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Research Article

Effect of Canola Oil Supplementation in Tris Extender on Short-Term Quality of Fresh Goat Semen at Room Temperature

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ABSTRACT
The preservation of semen quality is essential for success of artificial insemination programs in small ruminants. Conventional semen extenders commonly utilize egg yolk as a protective component; however, variability in composition and the risk of microbial contamination have encouraged the search for plant-based alternatives. This study evaluated the effectiveness of canola oil supplementation in a Tris-based extender for maintaining the short-term quality of fresh Boer buck semen stored at room temperature. Semen was collected from four to six healthy adult Boer bucks using an artificial vagina. The pooled semen was diluted (1:9) with Tris–citric–fructose extenders containing either egg yolk (control) or different concentrations of canola oil: 2 ml (C1), 4 ml (C2), and 6 ml (C3). Diluted samples were stored at ≤20 °C and evaluated at 0, 1, and 2 hours for progressive sperm motility, viability, and morphology. Significant differences among extenders were observed at 1 and 2 hours for motility ($P = 0.049$ and $P = 0.008$, respectively) and viability ($P = 0.003$). The C3 extender consistently maintained higher progressive motility (46.33 ± 4.72%) and viability (71.17 ± 0.23%) after 2 hours compared with other treatments. Extenders containing canola oil demonstrated relatively stable sperm morphology throughout storage. These findings indicate that canola oil at higher concentrations, can effectively support sperm membrane stability and reduce oxidative deterioration during short-term storage. Therefore, canola oil-supplemented Tris extenders may serve as a practical and cost-effective plant-based alternative to traditional egg yolk extenders for preserving fresh goat semen prior to artificial insemination.

Keywords: Canola Oil; Goat Semen; Semen Extender; Semen Preservation; Sperm Motility; Sperm Viability

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INTRODUCTION

Semen extenders are solutions used for preserving spermatozoa for fertilization purposes in the artificial insemination (AI) process in most female mammals. They function by maintaining sperm metabolic processes, stabilizing osmotic pressure, regulating the pH of the medium during storage and post-thawing, minimizing cryogenic damage, and preventing bacterial contamination (Kumar Yata *et al.*, 2020). The use of appropriate semen extenders is therefore essential for maintaining sperm viability, motility, membrane integrity, and fertilizing capacity during storage and transportation.

Among the commonly used extenders, Tris-based extenders supplemented with egg yolk have been widely applied for the cryopreservation of buck

semen because egg yolk contains low-density lipoproteins (LDL) and phospholipids that protect spermatozoa from cold shock and membrane damage during cooling and freezing. However, despite its protective properties, egg yolk presents several limitations. The use of egg yolk may negatively affect sperm cells during cryopreservation (Ajadi *et al*., 2012) and may represent a potential microbiological risk because it can serve as a medium for bacterial contamination, thereby compromising semen quality and biosecurity. Additionally, the biological composition of egg yolk varies between batches, making standardization difficult in semen processing protocols (Fernández-Santos *et al*., 2006).

Furthermore, egg yolk interacts with enzymes originating from the bulbourethral glands, particularly the egg yolk coagulating enzyme (EYCE), later identified as phospholipase A, which hydrolyzes egg yolk phospholipids and produces toxic compounds detrimental to sperm membrane integrity (Ferreira *et al*., 2014). These limitations have encouraged the search for alternative extender components that are safer, more consistent, and easier to standardize for semen preservation.

In recent years, plant-based semen extenders have gained considerable attention as alternatives to egg yolk in semen cryopreservation. Plant-derived phospholipids such as soybean lecithin and sunflower lecithin have been widely investigated because they provide membrane-stabilizing phospholipids without the biosecurity risks associated with animal-derived components. Studies have shown that plant-based extenders can effectively maintain sperm motility, membrane integrity, and post-thaw viability in several livestock species (Mousavi *et al*., 2023; Mujitaba *et al*., 2024). These extenders are easier to standardize, reduce microbial contamination risks, and eliminate the variability associated with egg yolk components. For example, soybean lecithin-based extenders have demonstrated comparable or improved sperm quality compared with traditional egg yolk extenders in small ruminants and cattle semen cryopreservation. Similarly, sunflower lecithin has been successfully evaluated as a plant-derived alternative to egg yolk in buck semen extenders, showing promising cryoprotective properties due to its phospholipid composition.

Another important factor influencing sperm survival during semen storage and cryopreservation is oxidative stress. Sperm plasma membranes are rich in polyunsaturated fatty acids, which makes them highly susceptible to lipid peroxidation caused by reactive oxygen species (ROS). Excessive ROS production during semen processing, cooling, and freezing can impair sperm motility, membrane integrity, mitochondrial function, and DNA stability. Consequently, oxidative stress is considered one of the major causes of reduced sperm fertility following cryopreservation. The inclusion of antioxidants in semen extenders has therefore been widely explored to counteract ROS and protect sperm membranes. Natural antioxidant compounds such as tocopherols, polyphenols, and plant-derived bioactive molecules have been reported to improve sperm quality by reducing lipid peroxidation and preserving membrane functionality. For instance, supplementation of plant-based semen extenders with antioxidant compounds such as Coenzyme Q10 has been shown to reduce ROS production and lipid peroxidation while improving motility, membrane integrity, and viability of cryopreserved buck spermatozoa. Similarly, other natural antioxidant additives have been reported to enhance post-thaw sperm quality and fertilizing capacity in goat semen preservation.

Canola (Brassica napus), a flowering plant belonging to the Brassicaceae family, produces bright yellow flowers and seed pods from which oil-rich seeds are harvested. The crop originated in the Mediterranean region and Northern Europe (Baker & Preston, 2008). Canola seeds contain approximately 40–45% oil and are characterized by a favorable fatty acid composition rich in monounsaturated and polyunsaturated fatty acids. Canola oil is also known to contain important bioactive compounds including tocopherols, phytosterols, and polyphenols, which possess antioxidant properties.

Importantly, canola oil contains phospholipids and natural antioxidants such as tocopherols that may contribute to sperm protection during semen storage. These compounds may help stabilize sperm plasma membranes by forming a protective lipid layer around the sperm surface, replacing phospholipids lost or damaged during cryopreservation, and reducing oxidative stress-induced damage. Consequently, plant-derived oils such as canola oil may serve as potential components of semen extenders capable of improving sperm survival, motility, and membrane integrity during preservation.

Therefore, the present study aimed to evaluate the efficiency of different concentrations of canola oil in improving the quality, viability, and shelf life of goat semen stored at room temperature.

MATERIALS AND METHODS

Experimental Animals and Management

The study was conducted using four to six healthy, sexually mature Boer bucks maintained under standard management conditions. The bucks were kept in well-ventilated housing and provided with adequate nutrition, clean drinking water, and routine veterinary care throughout the study period. All animals were allowed a period of adaptation before semen collection began. Only bucks with good libido and no history of reproductive disorders were selected for the experiment.

Preparation of Semen Extender

The semen extender will be the following substances used in this preparation: per ml of diluent, and the pH will be adjusted to 6.8. There are two groups of the extender which is the control TRIS extender that mixed with egg yolk and canola oil concentration extender. In this experiment, a TRIS buffer made of tris, citric acid, fructose, and penicillin and 4ml of egg yolk (TCFY) were utilised as a control extender. For the canola oil extender, there is a different percentage of canola oil in each concentration. The treatment was divided into three groups at 2 ml (C1), 4 ml (C2), and 6 ml (C3) per 20 ml of diluent, respectively, with the addition of 6% of glycerol. A total of 3.028 g of Tris, 1.675 g of citric acid, 1.250 g of fructose, and 0.10 g of penicillin make up the tris buffer, which was made by dissolving all of the components in 100 ml of distilled water. Based on the analyses performed for this study, 9.4 ml of distilled water and 0.6 ml of glycerol were combined to create 6% of the glycerol needed. In a semen extender, 1.2 ml of Tris buffer, 1.5 ml of 6% glycerol, and 0.3 ml of semen sample were combined. A total of 3 ml semen extender solutions were made for each treatment group.

Semen Collection

Semen was collected using an artificial vagina (AV) following standard procedures described for goat semen collection. Each buck provided three to five ejaculates during the experimental period, with collections performed once per week to allow sufficient recovery time between ejaculates and maintain semen quality. A teaser doe in oestrus was used to stimulate sexual behaviour and facilitate ejaculation. The doe's oestrus could occur naturally or be induced using a prostaglandin-based hormonal treatment to ensure the presence of behavioural and pheromonal cues that stimulate mounting behaviour in the buck. The teaser doe was restrained in a neck clamp or tethered position to allow controlled mounting.

During semen collection, the buck was allowed to approach and mount the doe. At the moment of mounting, the penis was gently diverted into the artificial vagina, allowing ejaculation to occur into the sterile collection tube attached to the AV. The AV was prepared using warm water (approximately 40–42°C) to simulate the temperature and mechanical pressure of the female reproductive tract. This thermal and physical stimulation helps trigger ejaculation when the buck's glans penis contacts the AV liner (Donovan *et al.*, 2001; Matshaba, 2010; Munyai, 2012). Immediately after ejaculation, the semen sample was protected from direct sunlight and temperature shock and transported promptly to the laboratory for evaluation.

Semen Handling and Initial Processing

Freshly collected semen samples were kept in a water bath maintained at approximately 37°C during transport and evaluation to preserve sperm viability. Only semen samples free from visible contamination such as urine, blood, or debris were used for further analysis. Samples with abnormal colour or odour were discarded.

Fresh Semen Evaluation

Following semen collection, the amount of fresh semen fluid was measured using a graduated measuring cylinder. The average amount of semen fluid produced by one ejaculation is 1.35 ml. In addition, the pH of the semen was measured using pH paper with a range of 0 to 14 (Shabani *et al.*, 2017).

Semen Dilution

The experiment was arranged in a Completely Randomized Design (CRD) to evaluate the effect of different semen extenders on Boer buck semen quality. Each treatment was replicated three times, and semen samples collected from four to six sexually mature Boer bucks, with multiple ejaculates per buck, served as experimental units.

Semen Allocation Procedure

Immediately after semen collection and preliminary evaluation, only ejaculates that met the minimum quality standards (acceptable motility, normal morphology, and absence of contamination) were used. To minimize individual buck variation, qualifying ejaculates were pooled and thoroughly mixed before allocation to treatments. The pooled semen was then randomly divided into equal portions and assigned to the respective treatment groups.

Semen samples were diluted at a 1:9 (semen: extender) ratio, meaning one part of semen was mixed with nine parts of the prepared extender solution. Dilution was carried out gradually at

approximately 37°C to prevent temperature shock and to maintain sperm viability.

The extender used in this study consisted of Tris buffer and glycerol as the primary components. For each treatment sample, the final extended semen mixture was prepared as follows:

0.3 ml of fresh semen

2.7 ml of prepared extender

The 2.7 ml extender fraction contained:

1.5 ml of 6% glycerol (serving as the cryoprotectant)

1.2 ml of Tris buffer (acting as a buffering agent to maintain pH stability)

This resulted in a total volume of 3 ml of diluted semen per treatment sample. Each treatment group received 3 ml of the diluted semen mixture, and the entire procedure was replicated three times using different ejaculates collected during the study period. These replicates served as independent observations for statistical analysis.

Semen Storage

In this experiment, diluted semen was kept at room temperature (<20℃). Additionally, because sperm are harmed by light, it is essential to keep diluted semen in a Styrofoam box during the entire storage period.

Assessment of Sperm Motility

During the sperm motility period, a glass slide was gently warmed up to assess the motility under the microscope. On a clean glass slide, 3µl of semen was dropped using a micropipette, and the slide was then covered with a coverslip. The percentage of spermatozoa motility was measured after the motility of sperm was studied under a microscope at a 40x magnification with three repetitions for each extender in one experiment cycle. In order to avoid the alternation of sperm motility, the observation was conducted in a laboratory at a set room temperature (18 and 24°C). The microscopic field may detect the degree of motility, such as progressive, slow, vibration, or static (Shabani *et al.*, 2017). However, only motility for progressive sperm mean was recorded in this study.

Assessment of Sperm Viability

A modified eosin-nigrosine stain method was used to determine the proportion of living sperm. On a glass slide, three drops of semen, one of eosin, and two of nigrosine stain were applied, and the slides were left to air dry for some time. Under 40x magnification with three repetitions for each extender in one experiment cycle, the viability of sperm was evaluated (living or dead). Dead sperm may be distinguished from living sperm by their pink or purple stain, whereas dead sperm cannot. However, only viability for live sperm mean was recorded in this study.

Assessment of Sperm Morphology

The term "sperm morphology" describes the sperm's size and form. One of the traits that were looked at as part of a semen study to assess male infertility was sperm morphology. A drop of semen, a drop of eosin, and two drops of the nigrosine stain were applied to a glass slide with three repetitions for each extender and left to air dry while measurements of normal and aberrant sperm were made. However, only morphology for normal sperm mean was recorded in this study.

Data Analysis

A two-way ANOVA was performed to evaluate the effects of extender type, storage time, and their interaction on progressive sperm motility. A post-hoc comparison using Tukey's test was also carried out. IBM Statistical Package for the Social Sciences (SPSS) was used to verify the differences between all parameters. A P-value of <0.005 was be considered significant. All of the data was expressed as mean ± standard deviation (SD).

RESULTS

Progressive Sperm Motility

The results in Table 1 indicated that storage time significantly reduced sperm motility, while extender composition modified the degree of decline. Post-hoc comparison using Tukey's test revealed significant differences among extenders at 1 h ($P = 0.049$) and 2 h ($P = 0.008$), whereas no significant difference was observed at 0 h ($P = 0.500$). At 1 hour of storage, the C1 extender showed significantly lower motility than the control and C3 treatments. At 2 hours, C1 and C2 demonstrated significantly reduced motility compared with the control and C3 groups. The C3 extender (Tris + 6 ml canola oil) consistently maintained the highest motility values after prolonged storage.

Viability of Live Sperm

The results in Table 2 showed that both extender type and storage time significantly influenced sperm viability, with a strong decline observed as storage duration increased. Significant differences among extenders were detected at 1 h and 2 h ($P = 0.003$), while no significant difference occurred at 0 h ($P = 0.595$). At 1 hour, the control and C3 extenders maintained significantly higher sperm viability than C1 and C2. At 2 hours, the lowest viability was observed in C1, whereas control and C3 maintained the highest values, indicating better protective capacity during storage.

Morphology of Normal Sperm

Table 3 shows significant effects of extender type during early storage, with significant differences observed at 0 h (P = 0.003) and 1 h (P = 0.001). However, at 2 h of preservation, the difference among extenders was not statistically significant (P = 0.054). At 0 and 1 hours, the C1 extender exhibited significantly higher percentages of morphologically normal sperm compared with other treatments. Although the statistical difference at 2 hours was marginal, extenders containing canola oil (C1, C2, and C3) generally showed smaller reductions in normal sperm morphology compared with the control.

Table 1. Effect of Canola Oil Extenders on Progressive Sperm Motility at Different Storage Times (n = 5)

Storage Time (h)	Extender	Mean ± SD	95% CI	Tukey Group	P-value
0	Control	56.50 ± 5.42	49.77 – 63.23	A	0.500
	C1	50.84 ± 11.08	37.08 – 64.60	A	
	C2	43.17 ± 18.61	20.06 – 66.28	A	
	C3	60.67 ± 0.00	60.67 – 60.67	A	
1	Control	48.00 ± 0.95	46.82 – 49.18	B	0.049
	C1	23.00 ± 8.01	13.06 – 32.94	A	
	C2	32.67 ± 7.54	23.30 – 42.04	Ab	
	C3	42.00 ± 4.71	36.15 – 47.85	B	
2	Control	44.50 ± 5.89	37.19 – 51.81	B	0.008
	C1	11.34 ± 6.13	3.73 – 18.95	A	
	C2	20.84 ± 5.42	14.11 – 27.57	A	
	C3	46.33 ± 4.72	40.47 – 52.19	B	

Values with different superscripts within the same row differ significantly (**P < 0.05**, Tukey test)

Table 2. Effect of Canola Oil Extenders on Sperm Viability at Different Storage Times (n = 5)

Storage Time (h)	Extender	Mean ± SD	95% CI	Tukey Group	P-value
0	Control	84.50 ± 0.71	83.62 – 85.38	a	0.595
	C1	85.50 ± 6.36	77.60 – 93.40	a	
	C2	79.34 ± 5.18	72.91 – 85.77	a	
	C3	82.00 ± 4.24	76.74 – 87.26	a	
1	Control	80.17 ± 2.12	77.54 – 82.80	b	0.003
	C1	62.16 ± 2.59	58.94 – 65.38	a	
	C2	60.34 ± 2.35	57.42 – 63.26	a	
	C3	75.50 ± 2.59	72.28 – 78.72	b	
2	Control	71.17 ± 0.23	70.88 – 71.46	C	0.003
	C1	50.17 ± 4.95	44.03 – 56.31	A	
	C2	58.67 ± 0.47	58.09 – 59.25	B	
	C3	71.17 ± 0.23	70.88 – 71.46	C	

Values with different superscripts within the same row differ significantly (**P < 0.05**, Tukey test)

Table 3. Effect of Canola Oil Extenders on Sperm Morphology at Different Storage Times (n = 5)

Storage Time (h)	Extender	Mean ± SD	95% CI	Tukey Group	P-value
0	Control	72.00 ± 0.00	72.00 – 72.00	A	0.003
	C1	84.00 ± 1.89	81.65 – 86.35	B	
	C2	73.33 ± 0.00	73.33 – 73.33	A	
	C3	73.17 ± 2.12	70.54 – 75.80	A	
1	Control	73.00 ± 1.41	71.25 – 74.75	A	0.001
	C1	82.17 ± 0.23	81.89 – 82.45	B	
	C2	73.67 ± 0.00	73.67 – 73.67	A	
	C3	74.84 ± 0.23	74.56 – 75.12	A	
2	Control	66.84 ± 3.06	63.04 – 70.64	A	0.054
	C1	75.84 ± 2.59	72.62 – 79.06	B	
	C2	70.67 ± 0.00	70.67 – 70.67	Ab	
	C3	71.84 ± 1.18	70.37 – 73.31	Ab	

Values with different superscripts within the same row differ significantly (**P < 0.05**, Tukey test)

DISCUSSION

The results obtained in this study demonstrate that storage time significantly influenced sperm quality

parameters, while the composition of the extender modified the magnitude of decline during preservation. Progressive sperm motility decreased gradually as storage time increased, with the most significant difference observed at 2 hours of preservation (P = 0.008), whereas no significant difference occurred at 0 hours (P = 0.500). This indicates that immediately after dilution, sperm cells were physiologically stable across treatments. However, as preservation time increased, the protective capacity of the extenders became more evident. Among the extenders evaluated, the C3 formulation (Tris + 6 ml canola oil) consistently maintained the highest progressive sperm motility after prolonged storage, indicating that higher concentrations of canola oil may enhance sperm stability during short-term preservation.

The progressive reduction in sperm motility observed in this study agrees with earlier reports that sperm motility generally declines during storage due to metabolic exhaustion and structural damage to the sperm plasma membrane. José A. Yániz *et al.* (2017) reported that sperm progressive motility is typically highest during the early period of storage and decreases as storage time increases. Similarly, Acharya *et al.* (2016) demonstrated that progressive sperm motility declines significantly during semen storage regardless of extender type or storage temperature. These findings suggest that the decline in motility is primarily associated with physiological and biochemical changes occurring in sperm cells during preservation.

One of the main factors responsible for the reduction in sperm motility during storage is oxidative stress. Mammalian sperm membranes contain high concentrations of polyunsaturated fatty acids (PUFAs) that are essential for maintaining membrane fluidity and fertilizing ability. However, these lipids are highly susceptible to lipid peroxidation caused by reactive oxygen species (ROS) (Ochsendorf, 1999; Mansour *et al.*, 2013). When ROS production exceeds the antioxidant capacity of the semen extender or seminal plasma, oxidative stress occurs, leading to membrane damage, mitochondrial dysfunction, and decreased sperm motility and viability (Agarwal *et al.*, 2014; Aitken & Baker, 2020). Furthermore, dilution of semen during extender preparation reduces the natural antioxidant defenses present in seminal plasma, thereby increasing the vulnerability of spermatozoa to oxidative damage during storage. Consequently, supplementation of semen extenders with antioxidant compounds has become an important strategy to maintain sperm quality during preservation (Bustani & Baiee, 2021; Selvaraju *et al.*, 2021).

The improved performance of the canola oil–based extenders observed in the present study may be attributed to the antioxidant and membrane-stabilizing properties of canola oil. Canola oil is rich in unsaturated fatty acids such as oleic acid and α-linolenic acid, which possess antioxidant activity and play an important role in maintaining membrane integrity (Ellwood, 2006). These fatty acids may reduce oxidative stress by scavenging free radicals and limiting lipid peroxidation of sperm membrane phospholipids. As a result, sperm cells preserved in extenders containing higher concentrations of canola oil, particularly the C3 formulation, were better protected against oxidative damage, thereby maintaining higher progressive motility during storage. Another mechanism that may explain the beneficial effect of canola oil is membrane phospholipid replacement and stabilization. During semen storage, sperm membranes may lose phospholipids due to oxidative degradation and enzymatic hydrolysis. Lipid-rich extenders may supply exogenous phospholipids that interact with or stabilize the sperm plasma membrane, helping maintain membrane fluidity and functional integrity. This phospholipid replacement mechanism has been reported to protect spermatozoa against cold shock, osmotic stress, and membrane destabilization during semen preservation (Medeiros *et al.*, 2002; Bucak *et al.*, 2020). Therefore, the lipid components present in plant-derived oils such as canola oil may help maintain sperm membrane stability and delay the decline in sperm motility observed during storage.

The findings of this study can also be discussed in relation to the growing interest in plant-based semen extenders, particularly those containing soy lecithin. Soy lecithin extenders have been widely investigated as alternatives to traditional egg yolk–based extenders because they provide phospholipids without the sanitary risks associated with animal-derived products. Previous studies have demonstrated that soy lecithin extenders can protect sperm membranes by forming a protective phospholipid layer around sperm cells and reducing oxidative damage (Amirat *et al*., 2004; Singh *et al.*, 2022). However, recent studies indicate that vegetable oils and plant-derived additives rich in natural antioxidants may provide similar or even improved protective effects during semen preservation (Leão *et al.*, 2021).

The results obtained in this study support this perspective, as the canola oil–based extender (C3)

effectively maintained sperm motility and viability during short-term storage at room temperature. In addition to motility, sperm viability was also significantly affected by both extender type and storage time. A gradual reduction in viability was observed as storage duration increased, reflecting progressive deterioration of sperm membrane integrity. Significant differences among extenders were detected at 1 hour and 2 hours of storage (P = 0.003), with the control and C3 treatments maintaining significantly higher viability compared with C1 and C2. Although the control extender exhibited slightly higher viability at 1 hour, the C3 extender maintained a relatively stable viability profile throughout the storage period, suggesting that the higher concentration of canola oil provided improved protection against sperm cell death. The maintenance of sperm viability during preservation is closely related to the pH stability of the semen extender. Appropriate pH levels regulate sperm metabolic activity and help maintain intracellular homeostasis. Slight reductions in pH can temporarily suppress metabolic activity and prolong sperm survival, whereas excessive acidity may impair sperm motility and viability by causing intracellular acidification (Jones *et al.,* 1995). Liu *et al.* (2016) demonstrated that increasing the initial pH of a semen extender improved sperm viability during liquid storage of goat semen. Similarly, Xu *et al.* (2009) reported that sperm motility and viability declined when extender pH decreased from 6.04 to 5.54. Therefore, maintaining an optimal pH environment within semen extenders is essential for preserving sperm viability during storage.

Regarding sperm morphology, significant differences among extenders were observed at 0 hours (P = 0.003) and 1 hour (P = 0.001), while the differences became statistically insignificant at 2 hours (P = 0.054). The C1 extender exhibited the highest percentage of morphologically normal sperm during early storage. Nevertheless, extenders containing canola oil (C1, C2, and C3) generally showed smaller reductions in normal sperm morphology compared with the control treatment. These results suggest that the lipid components of canola oil may contribute to maintaining structural stability of sperm membranes and protecting sperm cells from morphological abnormalities during storage.

Traditional egg yolk–based extenders have long been used in semen preservation because egg yolk phospholipids protect sperm cells against cold shock and osmotic stress. However, egg yolk extenders have several limitations, including microbial contamination risks, variability in composition, and biochemical interactions with enzymes present in seminal plasma (Baruah *et al*., 2019). One such enzyme, known as egg yolk coagulating enzyme (phospholipase A), can hydrolyze egg yolk phospholipids and produce toxic compounds that negatively affect sperm viability and motility (Ferreira *et al.*, 2014). Consequently, there has been increasing interest in plant-derived alternatives such as soy lecithin and vegetable oils that provide phospholipids without these sanitary and biochemical limitations. Overall, the results of this study indicate that canola oil–based semen extenders, particularly the C3 formulation, provide improved protection against oxidative stress, support membrane phospholipid stability, and maintain sperm motility and viability during short-term storage at room temperature. These findings highlight the potential of canola oil as a promising plant-derived component for semen extender formulations, which may serve as an effective alternative to conventional egg yolk–based extenders in artificial insemination programs.

CONCLUSION

In conclusion, the current investigation showed that, at room temperature, the concentration of the Tris-based canola oil extender was superior to the Tris-based egg yolk extender. Although the ideal canola oil concentration varies for each extender, the current investigation shows that tris + 6 ml of canola oil extender performed much better than the TCFC extender (control) in every parameter assessed at room temperature. In order to improve the quality of fresh semen and lengthen its shelf life before being inseminated during the AI process, the Tris + canola oil extender is ideal for the small-scale breeder, which is a less expensive replacement for the expensive commercial extenders that were previously in use. Utilising canola oil, a plant-based component in the extender, was safer than using egg yolk to preserve sperm morphology. Using a lower grade of egg yolk for the semen extender led to coagulation in the dilution, which was discovered throughout this trial. However, it is strongly advised that the effects of employing a canola oil extender in AI be discovered first to evaluate whether or not it improves embryo development within the uterus.

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