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Research Article

Addition of Sugarcane Juice to Tris Egg Yolk Buffer Diluent Improves the Quality of the Stored Diluted Semen of Kacang Goats at 5°C

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Abstract | This study aimed to determine the effect of sugarcane juice addition to egg yolk tris-buffer diluent on the semen quality of Kacang goats at 5°C storage. Semen samples were collected twice a week from a 2-year-old Kacang goat using an artificial vagina. The study design consisted of three treatments; T0, T1, T2 and T3, consisting of 0%, 10%, 15%, and 20 % of sugarcane juice, respectively. The measured parameters included progressive motility, viability, and abnormalities of spermatozoa. The data obtained were analyzed using the analysis of variance (ANOVA) followed by Duncan's multiple range test to determine the difference among the treatments. The current study showed that adding 15% sugarcane (T2) improved the semen parameters compared to the other treatments with 54% progressive motility, 74.8% viability, and 8.8% total abnormalities at 5°C for 4 days of storage. 15% sugarcane juice in egg yolk tris-buffer diluent preserves Kacang goat diluted semen at 5°C for 4 days.

Keywords | Egg yolk tris, Kacang goat, Semen quality, Sugarcane juice

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INTRODUCTION

Goat is one of the small ruminant livestock commonly raised by Indonesians due to several advantages, such as the ability to reproduce prolifically. Generally, goat can produce more than one kid per kidding, a desirable trait for breeders (Nafiu et al., 2020). Goats are also quite popular due to their traditional rearing system and good adaptations to the circumstances (Damanik and Depison, 2016). Kacang goat is a native Indonesian breed that needs to be preserved as a variety of the native livestock of the country. This goat breed is known for its fast reproduction rate, as it can produce offspring at 15-18 months of age.

Kacang goat is a local Indonesian goat with a small body weight compared to other goat breeds. But, the Kacang goat has excessive adaptability to the nearby surroundings, reproductive ability and disease resistance (Susilo, 2016). The disadvantages of the Kacang goat are its relatively small body size and relatively low live weight gain rate. Kacang goat has a particularly small body size, a mild and small head, short ears pointing immediately up, high adaptability to unfavorable natural conditions and very high reproductive overall performance. It's also a noticeably low rate of day-by-day weight advantage (Abadi et al., 2015). Body weight of female Kacang goats at adulthood is around 19.14 kg to 24.31±4.53 kg (Abadi et al., 2015; Tmaneak et al., 2016). Goat, in addition, is an essential source of income for small farmers in Indonesia (Zaenuri et al., 2023).

Kacang goat is suitable for meat and leather production because it is prolific, resistant to various conditions, and well-adapted to different rearing environments, with the main product being meat (Nafiu et al., 2020). To increase their productivity, appropriate reproductive technology, such as artificial insemination (AI) can be used (LukmanHy et al., 2020). This technique aims to minimize the potential danger of disease transmission through natural mating; moreover, the used semen is selected from superior males. The success of AI is significantly influenced by semen quality. The quality of the stored semen largely decreases when it is not immediately incubated. However, adding a sugarcane juice diluent can maintain semen quality during storage (Tethool et al., 2022).

Many researchers have gone through research by utilizing various types of fruit filtrate in semen diluents. So far, the results of these studies show a positive trend. Examples of studies using fruit filtrate include fig fruit filtrate (Zaenuri et al., 2013, 2017, 2023), guava fruit filtrate (Sumadiasa et al., 2015), rock melon (Lukmanhy, 2022), Fomagrande (Lukmanhy et al., 2022), Sugarcane filtrate (Manehat et al., 2021). Sugarcane juice contains 20–25% dry matter and contains elements like starch (carbohydrate) in the form of sucrose (cane sugar), which consists of glucose and fructose (Tanii et al., 2022). The carbohydrates contained in sugar cane juice function as an energy source for spermatozoa. Sugarcane juice extract is a natural diluent that is easily obtained, provides the required nutrients, and meets the standard requirements for semen diluent. Pure sugarcane extract contains 18.08% sucrose and 0.54% sugar invest (Erwinda and Susanto, 2014). The research results of Manehat et al. (2021), show the diluent with the composition of sugarcane juice: Egg yolk is 40 ml: 20 ml plus 0.5 gr of penicillin and 0.5 gr of streptomycin in 40 ml of distilled water can maintain the motility of individual bovine spermatozoa by 45 ± 5.0% in 96 hours after dilution and storage at 5°C.

Sucrose is a substrate for energy and an extracellular cryoprotectant against temperature changes to protect and support the motility of spermatozoa during processing and storage (Khaeruddin and Kurniawan, 2020). The main advantage of sugarcane juice is that it contains sucrose, a disaccharide (double sugar), composed of glucose and fructose subunits linked by glycolytic bonds. Sucrose breakdown produces ATP and ADP that function as driving energy for spermatozoa and as an intracellular cryoprotectant (Manehat et al., 2021). Preserving semen is a part of the AI program that allows long-term storage of diluted semen. This makes it necessary to investigate the addition of sugarcane juice to egg yolk tris-buffer diluent to maintain the quality of Kacang goat semen at 5°C.

MATERIALS AND METHODS

Animal and management

In the current study, semen samples of a 2-year-old Kacang goat. A total 7 Kacang Bucks, 1 of the best health and libido, was selected for this study. The buck feed is a mixture of 50:50 native grass and Taramba leaf. The amount of feed is at least 10% of body weight. Water was available ad libitum. All data were collected in five repetitions at 14-day intervals.

Sugarcane juice preparation

The sugarcane used in the study was a type of yellow sugarcane aged 11 months, amounting to 1 stalk with a length of 45 cm and a stem diameter of 10-12 cm. Sugarcane harvesting is carried out at the optimum level of maturity, namely at 11–12 months of age, when the sugarcane is at its highest sugar content. Sugarcane is harvested by cutting it from the clump on the first segment above the ground. After the sugarcane is cut, the next step is to clean the sugarcane by washing it using clean water. After cleaning, the next step is to separate the cane skin using a knife. After that, chopped sugarcane with a length of ± 1.5 cm and blended. After blending the water is filtered using a filter and put into the container that has been prepared. Sugarcane juice is ready to be used as a diluent (Manehat et al., 2021).

Treatment diluents

The control diluent (T0) was a Tris egg yolk diluent (Evans and Maxwell, 1989; Zaenuri et al., 2023; Sumadiasa et al., 2023). Treatment diluents were T1 = T0 + 10% sugarcane, T2 = T0 + 15% sugarcane, and T3 = T0 + 20% sugarcane. Control and treated diluents were homogenized with a magnetic stirrer before diluting the semen (Zaenuri et al., 2023).

Semen collection and processing

The semen was collected twice weekly for two weeks before semen samples collection. The semen samples were collected for 10 consecutive ejaculates using an artificial vagina by a trained technician. The semen samples were initially evaluated at the Central Laboratory for Bioscience and Biotechnology, Faculty of Mathematics and Natural Sciences, University of Mataram, Indonesia. Initial evaluation for fresh semen included volume, pH, consistency, colour, mass motility, and concentration. Semen that meet the requirements are saved for further processing.

Collected semen that passed the minimum requirement, then divided into 4 according to the treatments. Spermatozoa concentration per treatment was 100×10^6/ml diluents. Dilution ratio is calculated= (% motility sperm ×

concentration/mL × semen volume)/(50 ×100^6)= A. If the volume of fresh semen is 1 ml,1/A= b ml (fresh sperm). Then, b ml fresh sperm dilute up with 1 ml dilution to achieve concentration. The number of sperm per ml of extender is 100 x 10^6 (Zaenuri et al., 2013).

The diluent is gradually added to the tube that contains fresh semen. Lightly shake the tube to even out the semen and extenders. The semen was then divided into 4 tubes according to the observation plan. Additionally, the tube Incubated for 30 minutes in a 37˚C water bath allows the antibiotics in the bulking agent to work optimally. Sperm cultured to avoid cold stress was transferred to a 50ml beaker glass filled up with distilled water at a temperature of 18-21˚C. Stored in the refrigerator so that the temperature of the processed material is kept constant. The semen temperature gradually drops to 5˚C after 1 hour (Zaenuri et al., 2023).

The semen was declared good and processed according to the different treatments, and then diluted semen samples were assessed based on the individual motility, viability, and abnormalities of spermatozoa at days 0, 1, 2, 3, and 4.

Data analysis

All obtained data were statistically analyzed using variance (ANOVA), and significantly different results (P <0.05) were further tested with Duncan's test using the SPSS program version 20 (Sawyer, 2009).

Treatment semen evaluation

The examination of semen treatment included the percentages of progressive motility, viability and abnormality. A semen examination was performed at 0 h, 24, 48, and 72 h after extended. A Progressive motility assessment was assessed by placing a drop of semen on a slide, covering it with a coverslip, and observing it with a light microscope (Nikon, Japan) at 400x magnificence. Two hundred spermatozoa were observed in several batches of field of view. The percentage of progressively motile Spermatozoa count is the number of gradually motile spermatozoa divided by 200 (Mayasula et al., 2021).

Sperm viability was assessed using a swab preparation. One drop of semen was mixed with one drop of eosin nigrosine, homogenized through the tip of another slide, pushed to the edge of the slide and left on the glass slide for 30 seconds. A smear preparation was then performed. Smears were observed under a light microscope (Nikon, Japan) at 400x magnification. Sperm that absorbed colour were dead, whereas sperm that did not absorb or were clear were viable. The percentage of viable sperm is the number of viable sperm divided by the number of observed sperm multiplied by 100 (Zaenuri et al., 2017).

Normal morphology was examined using a phase-contrast microscope (Olympus CX 43, Optical Co., Ltd., Tokyo, Japan) with a magnification of 400x connected to a monitor (Saraswat, 2012). Headless sperm, double-headed sperm, tailed sperm, and sperm with a curled tail were counted and calculated as abnormal sperm (Ibrahim et al., 2008). Data on abnormal sperm were presented as the accumulation of Headless sperm, double-headed sperm, tailed sperm, and sperm with a curled tail.

RESULTS AND DISCUSSION

Semen quality remarkably determined the success of AI in livestock. In this study, the fresh semen of the Kacang goat was evaluated for macroscopic and microscopic testing; the results are presented in Table 1.

Table 1: Characteristics of fresh semen of Kacang goat.

Variables	Mean±SD
Volume	0.64±0.14
Color	milk white
Smell	Typical
Consistency	Thick
pH	7±0.90
Mass Motility	+++
Concentration spermatozoa (×10^7/ml)	236±21.07
Viability (%)	98.2±1.78
Abnormality (%)	6.3±1.90

Table 1 shows that the semen volume of the Kacang goat was 0.64 ml. Similarly, Anwar et al. (2019) stated that the volume of goat semen per ejaculation ranged from 0.5-1.5 ml. However, semen volume in this study was lower than that reported by three previous studies, which were 1.54±0.32 ml (Zaenuri et al., 2023), 2.64 ml (Pratiwi, 2007) and 2.44 ml (Armansyah et al., 2018). The reported value of the semen volume is still within the normal range of goat semen as written by some researchers, such as 0.96 ml/ejaculate (Putranti et al.,2020), 0.70-1.50 ml (Suyadi et al., 2004), 0.640 (Rizal M., 2009) and 0.80 ml (Pamungkas et al., 2008). Different volumes among studies would be affected by the number of factors such as the frequency of ejaculation and variance of individual livestock breeds, age, season, libido, and the variation in the condition of livestock (LukmanHy et al., 2022b). In addition, semen volume correlated with spermatozoa concentration and technician who did semen collection (Zaenuri et al., 2023).

In the present study, the colour of the collected fresh semen was milky white, which is considered normal (Nahriyanti et al., 2017). According to Sumadiasa et al. (2019), the reddish colour indicates that the semen is contaminated with fresh blood, while the greenish colour of semen

suggests the presence of putrefactive bacteria in the semen samples.

The smell of collected semen in this study was typical, which indicated normal semen with an absence of contamination. Sumadiasa et al. (2022) stated that normal semen has a distinctive fishy odour accompanied by the smell of the animal. However, a foul odor can occur when the semen contains pus caused by an infection of male reflux organs (Lopes, 2002). The addition of 15% sugarcane juice to egg yolk tris-buffer diluent improves the quality of the stored diluted semen of Kacang goats at 5 ˚C, caused by an infection in the reproductive tract of male animals.

In this study, the semen viscosity was thick, and the degree of viscosity had a positive correlation with the content of the semen. Therefore, when a low viscosity was observed, it was assumed that the semen had a low concentration of spermatozoa (Sumadiasa et al., 2018). The pH of the collected semen was neutral, with a value of 7.00, which was within the normal range of 6-7 (LukmanHy et al., 2020; Dhumal et al., 2021).

The mass motility of the collected fresh semen in this study was excellent (+++). Moreover, the best mass motility was 3+, which indicated progressive forward movement of the spermatozoa. The thicker and more prominent the wave, the more progressively motile spermatozoa (Tambing et al., 2003). Despite this, the subjectivity of mass motility assessment limits its usefulness for semen quality assurance in industry (Rodríguez-Martínez, 2003). The progressive movement obtained from this study was 80 ±2.04%. Similarly, (Zaenuri and Rodiah, 2016) stated that the progressive percentage of normal spermatozoa motility suitable for further processing ranged from 70 - 90%. According to LukmanHy et al. (2022a), the motility of individual fresh sperm ideal for processing to the next stage was at least 65% because high motility increased the ability of spermatozoa to reproduce. This value was higher than the result of (Zaenuri and Rodiah, 2016), where the average percentage of fresh semen individual motility of Kacang goat was 75.2%.

Spermatozoa motility was highly dependent on the energy supply produced by metabolism, which took place well when the plasma membrane was intact to regulate the substrates or electrolytes needed for metabolism. Furthermore, the feed was also an important factor affecting spermatozoa motility (Sumadiasa, 2023). Good semen has individual motility ranging from 70-85%. Factors that affect spermatozoa motility are the method of storing semen, the storage environment and the treatment of semen after shelter (Zaenuri et al., 2013).

The concentration of spermatozoa was related to the quality of the semen to be diluted. In this study, the concentration value was 236 ± 21.07/ml, which was lower than that of the $424x10^7$/ml obtained by (Zaenuri et al., 2023). Sperm concentration in goat AI with refrigerated semen is not well established, and a wide range of sperm concentrations, from 800 to 100 × 10^6 sperm/mL for cooled semen, have been used (Arrebola et al., 2014; Mara et al., 2007). According to (LukmanHy et al., 2022), fresh semen that can be processed into liquid semen must meet a concentration of ≥ $200x10^6$ cells/ml. Differences in the type of goat, breed, age, physical condition, feed quality, temperature, reproductive health, and ejaculation frequency also caused the variation in concentration (Lemma and Shemsu, 2015). Zaenuri et al. (2023) also explained that the process of semen collection also causes many negative impacts on semen quality and quantity.

In this study, the percentage of viability was 98.2±1.78%; this met the requirements stated (Kaiin and Gunawan, 2017) that fresh semen suitable for processing must have viability ≥ 80%. Furthermore, Mayasula et al.(2021) stated that semen with viability ≥70% was categorized as good. The loss of sperm viability after freezing prevents caprine reproduction from using frozen semen more widely (Batista et al., 2009). The sperm concentration of at least 2 x 10^9/mL, a minimum sperm viability of 80%, and a maximum sperm abnormality rate of 15% (Tambing et al., 2003).

High spermatozoa abnormalities can interfere with fertility. According to Sumadiasa (2023), spermatozoa with normal morphology are required for fertilization. Abnormality is a condition in which spermatozoa experience defects in one or all parts of the body of spermatozoa. The percentage of abnormal spermatozoa in this study was 6.3±1.90, which was categorized as good quality (Yuliani and LukmanHy, 2013); good spermatozoa abnormalities ranged from 5-15%. According to (LukmanHY et al., 2022a), fresh semen that can be processed into liquid semen must meet the requirements with an abnormality of ≤20%.

High progressive motility was one of the determining factors for good semen quality, where the active forward movement was categorized as the best. When most of the spermatozoa stopped moving, they were considered dead. Meanwhile, the results of observing spermatozoa motility of Kacang goat diluted with sugarcane juice at various concentrations were presented in Table 2.

The results shown in Table 2 indicated that adding sugarcane juice to egg yolk tris diluent can maintain spermatozoa motility of Kacang goat at 5˚C. The addition of sugarcane juice at concentrations of 10% (T1), 15% (T2), and 20% (T3) improved the spermatozoa motility

to 44±5.47, 55±5.00, and 47±6.70, respectively (P<0.05). The values were higher than the control treatment (T0), which produced a spermatozoa motility of 24±5.47. These results were better compared to the 50 ± 2.68% obtained by (LukmanHy et al., 2022) using 2% red watermelon fruit extract at the same temperature storage. Furthermore, Amrillah, 2017 used kiwi fruit juice (*Actinidia deliciosa*) at room temperature on Boer goat at 46.00±5.48% to maintain progressive motile.

Table 2: Average progressive motility of Kacang Goat semen in tris-egg yolk added by sugarcane juice at 5°C.

Storage time (Days)	Treatment			
	T0	T1	T2	T3
0	56±5.47a	65±5.00b	74±2.23c	68±2.73b
1	50±0.00a	64±5.47b	70±0.00c	67±2.73bc
2	48±4.47a	64±5.47b	67±2.73b	65±5.00b
3	44±5.47a	53±2.73b	62±4.47c	58±4.47bc
4	24±5.47a	44±5.47b	55±5.00c	47±6.70b

Different superscripts in the same column show a significant difference (P<0.05) and the same superscript in the same row shows no significant difference (P>0.05).

The average percentage of progressive spermatozoa motility after 4 days of storage had the best results at T2 with the addition of 15% sugarcane juice and a motility value of 55±5.00% (Table 2). This high value was due to sugarcane juice containing carbohydrates in sucrose, an energy source for spermatozoa during processing and storage. According to (Erwinda and Susanto, 2014), pure sugarcane juice extract contained 18.08% sucrose and 0.54% sugar, which were higher than other nutrients in sugarcane juice. The sucrose functioned as an extracellular cryoprotectant against temperature changes to protect and support spermatozoa life during processing and storage (Khaeruddin and Kurniawan, 2020). This study found that, at the low concentration of sugar cane (15%), the low percentages of spermatozoa motility. In the same line, with the high (20%) concentration of sugar cane, the spermatozoa motility will also be decreased, this could be due to the less or excessive energy available for spermatozoa. If the energy from sugar cane is less, the spermatozoa motility will decrease, and if there is too high a concentration of sugarcane, it could be poison for spermatozoa.

Carbohydrates are compounds that act as extracellular cryoprotectants and protect the plasma membrane of spermatozoa cells from damage during the sperm preservation. Cell plasma membranes that remained intact positively influence spermatozoa motility and vitality. Meanwhile, spermatozoa motility highly depended on the energy provided in the form of adenosine triphosphate (ATP) from metabolism, which was adequately carried out when the cell plasma membrane is intact. The cell plasma membrane regulates traffic in and out of all substrates and electrolytes needed for cell metabolism (Fitri and Fitriana, 2020).

In addition to high sucrose contents, sugarcane also contains protein and vitamins beneficial to spermatozoa (Tkachev et al., 2020). Sugarcane juice combined with egg yolk helped maintain spermatozoa's survival capacity. Moreover, egg yolk contained lipoprotein and lecithin, which played a role in maintaining and protecting the intensity of the lipoprotein sheath and spermatozoa cells from a sudden decrease in cold temperatures and steadied the plasma membrane (Berek et al., 2021).

Storage at cold temperatures for a long time damaged the spermatozoa by lipid peroxidation. This process changed the structure of spermatozoa, specifically in the acrosome membrane, causing loss of motility, rapid metabolic changes, and release of intracellular components. In this study, treatments T1, T2, and T3 containing sugarcane juice had a motility value above 40%, with T2 being the best treatment recommended for AI material requirements. Meanwhile, liquid semen with a motility percentage of not less than 40% can be used for AI (LukmanHy et al., 2022b)

The viability percentage was one of the most critical tests to determine the number of viable and dead spermatozoa, which can be identified using eosin staining methods. The results of observations of spermatozoa viability of Kacang goat diluted with egg yolk tris diluent combined with sugarcane juice at various concentrations were presented in Table 3.

Table 3: Average spermatozoa viability of Kacang Goat semen in tris-egg yolk added by sugar cane juice at 5°C.

Storage time (Days)	Treatment			
	T0	T1	T2	T3
0	83.2±0.83^{a}	86.6±0.54^{b}	90.0±0.70^{c}	87.4±0.89^{b}
1	80.4±1.14^{a}	84.0±1.22^{b}	88.2±0.83^{c}	85.2±1.30^{b}
2	77.0±1.22^{a}	80.0±0.70^{b}	85.8±0.83^{d}	83.2±1.30^{c}
3	73.0±1.22^{a}	78.2±0.83^{b}	83.6±0.89^{d}	79.8±1.48^{c}
4	67.4±1.81^{a}	74.2±0.83^{b}	79.4±1.14^{c}	75.8±2.38^{b}

Different superscript values in the same line showed a significant difference (P<0.05) and the same superscript showed no significant difference (P>0.05).

Table 3 showed that adding sugarcane juice to egg yolk tris maintained the viability of Kacang goat spermatozoa at 5°C. In this study, the viability percentage obtained after 4 days of storage was slightly better than the result of, which used 10% pomegranate juice, which was 74.8 with a viability value of 56.85% (LukmanHY et al., 2022), 66.0±7.9 when extender was enriched with 7% fig fruit filtrate (Zaenuri et al., 2013). This difference was caused

by the physical and chemical properties and levels of diluent in the treatment, osmotic pressure, electrolytes, and non-electrolytes (Laos et al., 2021). Membrane structure eventually reduces its viability when liquid semen is stored at 5°C due to oxidative stress, triggering the formation of reactive oxygen (Zaenuri et al., 2014). The high viability of all treatments with the addition of the sugarcane juice extract was because the nutrient in the sugarcane juice effectively maintained the viability of the spermatozoa of the Kacang goat. Laos et al. (2021) stated that spermatozoa utilized sucrose from sugarcane extract in metabolism to support the energy needed for movement. The average percentage of spermatozoa viability after 4 days of storage had the best results at T2 treatment, with a value of 79.4±1.14%. This is likely because the sugarcane-egg yolk diluent in this treatment had a more complete composition in terms of nutrients such as amino acids, carbohydrates, vitamins, and minerals. These nutrients maintained the vitality of spermatozoa, specifically lipoprotein, lecithin, and fructose contained in sugarcane-yolk diluent can protect spermatozoa from damage to the cell sheath due to cold shock. Moreover, the results showed that semen stored longer at both room temperature and 5°C experienced a decrease in the percentage of viability. This was because changes in the structure of the phospholipids plasma membrane occurred during storage, which disrupted the function and permeability of the cell plasma membrane (Laos et al., 2021). When damage to the cell plasma membrane occurred, it interfered with metabolic processes, and the synthesis process of ATP was inhibited, causing decreased viability of spermatozoa (Sukmawati et al., 2015).

The decrease in the percentage of viability of spermatozoa during the storage period at cold temperatures was also due to the reduced energy contained in the diluent, while the energy required by the spermatozoa was higher, and the concentration of lactic acid in the diluent increased (Kusumawati et al., 2016). According to (Sartika et al., 2022), the substances contained in the diluent used became toxic because they passed through an oxidation reaction during storage and increased free radicals, thereby damaging the integrity of the spermatozoa plasma membrane. During storage at cold temperatures, spermatozoa experienced damage due to oxidative stress, which caused a decrease in viability (Amtiran et al., 2020).

The viability percentage from adding sugarcane juice with a concentration treatment of 15% (T2) at 4 days of storage obtained the best results (79.4). According to (Varasofiari et al., 2013), the percentage used for AI was at least 50% of live and motile spermatozoa.

Spermatozoa with normal morphology is required for fertilization to occur. This is because abnormalities in cell structure cause disturbances during fertilization; therefore, the percentage of abnormal spermatozoa is used to determine the quality of semen (Rodiah et al., 2015; Zaenuri et al., 2017). The results of observing the spermatozoa abnormalities of Kacang goat diluted with egg yolk tris diluent combined with sugarcane juice at various concentrations are presented in Table 4.

Table 4: Average spermatozoa abnormalities in Kacang goat.

Storage time (Days)	Treatment			
	T0	T1	T2	T3
0	4.4±1.14^{b}	4.8±0.83^{b}	2.4±0.54^{a}	3.8±0.83^{b}
1	6.4±0.89^{b}	6.2±1.30^{b}	3.8±0.83^{a}	5.0±1.09^{ab}
2	8.0±1.00^{c}	7.6±1.14^{bc}	5.6±0.89^{a}	6.2±1.30^{ab}
3	9.4±0.54^{bc}	9.8±1.09^{c}	7.0±1.00^{a}	8.4±1.14^{b}
4	10.8±1.30^{b}	11.0±0.70^{b}	8.8±0.83^{a}	9.8±0.83^{ab}

Different superscript values in the same line showed a significant difference (P<0.05) and the same superscript showed no significant difference (P>0.05).

The results showed that adding sugarcane juice to egg yolk tris diluent maintained the abnormal spermatozoa of Kacang goat at a temperature of 5°C. Unfortunately, the abnormality of spermatozoa in this study was not explicitly classified. The average percentage of abnormal spermatozoa after 4 days of storage obtained at T0, T1, T2, and T3 were 10.8%, 11.0%, 8.8%, and 9.8%, respectively (P<0.05). The treatment with the lowest abnormality was T2, which involved adding 15% sugarcane juice, resulting in an 8.8% abnormality. Furthermore, the average abnormality at 4 days of storage at 5°C for all sugarcane juice treatments had a significantly bigger difference than the T0 treatment without sugarcane juice. The percentages of abnormal spermatozoa should not be more than 15% (Sariozkan et al., 2019) to 20% (Wongtawan et al., 2006).

Cell plasma membranes that remained intact positively affected spermatozoa abnormalities based on the energy provided in the form of ATP from metabolism. The metabolism process was carried out effectively when the cell plasma membrane was intact. The cell plasma membrane regulates traffic in and out of all substrates and electrolytes needed for cell metabolism (Labetubun and Siwa, 2012).

Sugarcane juice combined with egg yolk was very good at maintaining abnormal spermatozoa (Mahfud et al., 2019). The benefits of egg yolk were found in lipoprotein and lecithin, which played a role in maintaining and protecting the intensity of the lipoprotein sheath and spermatozoa cells from decreasing temperature and stabilizing the plasma membrane (Sumadiasa et al., 2022).

According to (Manehat et al., 2021), the longer the storage of liquid semen, the lower nutrients available for

spermatozoa, leading to an imbalance in osmotic pressure metabolic processes during storage. These processes affected physical changes in semen and caused higher spermatozoa abnormalities due to decreased semen pH. Moreover, (Berek et al., 2021) stated that abnormalities increased due to lipid peroxidase, changes in osmotic pressure from free radicals, and lactic acid resulting from metabolic processes. This condition damaged the plasma membrane and increased abnormalities of the spermatozoa.

Lipoprotein and lecithin in egg yolk maintained and protected the integrity of the plasma membrane sheath of spermatozoa from cold shock to suppress damage to the plasma membrane in spermatozoa. Yuliani and LukmanHy (2013) stated that lipoprotein and lecithin maintained and protected the integrity of the plasma membrane sheath of spermatozoa to suppress damage to the plasma membrane in spermatozoa. Pribadi et al. (2022) also found that combining egg yolk with sucrose, protein, and vitamins in sugar cane was beneficial for spermatozoa. Furthermore, (Mahfud et al., 2019) reported that egg yolk protected the integrity of the spermatozoa membrane from the effects of cold shock at 5°C storage.

According to (Kusumawati et al., 2016), abnormal sperm for AI purposes should not contain more than 20%, which was consistent with abnormal values at T2 based on AI standards. Ama et al. (2017) also added that the standard percentage of abnormal spermatozoa in goat for AI purposes was ≤ 15%.

CONCLUSIONS AND RECOMMENDATIONS

The addition of 15% sugarcane juice is recommended as the best concentration when sugarcane juice is added to egg yolk tris-buffer diluent to maintain the motility, viability, and abnormalities of Kacang goat diluted semen at 5°C for 4 days of storage.

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NOVELTY STATEMENT

The addition of sugarcane juice to egg yolk tris diluent was able to maintain the motility, viability, and abnormality of spermatozoa of Kacang goat at 5°C storage.

AUTHOR'S CONTRIBUTION

All authors developed the theory and supervised the study. Lukman HY, I Wayan Lanus Sumadiasa, Enny Yuliani, Lalu Ahmad Zaenuri, Mardiansyah, and Ryan Aryadin Putra contributed to the sample collection, analysis calculations, and writing of the final version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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