biochemical substances, both beneficial for life functions and toxic substances that can damage spermatozoa, such as metabolic wastes and free radicals. Semen washing will optimize spermatozoa fertility, as harmful substances and the main energy source are removed. Therefore, giving fructose or glucose is necessary for energy recovery. The washed Boer Bucks semen was stored in Tris-egg yolk (TEY) without glucose or fructose (T1), TEY plus 1% glucose (TEYG, T2), and TEY plus 1% fructose (TEYF, T3). Each treatment was repeated 5 times to observe motility, viability, abnormalities, and intact plasma membrane (IPM) spermatozoa. We analyzed the data using analysis of variance (ANOVA) and the SPSS-20 application. In 8 hours of storage, the results indicated that the average motility of spermatozoa in T1, T2, and T3 was 42.00 ± 1.67%, 44.33 ± 2.88%, and 49.00 ± 2.53%. The viability percentages were 45.67 ± 1.21%, 48.00 ± 1.41%, and 53.50 ± 2.59%, while the abnormality percentages were 15.33 ± 0.52%, 14.50 ± 1.05%, and 12.17 ± 1.17%. The IPM was 42.67 ± 1.21%, 44.50 ± 1.05%, and 49.33 ± 1.75%. In conclusion, glucose or fructose can maintain the quality of spermatozoa post-washed, with the best quality obtained from 1% fructose/100 ml of diluent.

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Semen is a male genital secretion fluid produced by the testes and then ejaculated into the female reproductive tract during copulation. Semen consists of seminal plasma and spermatozoa. Quality of the spermatozoa is one of the contributing factors to the success of the artificial insemination program; only good-quality spermatozoa can reach the egg cell for oocyte penetration in fertilization. Spermatozoa are suspended in seminal plasma, a complex fluid that mediates the chemical function of the ejaculate. Seminal plasma consists of various biochemical constituents, such as ions (Na+, K+, Zn+, Ca++, Mg++, Cl2), energy substrates (fructose, sorbitol, glycerophosphocholine), organic components (citric acid, amino acids, peptides, proteins with low and high molecular weight, lipids, various hormones, cytokines), and so on. Apart from that, there are also nitrogenous components and reducing substances such as ammonia, urea, uric acid, creatinine, ascorbic acid, and hypotaurine [1]. Some chemical components in seminal plasma can be toxic to spermatozoa, so they need to be removed by washing the semen.

1.2. Literature Review

Washing is one way to remove or separate seminal plasma, which contains various toxic debris materials, to improve the quality of spermatozoa. Various strategies have been tried to minimize the

adverse effects of infertility mediated by anti-sperm antibodies (ASA) [2]. Washing is an efficient technique that reduces toxins from colloidal gradients and selects the best spermatozoa fraction for assisted reproductive techniques (ART). However, washing semen can eliminate the primary energy source for stored spermatozoa, namely fructose or other sugar substances contained in seminal plasma. This causes the energy source, nutrition, and other needs of spermatozoa to be lost. In this way, metabolism does not occur, and the vitality and motility of spermatozoa will immediately weaken.

On the other hand, washing is an action that enables spermatozoa to support the success of in vitro fertilization (IVF). Therefore, it is necessary to add exogenous fructose or glucose to thin the semen after washing. This is intended to restore the nutrition and energy of spermatozoa to maintain vitality, motility, and fertility for IVF and artificial insemination (AI), as agreed with [3]. Based on research, there have not been many reports about efforts to restore spermatozoa's nutrition and energy after washing semen. For this reason, research was conducted on giving glucose or fructose for energy recovery in Boer goat spermatozoa after washing.

1.3. Research Objective

This research was conducted to determine the effect of glucose or fructose supplementation in the Tris-egg yolk diluents on the quality of Boer Goat spermatozoa after washing.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The research material was semen from 3 male Boer goats aged 2 – 4 years which were accommodated alternately once per week using an artificial vagina. Goat rearing and semen collection are carried out in Baturinggit Village, Ampenan District, Mataram City. Research was carried out at the Animal Breeding and Reproduction Laboratory, Faculty of Animal Sciences, University of Mataram.

2.2. Research Method

Three semen diluents, namely Tris-egg yolk (TEY) without glucose or fructose (T1), TEY plus 1% glucose (TEYG, T2), and TEY plus 1% fructose (TEYF, T3), were applied in this experiment. Washing of semen was performed using the centrifugation method at 3000 rpm for 10 minutes in 0.9% physiological NaCl solution (v/v = 1/2). All treatments were repeated 5 times.

The collected ejaculate is immediately evaluated macroscopically, including volume, color, consistency, and semen odor. Microscopic evaluation, including concentration, motility, sperm life and death, and abnormalities. This is intended to determine whether the ejaculate is suitable as research material. The semen used in the study has a volume of 0.5-1 mL, a least life and motility of sperm 70%, a maximum abnormality of 20%, and a minimum concentration of 1000 million/mL of semen [4; 5].

Semen samples that meet the process requirements are diluted with isotonic medium (physiological NaCl) for washing. A total of 0.6 mL of semen was put into a 10 mL test tube and then dissolved in 1.2 mL of physiological NaCl. The semen solution was washed once by centrifuging at 3000 rpm for 10 minutes. The washing supernatant was discarded, and 0.2 mL of each spermatozoa pellet was resuspended in 1.2 mL of diluent C, TEYG, and TEYF [Modification from 6; 7]. The diluted washed spermatozoa samples were observed under an Olympus phase contrast microscope at 400x magnification. The observations began immediately after dilution and were repeated every hour until individual progressive motility reached at least 40%.

2.3. Research Variables

The variables observed were motility, viability, abnormalities, and intact plasma membrane (IPM) of spermatozoa.

2.4. Data Analysis

The data results were analyzed using one-way pattern analysis of variance (One-way ANOVA) based on a Complete Randomized Design and continued with Duncan's Multiple Range Test at a 0.05 confidence level using the SPSS V.20 application tool.

3. RESULTS AND DISCUSSION

3.1. Quality of Fresh Semen

The quality of inseminated spermatozoa is an important factor for the success of the AI program. Therefore, immediately after storage, the quality and quantity of fresh semen must be checked or assessed macroscopically and microscopically. Macroscopic assessment includes volume, color, odor, and consistency of semen; microscopic assessment includes motility, life and death of spermatozoa, abnormalities, and concentration of spermatozoa. In general, semen that is suitable for processing must have a minimum volume of 0.5 – 1 mL, live and motile spermatozoa of at least 70%, maximum abnormalities of 20%, and a minimum concentration of 1000 million/mL of semen [4]. Data on the average quality of fresh semen from the assessment used in this study are presented in Table 1.

Table 1. The average quality of Boer goat fresh semen as a research material

No	Variables assessed	Assessment results	References
1	Volume (mL)	0.75 ± 0.15	0.82[a]; 1.67[b]
2	pH	6.80	7.04 ± 0.62[b]
3	Individual motility (%)	73.33 ± 2.58	89.22 ± 0.60[b]
4	Viability (%)	83.67 ± 3.50	88.42 ± 1.94[b]
5	Abnormality (%)	2.33 ± 0.82	Normal: 88.42 ± 1.94[b]
6	Concentration (10^9/mL)	1.87 ± 0.08	1.567[a]; 1.67 ± 0.19[b]

Sources: a = [8]; b = [9]

The assessment results in Table 1 show that the fresh semen used as the material in this study is very feasible. The volume of Boer goat semen obtained is under normal provisions according to several previous researchers, which is in the range of 0.6 to 1.14 mL with an average of 0.64 ± 0.11 mL [10]. The volume of semen from the collection is influenced by the variation of goat herds, collection methods, collection frequency, goat age, goat condition, and operator expertise [11]). The normal concentration, motility, and morphology of sperm in these studies were lower than those obtained by [12], 3.5 to 6.0 billion, 80 – 90%, and 70 – 80%, respectively.

Good quality semen has a yellowish-cream color. Yellowish-green semen indicates the presence of Pseudomonas aeruginosa bacteria, and red or brownish semen suggests the presence of fresh or rotting blood [13; 14]. In addition, the aroma, consistency, and pH of semen and the results of microscopic examinations also meet the eligibility standards for use in artificial insemination [15].

Semen from the collection consists of spermatozoa and seminal plasma containing various substances needed by spermatozoa to live and have energy for motility. However, these substances are also toxic to spermatozoa, so the survival of spermatozoa from ejaculation in seminal plasma is limited to only a few hours [16]. Seminal plasma has a detrimental effect on the survival of spermatozoa during cryopreservation if egg yolk is added to the diluent [17].

3.2. Motility of Washed Spermatozoa

One important method for improving the quality of spermatozoa for AI purposes is washing semen. Sperm are separated from seminal plasma and other contaminants, so healthy and high-quality spermatozoa are obtained through AI [3]. The washing method can remove microbes in sperm (human semen), where electron microscopic examination shows no microbes attached to any part of the spermatozoa [18; 19]. Semen plasma in chickens has DNAase activity, so it needs to be removed for spermatozoa-mediated gene transfer [20].

Carbohydrates are the primary energy source for spermatozoa after leaving the reproductive tract. Semen washing not only removes dirt and various toxic components that are harmful to spermatozoa, but also all preservative and protective substances (carbohydrates and antioxidants) that are essential for spermatozoa will be lost [5]. Therefore, a diluent containing various exogenous substrates is needed to restore nutrition, energy, and protective components for spermatozoa.

After washing, spermatozoa must regain their nutrient and energy sources to maintain their quality for a longer period, namely through dilution [16]. Diluents are needed to preserve and protect spermatozoa's fertilization capacity during in vitro storage at low temperatures. The effects of the type of diluent (Tris or Bioxcell) and preliminary processes such as centrifugation or washing before cryopreservation are thought to affect the motility, morphology, and functional integrity of spermatozoa. Semen management will also affect lipid peroxidation (LPO) and superoxide dismutase (SOD) activity of goat spermatozoa during the freezing-thawing process [17]. The swim-up technique can separate and throw away non-sperm components and the diffusion of other useless substances. Likewise, the presence and production of reactive oxygen species (ROS) and sperm DNA damage caused by high levels of ROS after washing [7].
Supplementation of fructose or glucose in the diluent is thought to maintain the quality of spermatozoa after washing so that spermatozoa still have good fertility for in vitro fertilization and artificial insemination. Fructose or glucose is the largest source of energy for spermatozoa after storage (outside the body). The average motility of washed spermatozoa after fructose or glucose supplementation is shown in Table 2.

Table 2. Average motility of washed spermatozoa during storage at room temperature in Tris-Egg Yolk diluent supplemented with fructose or glucose

Storage time at room temperature (hours)	Diluent		
	TEY	TEYG	TEYF
0	72.50 ± 2.74	73.33 ± 2.58	75.00 ± 0.00
1	69.17 ± 1.83^{a}	71.33 ± 1.03^{ab}	72.83 ± 2.48^{b}
2	65.67 ± 1.75^{a}	66.50 ± 1.87^{a}	70.17 ± 1.83^{b}
3	61.83 ± 1.60^{a}	63.17 ± 1.94^{a}	67.33 ± 1.97^{b}
4	57.00 ± 0.89^{a}	60.00 ± 1.26^{b}	64.83 ± 1.47^{c}
5	53.67 ± 1.21^{a}	56.17 ± 1.47^{b}	60.67 ± 2.16^{c}
6	49.17 ± 1.83^{a}	51.67 ± 2.42^{a}	57.00 ± 2.37^{b}
7	45.50 ± 1.76^{a}	51.67 ± 2.23^{a}	53.67 ± 2.50^{b}
8	42.00 ± 1.67^{a}	44.33 ± 2.88^{a}	49.00 ± 2.53^{b}

^{ab}Different superscripts within each row indicate significant differences ($P < 0.05$)

The results in Table 2 show that glucose or fructose supplementation into the tris-egg yolk diluent has been shown to have a positive impact on spermatozoa motility after washing. Fructose supplementation significantly ($P < 0.05$) gave the best effect. Still, glucose and fructose supplementation can maintain spermatozoa quality for up to 8 hours of storage at room temperature, better than the control. According to the study by [21], 70 mM fructose supplementation in the tris-egg yolk diluent is the best for long-term cold preservation of dog semen compared to glucose and control. Spermatozoa (dogs) consume more glucose than fructose because spermatozoa prefers glucose to fructose.

https://doi.org/10.29165/ajarcde.v9i2.658

Carbohydrates are the primary energy source for spermatozoa after leaving the reproductive tract. Semen washing not only removes dirt and various toxic components that are harmful to spermatozoa but also removes all preservatives and protective substances (carbohydrates and antioxidants) that are essential for the life and movement of spermatozoa [22]. Therefore, a diluent containing various exogenous substrates is needed to restore nutrition, energy, and protective components for spermatozoa. The addition of sugar in semen diluents has varying protective effects on spermatozoa quality, depending on the type of sugar [23].

Various studies have used fructose or glucose in tris-citric acid diluents to freeze mammalian sperm. Fructose is the main ingredient of glycolysis in seminal plasma and is an important substrate for spermatozoa metabolism, which is needed as an energy supply for spermatozoa to function normally. Fructose and glucose have low molecular weights, so they easily cross the spermatozoa's plasma membrane for metabolic activities and their use as an energy source for the spermatozoa [24]. Seminal plasma removal or cleaning by washing harms motility (48.4 ± 2.34%) and the percentage of abnormal spermatozoa (3.20 ± 4.41%) in diluents containing 5% egg yolk. An increase in egg yolk levels by 10% can significantly maintain motility at 65.65 ± 2.80% and abnormalities at 22.65 ± 3.70% [25].

3.3. Viability of Washed Spermatozoa

Supplementation of glucose or fructose into the tris-egg yolk diluent has also been shown to maintain the vitality of washed spermatozoa. Semen washing using the centrifugation method at a speed of 3,000 rpm for 20 minutes and diluted with 80% palm sugar juice plus 20% egg yolk provided motility, viability, and intact plasma membranes of 72, 82.6, and 82.2%, respectively, on the 2nd day of storage [10]. The viability of washed spermatozoa after the diluent was supplemented with fructose and glucose can be seen in Table 3.

Table 3. Average viability of washed spermatozoa during storage at room temperature in Tris-Egg Yolk diluent supplemented with fructose or glucose

Storage time at room temperature (hours)	Diluent		
	TEY	TEYG	TEYF
0	77.17 ± 2.23^{a}	78.33 ± 1.03^{ab}	80.00 ± 1.41^{b}
1	72.00 ± 1.41^{a}	75.33 ± 1.63^{b}	77.17 ± 1.72^{b}
2	68.67 ± 1.63^{a}	70.33 ± 2.34^{a}	73.33 ± 1.63^{b}
3	65.17 ± 1.83^{a}	66.83 ± 2.14^{a}	70.17 ± 1.94^{b}
4	60.67 ± 1.51^{a}	63.17 ± 1.33^{b}	67.33 ± 1.21^{c}
5	56.83 ± 0.98^{a}	59.67 ± 1.37^{b}	64.00 ± 0.89^{c}
6	52.83 ± 1.33^{a}	55.83 ± 1.47^{b}	60.50 ± 2.07^{c}
7	49.00 ± 1.10^{a}	51.83 ± 1.17^{b}	58.83 ± 3.25^{c}
8	45.67 ± 1.21^{a}	48.00 ± 1.41^{b}	53.50 ± 2.59^{c}

^{ab}Different letters in the same row indicate significant differences ($P < 0.05$)

The results in Table 3 show that glucose or fructose supplementation has a significant impact ($P < 0.05$) on the viability of goat spermatozoa for up to 8 hours of storage at room temperature. The best impact was obtained from fructose supplementation. The combination of fructose, coconut water, and egg yolk gave the best effect compared to coconut water, egg yolk, and egg yolk-fructose on the viability of chicken spermatozoa for up to 7 days of storage at 5 °C [26]. Other types of sugar, such as trehalose combined with skim milk, significantly ($P < 0.05$) increased the motility of horse spermatozoa compared to raffinose and fructose, and trehalose and fructose were better than raffinose [27].

Glucose is very suitable for combination with various diluents, such as tris-egg yolk, citrate-egg yolk, coconut water-egg yolk, etc. The addition of 80 mM glucose in coconut water-egg yolk diluent can maintain progressive motility of more than 46.67 ± 2.89% and viability of local rooster spermatozoa of more than 74.68 ± 4.51% up to 72 hours of storage at 5 °C [28]. It can be seen here that egg yolk plays a significant role in various diluents. Egg yolk is needed to protect spermatozoa from cold stress and maintain spermatozoa membrane phospholipids during semen processing because egg yolk contains lecithin, phospholipids, and low-density lipoprotein (LDL). However, using egg yolk in a diluent can affect biochemical and metabolic tests, inhibit respiration, and potentially reduce spermatozoa motility [29].

3.4. Abnormality of Washed Spermatozoa

Supplementation of various types of sugar in semen diluent also reduces spermatozoa abnormalities during processing and storage because it can maintain the osmotic pressure of the diluent. The average abnormalities of washed spermatozoa obtained from the results of this study are presented in Table 4.

Table 4 shows that the lowest abnormalities were found in the tris-egg yolk-glucose treatment, followed by tris-egg yolk-glucose and tris-egg yolk. Fructose or glucose supplementation can reduce the rate of increase in spermatozoa abnormalities. The process of glycolysis and oxidative phosphorylation of sugar as an energy source can support the viability and motility of spermatozoa. When sugar interacts with phospholipids in the plasma membrane, membrane stabilization is better, and the survival rate of spermatozoa is higher after storage [30]. Abnormalities or total changes in spermatozoa morphology in the diluent combination of fructose with coconut water-egg yolk, citrate-egg yolk, and tris-egg yolk were 14.75 ± 8.76%, 15.25 ± 9.87%, and 16.29 ± 8.56% [31].

Table 4. Average abnormalities of washed spermatozoa during storage at room temperature in Tris-Egg Yolk diluent supplemented with fructose or glucose

Storage time at room temperature (hours)	Diluent		
	TEY	TEYG	TEYF
0	7.17 ± 0.75^{a}	6.17 ± 0.75^{a}	5.83 ± 0.41^{b}
1	7.67 ± 0.82^{a}	7.17 ± 0.98^{ab}	6.33 ± 0.52^{b}
2	8.17 ± 0.75^{a}	7.83 ± 0.75^{b}	6.67 ± 0.52^{b}
3	8.67 ± 0.52^{a}	8.33 ± 0.52^{b}	7.33 ± 0.82^{b}
4	9.50 ± 0.84^{a}	8.83 ± 0.75^{ab}	8.00 ± 0.63^{b}
5	11.00 ± 0.63^{a}	10.17 ± 0.75^{b}	8.83 ± 0.41^{c}
6	12.50 ± 0.55^{a}	12.00 ± 1.10^{b}	9.83 ± 0.75^{b}
7	13.67 ± 1.03^{a}	13.17 ± 0.75^{b}	10.83 ± 0.98^{b}
8	15.33 ± 0.52^{a}	14.50 ± 1.05^{b}	12.17 ± 1.17^{b}

^{ab}Different letters on the same line indicate significant differences ($P < 0.05$)

Goat semen contains the enzyme phospholipase-A, which is secreted by the bulbourethral gland, which is often also called Egg yolk coagulation enzyme (Eyce), which is an enzyme that causes coagulation of egg yolk or Bulbourethral secretory gland (BUSgp60). This enzyme is responsible for reducing the viability of spermatozoa cells that have been cooled or frozen in diluents containing egg yolk or milk [32].

3.5. Intact Plasma Membrane (IPM) of Washed Spermatozoa

The result of washing can also remove the antibody binding to the spermatozoa membrane so that the membrane becomes incomplete, and the spermatozoa will be damaged and unfit for use in AI [33]. The integrity of spermatozoa cell membranes in Tris-Egg Yolk diluent supplemented with fructose or glucose can be seen in Table 5.

Table 5. Average intact plasma membrane (IPM) of washed spermatozoa during storage at room temperature in Tris-Egg Yolk diluent supplemented with fructose or glucose

Storage time at room temperature (hours)	Diluent		
	TEY	TEYG	TEYF
0	74.83 ± 0.75^{a}	75.67 ± 0.82^{a}	78.17 ± 0.98^{b}
1	70.83 ± 1.60^{a}	73.17 ± 1.47^{b}	75.33 ± 1.21^{c}
2	66.67 ± 1.37^{a}	68.00 ± 1.55^{ab}	70.00 ± 2.10^{b}
3	63.00 ± 1.55^{a}	64.17 ± 1.17^{a}	66.83 ± 1.17^{b}
4	57.33 ± 0.82^{a}	60.00 ± 1.667^{b}	64.00 ± 1.26^{c}
5	53.33 ± 1.03^{a}	56.33 ± 1.03^{b}	60.50 ± 1.05^{c}
6	49.67 ± 1.51^{a}	51.50 ± 1.64^{a}	57.50 ± 1.87^{b}
7	45.50 ± 1.05^{a}	47.00 ± 0.89^{a}	52.17 ± 2.64^{b}
8	15.33 ± 0.52^{a}	14.50 ± 1.05^{b}	12.17 ± 1.17^{b}

^{ab}Different letters in the same row indicate significant differences ($P < 0.05$)

Table 5 shows that the percentage of intact plasma membrane (IPM) in the TEY-fructose diluent from this study was significantly ($P < 0.05$) greater than that of TEY-glucose and TEY (control). Carbohydrates are a source of glucose, fructose, and various other types of sugar, which can have a dual function, namely as an energy source, and large molecular carbohydrates can function as extracellular cryoprotectants. Fructose is the main ingredient of glycolysis in seminal plasma, while glucose is an important substrate for sperm metabolism and is required for energy supply so that spermatozoa can function normally. Fructose and glucose have a low molecular weight, so they can easily cross the spermatozoa's plasma membrane in their function of providing an energy source [34]. The average IPM in epididymal spermatozoa stored at NaCl concentrations ranging from 0.7% to 1.1% was 87.92%, 87.60%, 85.17%, 78.53%, and 75.79% [35], 83.19 ± 1.09 [9].

The highest percentage of motility, viability and acrosome integrity of sheep spermatozoa achieved in diluent containing fructose, namely 64.3 ± 7.9; 79.4 ± 11.0 and 79.6 ± 6.0 compared with glucose, namely 54.6 ± 5.9; 72.3 ± 12.3 and 74.3 ± 7.3; sucrose was 63.6 ± 8.7; 76.1 ± 9.0 and 78.3 ± 6.4 and trehalose was 55.9 ± 9.2; 71.8 ± 10.4 and 75.1 ± 6.2) [36]. The motility and viability of goat spermatozoa in liquid semen diluted with tris-citrate-fructose can be maintained at a minimum of 6 hours with a small reduction rate [37]. A higher quantity of motile sperm will be obtained when using double or Triple sperm washing [19].

Glucose is a type of carbohydrate that is usually added to mammalian semen as an energy source. Metabolizing glucose is

https://doi.org/10.29165/ajarcde.v9i2.658

carried out by spermatozoa during long-term storage of semen, both for goat semen and in other species [28]. Sheep spermatozoa were able to fertilize after cooling and preserving at 0℃ using 5 mM trehalose in Tris-fructose-egg yolk diluent. The preservation of sheep semen in this diluent is more effective than in conditions in other diluents [34].

Storage of liquid semen is cheaper than frozen semen, the liquid semen can be an alternative to frozen-thawed semen. However, several research results show that storing semen in liquid form can cause oxidative stress, which damages the plasma membrane and DNA of spermatozoa. Glucose and pyruvate are better than lactate in maintaining goat sperm motility. Mammalian spermatozoa obtain energy from monosaccharides via glycolysis alone or combined with the mitochondrial tricarboxylic acid (TCA) cycle. The TCA cycle and the pentose phosphate pathway (PPP) of spermatozoa can produce molecules with large reduction capacity [38; 39].

The pentose phosphate pathway (PPP) is more important than glycolysis in maintaining sperm motility. The pentose phosphate pathway reduces oxidative stress and provides glycolysis with more intermediate products, such as fructose-6-phosphate. Pyruvate enters goat spermatozoa via the monocarboxylate transporter and is then oxidized by the tricarboxylic acid cycle and electron transfer. It is used to maintain motility and optimally control the survival of spermatozoa during the handling and preservation of semen in all animal species [40].

Fructose is the main energy source and essential nutrient for spermatozoa in ejaculate (semen), which is metabolized and converted into pyruvate and lactate via the Embden–Meyerhof pathway. The main energy is stored as adenosine triphosphate (ATP), which is produced through mitochondrial oxidative phosphorylation and glycolysis. Meanwhile, sugar is an exogenous medium that spermatozoa can use in two ways. The first way, sugar is metabolized through the glycolysis pathway (main pathway), producing lactate/pyruvate, limited glycogen synthesis, and some incorporation of pyruvate into the Krebs Cycle. In the second way, sugar is stored as glycogen as a medium and long-term energy reserve to maintain motility when the environment does not provide external energy sources. The combination of glucose and fructose supplementation in diluent media (mTALP) can increase progressive motility, acrosome reaction, and fertilizing ability of pig spermatozoa [41].

Addition of low concentrations of fructose in the extender (10mM) can increase the viability of spermatozoa cells after thawing. Fructose is an efficient energy source for spermatozoa cells resulting from the cryopreservation process [42]. The addition of fructose to the coconut water semen diluent has a positive influence on the quality of spermatozoa. Addition of 1.75% fructose resulted in a spermatozoa motility percentage of 57.18 ± 1.43% and abnormalities of 5.76 ± 0.20% at 12 hours of cold storage (top layer of the albumin column). Meanwhile, in the bottom layer of the albumin column, 57.62 ± 0.65% and 4.35 ± 0.25%, compared to a concentration of 1%, 1.25%, and 1.50% [43].

4. CONCLUSIONS

Supplementation of glucose or fructose in Tris-egg yolk diluent has a good effect on the quality of Boer Goat spermatozoa after washing. Supplementation with 1% fructose is the most effective source of sugar energy for the quality of spermatozoa after washing. A type of carbohydrate (fructose, glucose, trehalose, raffinose, etc.) is very recommended for the energy recovery of washed semen.

ACKNOWLEDGEMENT

The authors thank the Institute for Research and Community Service, University of Mataram, for funding this research. The authors would like to thank Muhajirin for his help during the semen collection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

NOVELTY STATEMENT

Utilization of glucose and fructose in semen extender to improve the quality of Boer buck spermatozoa after washing. It is found that 1% glucose or fructose supplemented in the extender can recover the quality of washed Boer buck spermatozoa until 8 hours of storage.

REFERENCE

[1] Juyena N. S. and C. Stelletta, 2012. Seminal plasma: an essential attribute to spermatozoa. Journal of Andrology, Vol. 33, No. 4: 536-551. DOI: 10.2164/jandrol.110.012583.

[2] Schneider D., C. Feijo, S. Verza Jr. and S. Esteves, 2014. Effectiveness of sperm washing by discontinuous density gradient centrifugation to remove antibodies bound to the sperm membrane. Medical Express,1(3):123-126. DOI: 10.5935/MedicalExpress.2014.03.05

[3] Suherni S., F.S. Yosaliah; R. Yola dan S. Trilas, 2011. Kualitas spermatozoa domba setelah pencucian dengan medium brackett and oliphant's (bo) pada pengencer susu skim dan susu kuning telur. The quality of ram sperm after washing with Brackett and Oliphant's (BO) medium at skim milk and egg yolk diluent. VETERINARIA Vol. 4 No. 3: 181-186.

[4] Asadpour R., Razi Jafari, and Hossein Tayefi-Nasrabadi, 2011.Influence of added vitamin C and E on frozen-thawed bovine sperm cryopreserved in citrate and Tris-base extenders. Veterinary Research Forum, 2(1): 37–44. https://vrf.iranjournals.ir/ article_1524.html

[5] Sumadiasa I W.L., Lukman, Rodiah, 2018. The role of guava fruit filtrate in maintaining the pre-freezing and post-thawed quality of Bali bull spermatozoa. Jurnal Kedokteran Hewan, 12(2):39-42. https://doi.org/10.21157/j.ked.hewan.v12i2.8801.

[6] Olivares C.C., J. F. da Fonseca, L. S. de A. Camargoc, J. M. Gon¸alves de Souza-Fabjana, A. L. R. Rodrigues, F. Z. Brandãoa, 2015. Comparison of different methods of goat sperm selection and capacitation for optimization of assisted reproductive technologies. Small Ruminant Research, 127(2015): 44–49. http://dx.doi.org/10.1016/j.smallrumres.2015.04.009

[7] Natali I., 2011. Sperm Preparation Techniques for Artificial Insemination - Comparison of Sperm Washing, Swim Up, and Density Gradient Centrifugation Methods. In: Artificial Insemination in Farm Animals. Edited by Milad Manafi. DOI: 10.5772/17026

[8] Sundararaman M.N., J. Kalatharan and M.J. Edwin, 2007. Attempts to achieve semen collections from incapacitated Boer Bucks by electro-ejaculation. Asian J. Anim. Vet. Adv., 2(4): 244-246. DOI:10.3923/ajava.2007.244.246

[9] Keller W.S., I.P. Kashoma and M.M. Kichuki, 2025. Quantitative semen characteristics of Malya and Boer bucks: Candidates for artificial insemination of goats in Tanzania. Appl. Vet. 3: 1–9.

[10] Rizal M., M. Riyadhi, and A. Sulaiman, 2018. The quality of boer goat semen preserved with sugar palm juice. Bulletin of Animal Science. Buletin Peternakan 42 (2): 97-102. http://buletinpeternakan.fapet. ugm.ac.id/

[11] Evans G., and W.M.C. Maxwell, 1987. Salamon's artificial insemination of sheep and goats, Butterworth, Sydney, Boston, London, Durban, Singapore, Willington.

[12] Faigl V., N. VASS, A. JÁVOR, M. KULCSÁR, L. SOLTI, G. AMIRIDIS and S. CSEH1, 2012. Artificial insemination of small ruminants – A review. Acta Veterinaria Hungarica 60 (1): 115–129. DOI: 10.1556/AVet.2012.010

[13] Ariantie O.S., T.L. Yusuf, D. Sajuthi, R. I. Arifiantini, 2014. Kualitas semen cair kambing peranakan etawah dalam modifikasi pengencer tris dengan trehalosa dan rafinosa. Jurnal Veteriner, 15 (1): 11-22. https://ojs.unud.ac.id/index.php/jvet/ article/view/8780/6560

[14] Inonie R. I., L. O. Baa dan T. Saili, 2016. Kualitas spermatozoa kambing Boerawa dan kambing kacang pada penggunaan tris-kuning telur berbeda. Jitro, 3(1): 52-64.

[15] Pamungkas F.A., A. Batubara and Sutoro, 2014. The quality of spermatozoa of gembrong goats during the cryopreservation process. Media Peternakan, 37(2): 95 – 100.

[16] Daramola J.O. and E.O. Adekunle, 2015. Cryosurvival of goat spermatozoa in Tris-egg yolk extender supplemented with vitamin C. Arch. Zootec. 64 (247): 261-268. http://www.redalyc.org/articulo.oa? id= 49541390009

[17] Sarıo¨zkan S., M.N. Bucak, P.B. Tuncer, U. Tasdemir, H. Kinet, P.A. Ulutas, 2010. Effects of different extenders and centrifugation/washing on post-thaw microscopic-oxidative stress parameters and fertilizing ability of Angora buck sperm. Theriogenology, 73 (2010): 316–323. http://www.elsevier. com/copyright

[18] Wong P. C., J. P. Balmaceda, J. D. Blanco, R. S. Gibbs, and R. H. Asch, 1986. Sperm washing and the swim-up technique using antibiotics remove microbes from human semen. Fertility and sterility, 45(1): 97-100. DOI: 10.1016/s0015-0282(16)49104-1

[19] Soyer-Calıskan C., K. Hatirnaz, S. Celik, A. Başbuğ, E. S. Hatirnaz, S. Hatirnaz and M. H. Dahan, 2022. A comparison of triple and double sperm washing for density gradient preparation in intrauterine insemination cycles when overnight incubation of specimens occurred. Clin. Exp. Obstet. Gynecol., 49(11): 250. https://doi.org/10.31083/j.ceog4911250

[20] Churchill R.R., P. E. Praveena and D. Sharma, 2014. Semen quality parameters, their inter-relationship, and post-washing sperm attributes of Rhode Island Red roosters. Semen quality parameters, their inter-relationship and post-washing sperm attributes of Rhode Island Red roosters, Veterinary World, 7(12): 1117-1122. www.veterinaryworld.org/Vol.7/December-2014/16.pdf

[21] Ponglowhapan S., B. Essén-Gustavsson and C.L. Forsberg, 2004. Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. Theriogenology, 62(Issue 8): 1498-1517. https://doi.org/10.1016/ j.theriogenology.2004.02.014Get rights and content

[22] Amaral A, C. Paiva, M. Baptista, A.P. Sousa and Jo~ao Ramalho-Santos, 2011. Exogenous glucose improves long-standing human sperm motility, viability, and mitochondrial function. Frtility and Sterility, 96(4): :848–50. doi:10.1016/j.fertnstert. 2011.07.1091

[23] Yildiz C., A. Kaya, M. Aksoy, and T. Tekeli, 2000. Influence of sugar supplementation of the extender on motility, viability, and acrosomal integrity of dog spermatozoa during freezing. Theriogenology, 54(4): 579-585. https://doi.org/10.1016/S0093-691X(00)00373-3Get rights and content

[24] Kamal M., E. Alam, S. K. Das, Most. S. Yeasmin, S. Ahmed, Mst. A. Rahman, D. K. Das, R. Gofur, A. Masum, 2023. Effects of glucose and trehalose on tris-citric acid-egg yolk-fructose diluents for semen cryopreservation in goat. J. Adv. Vet. Anim. Res., 10(2): 169–177. ttp://doi.org/10.5455/javar. 2023.j666

[25] Sen C.C., Tekin K, and AKÇAY E., 2015. Effect of egg yolk and removal of seminal fluid on semen cryopreservation in Norduz goat. Harran Üniv Vet Fak Derg, 4 (2): 64-67. https://www. researchgate.net/ publication/297140471

[26] Rochmi dan Sofyan, 2019. A diluent containing coconut water, fructose, and chicken egg yolk increases rooster sperm quality at 5°C. Veterinary World, 12: 1116-1120. www.veterinaryworld.org/Vol.12/ July-2019/28.pdf

[27] Arifiantini R.I., B. Puwantara, T. L. Yusuf dan D. Sajuthi, 2009. Peranan fruktosa, rafinosa, dan trehalosa pada kriopreservasi semen kuda. Media Peternakan, 32(3): 17 1–178. https://journal.ipb.ac.id/ index.php/mediapeternakan/article/view/8798

[28] Khaeruddin and M. Amir, 2019. The Effect of the combination of glucose concentration with the type of extenders on the quality of native rooster spermatozoa during storage. CJAH, 4(2): 36-43. http://usnsj .com/index.php/CJAH

[29] Sumadiasa I W.L., E. Yuliani dan L. Lukman, 2023. Effect of guava filtrate supplementation in tris and citrate-based extenders on spermatozoa quality of the Brangus bull after sex rest. Adv. Anim. Vet. Sci. 11(1): 150-158. http://dx.doi.org/10.17582/ journal.aavs/2023/11.1.150.158

[30] Rattanatabtimtong S., P. Satsook, S. Chomchai1 and V. Tongthaeng, 2018. Effects of sugar types in semen extender on sperm quality and longevity of frozen goat semen. ISSAAS, 24(1): 152-161. https://www.cabidigitallibrary.org/doi/pdf/ 10.5555/20183221283

[31] Matos-Brito, B.G., I.C.S. Lima, J.F. Pereira, F.M. Barboza, M.A.B. Linard, G.V. Aguiar, A.G.V. Catunda, A.A.A. Moura, J.F. Nunes, 2 and A.C.N. Campos, 2013. Effect of initial seminal plasma fructose concentration on goat semen storage at 5 °C. Arch. Zootec. 62 (237): 143-146.

[32] Aguiar G.V., M.F. van Tilburg, A.G.V. Catunda1, C.K.S. Celes, I.C.S. Lima, A.C.N. Campos, A.A.A. Moura, A.A. Araújo, 2013. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. Arq. Bras. Med. Vet. Zootec., 65(1): 6-12. https://www.researchgate.net/publication/262505841

[33] Ijaaz A. and A.G. Hunter, 1989. Effect of washing and capacitating media pH on bull sperm motility, acrosome integrity, and ability to penetrate Zona-Free Hamster oocytes. J Dairy Sci., 72:2691-2699.

[34] Zhao J., G. Xiao, W. Zhu, D. Fang, N. Li, C. Han and Q. Gao, 2020. Trehalose addition to a Tris-fructose egg yolk extender on the quality of ram sperm preserved at 0 °C. R. Bras. Zootec., 49: e20200061: 1 – 7. https://doi.org/10.37496/rbz4920200061

[35] Arsiwan, T. Saili, L.O. Baa dan S. Rahadi, 2014. Membran plasma utuh sprmatozoa epididimis kambing perranakan ettawa dalam natrium klorida dengan konsentrasi berbeda. JITRO, 1(1): 79-87. https://ojs.uho.ac.id/index.php/peternakan-tropis/article/view/364/223

[36] Panyaboriban S, J. Suwimonteerabutr, N. Phutikanit, T. Swangchan-Uthai, T. Tharasanit, M. Techakumphu, 2015. Effect of various combinations of sugar supplementation in the extender on frozen-thawed ram semen quality and fertility. Thai J Vet Med., 45(2): 229-237.

[37] Kostaman T. dan I Ketut Sutama, 2006. Studi motilitas dan daya hidup spermatozoa kambing Boer pada pengencer tris-sitrat-fruktosa. J. Sains Vet., 24(1): 58-63.

[38] De Preter G., N. Marie-Aline, P. Danhier, L. Brisson, V.L. Payen, P.E. Porporato, B.F. Jordan, P. Sonveaux2 and B. Gallez, 2015. Inhibition of the pentose phosphate pathway by dichloroacetate unravels a missing link between aerobic glycolysis and cancer cell proliferation. Oncotarget, 7(3): 2910-2920. www.impactjournals.com/oncotarget/

[39] Cho E.S., Y.H. Cha, H.S. Kim, N.H. Kim and J.I. Yook, 2018. The pentose phosphate pathway as a potential target for cancer therapy. Biomol Ther, 26(1): 29-38. DOI: 10.4062/biomolther 2017.179

[40] Pena F.J., J.M. Ortiz-Rodríguez, G.L. Gaitskell-Phillips, M.C. Gil, C. Ortega-Ferrusola, F.E. Martín-Cano, 2021. An integrated overview of the regulation of sperm metabolism (glycolysis-Krebs cycle-oxidative phosphorylation). Animal Reproduction Science. https://doi.org/10.1016/j.anireprosci.2021.106805

[41] Tsujii H., E. Ohta, A.G. Miah, S. Hossain and U. Salma, 2006. Effect of fructose on motility, acrosome reaction, and in vitro fertilization capability of boar spermatozoa. Reproductive Medicine and Biology, 5: 255–261. doi: 10.1111/j.1447-0578. 2006.00150x.

[42] Pappa A.Z., H.M. da Silva1, L. Valadão and F.M. da Silva,12019. Effect of fructose on thawed bull semen obtained by post-mortem collection. Biomed J Sci & Tech Res 19(3): 14319-14323. DOI: 10.26717/BJSTR.2019.19.003300

[43] Rasad dan Simanjuntak, 2009. The effect of fructose addition in semen extender on the quality of separation of garut ram sperm in several storage lengths. Animal Production, 11(3): 196-201. https://www.researchgate.net/publication/277730175