



Effect of Adding an Aqueous Extract of *Aloe Vera* (Aeav) to Tris Extender on some Characteristics of Goat Epididymal Spermatozoa at Different Cooling Times

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Abstract | Plant extracts have been used as alternative additives for the semen preservation process in different farm animal species to improve semen properties such as viability, motility, and membrane integrity, which thereby increase sperm fertility. The current study was conducted to investigate the effect of adding an aqueous extract of Aloe vera leaf gel (AEAV) to the epididymal spermatozoa of local goats during the cooling process for different periods. The AEA V was prepared and stored in the refrigerator until the time of use. Epididymal spermatozoa were diluted by a Tris extender and divided into three treatments: T1 with a Tris extender only (control group), T2: 0.02 AEA V, and T3: 0.03 AEA V. Epididymal sperm were cooled at 5 °C for 0, 24, 48, 72, 96, and 120 hours. The results show a significant effect ($P \leq 0.01$) of AEA V on sperm motility, abnormal sperm percentage, dead sperm percentage, and membrane integrity. The overall means were 51.11 %, 65.55 %, and 60.55%, for motility, 4.00 %, 4.33 %, and 1.5%, for abnormal sperm, 19.80 %, 17.64 %, and 19.33%, for dead sperm, and 74.25%, 78.63%, and 78.33%, for membrane integrity, respectively, for T1, T2, and T3. No significant differences were observed for dead sperm among 0.03 AEA V and the control group, abnormal sperm among 0.02 AEA V and the control, and also for HOST, between 0.02 and 0.03 AEA V. The best interaction between AEA V levels and cooling periods was reported for 0.03 of AEA V in fresh semen at 0 hrs. for all parameters of this study, except the HOST which did not differ at 0 hrs. of cooling among all AEA V levels. On the other hand, the results of the present study indicated significant differences ($P \leq 0.01$) for all six cooling periods (hrs.), except for abnormal sperm among 72, 96, and 120 hrs. post-cooling. It could be concluded that adding two levels (2% and 3%) of an aqueous extract of Aloe vera gel has improved some characteristics of goat epididymal sperm preserved under cooling conditions for different periods.

Keywords | Aloe vera, AEA V, Semen characteristics, Local buck, Cooling period

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Semen preservation by chilling is considered a fundamental step of artificial insemination (AI) (Rahman *et al.*, 2014) of farm animals. The harmful effects of sperm preservation in goats are due to the integrity of the cell membrane, as the cell membrane contains polyunsaturated fatty acids that are quickly oxidized, and the number of antioxidants in sperm cells is lower than in other cells (El-Hadary *et al.*, 2023) thus, the susceptibility to the effect of oxidative stress is greater on sperm cells (Khalique *et al.*, 2023). Preservation of semen using alternative additives such as plant extracts have been used in different farm animal species to improve semen quality, which thereby increases sperm fertility. Aloe vera is a medicinal plant that contains specific bioactive ingredients, including proteins, lipids, amino acids, alkaloids, saponins, flavonoids, enzymes, organic and inorganic compounds (Hamman, 2008; Akaberi *et al.*, 2016, Lopez-Cervantes *et al.*, 2018), vitamins C, B (1, 2, and 6), A, and E (Daniel, 2024), and mineral salts like sodium, calcium, iron, potassium, chloride, manganese, copper, and zinc (Sánchez *et al.*, 2020; Ebrahim *et al.*, 2020; Darzi *et al.*, 2021; Saleem *et al.*, 2022; Kamble *et al.*, 2022). The physical parameters of a fresh Aloe vera leaf, including length, width, and gel weight, were conducted by Talukdar *et al.*, (2023). The protective effect of Aloe vera on cooling semen has been determined in goats (Aguilar *et al.*, 2012), cattle (Farias *et al.*, 2019), dogs (Melo *et al.*, 2014), and peccaries (Souza *et al.*, 2016). Mona *et al.* (2023) indicated that adding Aloe vera raw gel extract at 10µg/ml to bovine spermatozoa showed increased sperm hyper-motility and acrosome reaction and produced the highest percentage of fertilized oocytes. According to Agbaye *et al.* (2023), adding 7.00 g/L of Aloe vera leaf gel to extended goat semen affected fertilizing capability and maintained spermatozoa motility and plasma membrane integrity by more than 60% for 72 hours. By enhancing sperm parameters and promoting spermatogenesis, Aloe vera showed strong spermatogenic activity. aloe vera seems to be an alternate plant-based agent for using in the preservation of sperm, particularly because of its rich compound and the existence of polysaccharides derived from it that have cryo-protective properties (Chauhan *et al.*, 2014; Zhang *et al.*, 2018), as well as a superior antibiotic that can enhance reproductive performance and animal development (Daniel, 2024). There are not many authors discussing how leaf extracts affect sperm properties. The aim of this study is to investigate the effect of adding aqueous extract of Aloe vera gel with two concentrations (2 and 3%) in goat epididymal spermatozoa cooled at 5 °C and preserved for six storage periods (24, 48, 72, 96, and 120 hrs.) on percentages of sperm motility, morphological abnormal sperm, dead sperm, and hypo-osmotic swelling test (HOST).

The current study was conducted in the graduate laboratories of the College of Agriculture, Al-Muthanna University, Department of Animal Production, from January 2021 until May 2022. This study was conducted in accordance with the instructions and procedures required in the postgraduate laboratory and approved by the Ethics Committee of Agriculture College, University of Al-Muthanna, ethical approval number 154LBI04006.

COLLECTION TESTES AND SEMEN PREPARATION

The testes were collected from adult bucks immediately after slaughter, where they were cut together with the scrotum and then transported to the laboratory using a container containing ice cubes. The testes were removed and the epididymis was dissected. By flushing, the sperm were extracted from the cauda (Martinez-Pastor *et al.*, 2006). After that, 100 ml of media was prepared, adding 63.3 g of Tris, 1.99 g of Citric Acid, 5 g of sucrose, and small percentages of antibiotics (Pwnicillin and Streptomycin). All of these materials are added to 80 ml of distilled water. After that, the medium is filtered in two batches using a Melbourne filter (22 and 45 mm), after which the pH of the medium is adjusted between 7.2 and 7.4.

PREPARATION OF AQUEOUS EXTRACT OF ALOE VERA (AEAV)

Fresh Aloe vera leaves were harvested and washed; the outer layers were carefully peeled with forceps to prevent staining of the colorless, viscous inner gel. The gel was carefully scooped with a spatula into a beaker, homogenized, covered with foil paper, and kept at a temperature of 37 °C in a water bath. After that, 100 grams of raw gel were collected and mixed with 25 ml of distilled water using an electric mixer for one minute. The mixture was left for 12 hours with constant stirring using an electric shaker, then placed in the incubator at 37 °C until it was completely dry and stored for use in the refrigerator. After preparing the Tris medium in the manner mentioned above, two concentrations of AEAV, 0.02 and 0.03, were added to 4 ml of Tris solution and 1 ml of egg yolk, and the pH of the medium was measured. Then the medium was left in the incubator to warm, and then the sperm were added and examined.

SEMEN EVALUATION

SPERM MOTILITY: The semen was diluted using Tris diluent, and individual motility was estimated according to what was stated by Chemineau *et al.* (1991) by taking a drop of the diluted semen, placing on a glass slide, and placing the cover of the slide on the sample at a temperature of 37 °C. It was examined under a microscope at 40x magnification, and the motility was calculated based on the ratio. The percentage of sperm with forward movement and the strength and speed of their movement

Table 1: Effect of *aloe vera* concentration, Cooling Periods (hrs.) and their interaction on the individual motility of sperms (%).

AEAV Con.(%)	Cooling Periods (hrs.) Mean± Stander Error						Mean	Sig.
	0	24	48	72	96	120		
0	76.66±1.66 A b	70.0±2.88 B c	61.66±4.40 C c	46.66±4.40 D c	31.66±4.40 E c	20.00±2.88 F c	51.11 c	**
0.02	86.66±1.66 A a	81.66±1.66 A a	73.33±1.66 B a	63.33±1.66 C a	50.00±0.0 D a	38.33±1.66 E a	65.55 a	
0.03	86.66±1.66 A a	76.66±1.66 B b	66.66±1.66 C b	56.66±1.66 D b	45.00±0.00 E b	31.66±1.66 F b	60.55 b	
Mean	83.33 a	76.11 b	67.22 c	55.55 d	42.22 e	30.00 f		
Sig.	**							

Averages with small letters within one column indicate a comparison between transactions, and averages with capital letters within one row indicate a comparison between different periods within one transaction. **: P≤0.01.

ABNORMAL SPERM PERCENTAGE: The percentage of morphological abnormal sperm was estimated based on [Hancock \(1951\)](#) using the same slide for estimating the percentage of dead sperm, and it was examined with a microscope at 40x magnification according to the following equation:

$$\text{Percentage of deformed sperm} = \frac{(\text{number of abnormal sperm})}{((200) \text{ calculated sperm count})} \times 100$$

DEAD SPERM PERCENTAGE: The percentage of dead sperm was estimated based on what was stated by [Swanson and Beardon \(1951\)](#) by taking a drop of fresh semen and placing it on a clean glass slide at a temperature of 37 °C. Then one drop of a mixture of eosin dye and necrocin (2:1) was added, and the two drops were mixed well and gently using a glass slide, made a smear on another glass slide at an angle of 45 degrees and examined it under a microscope with strong magnification (400x).

HYPOT-OSMOTIC SWELLING TEST (HOST): The percentage of plasma membrane integrity was estimated based on what was stated by [Lomeo and Giambersio \(1991\)](#) by taking 10µl of semen and 1 ml of distilled water (mOsm 0 /l, pH 7.02) and incubating the sample at a temperature of 37 °C for five minutes. Then, immediately post-incubation, we took a drop of semen and placed it on a clean glass slide at a temperature of 37 °C with a cover slide on the sample and examined under a microscope (400X).

STATISTICAL ANALYSIS

The data was analyzed using the Statistical Analysis System ([SAS, 2012](#)) to investigate the impact of various factors on the examined characteristics according to a factorial experiment (3×6) applied in a complete random design (CRD) according to the mathematical model below, and the significant differences between the means were compared with a polynomial test Duncan ([Duncan, 1955](#)) with a 0.01 probability of error. mathematical model:

$$y_{ij} = A_i + B_j + AB + e_{ij}$$

RESULTS AND DISCUSSION

SPERM MOTILITY

Table 1 shows the effects of AEA, storage time, and the interaction between them on the individual motility of spermatozoa in goat semen chilled at 5 °C. Results indicate a significant effect (P≤0.01); the highest rate was noticed in the first concentration of AV. Namely, 65.55% compared with the lowest rate that was noticed in the control group namely, 51.11%. The storage period affected individual motility, the least rate was reported in the 6th period (30.0%) compared with the highest rate reported in fresh semen (83.33%). The results indicated that the best interaction was reported in fresh semen at 0.02 and 0.03 of AV. namely 86.66%, while the lowest rate was reported in sperm motility at control at the 6th storage period, about 20%. The enhancement of sperm motility continues at 48, 72, 96, and 120 hours of storage by adding 0.02 and 0.03 AV compared with the control group.

ABNORMAL SPERM PERCENTAGE

The research results demonstrated that 3% AEA had a significant effect (P<0.01) on the morphological abnormal sperm percentage. While there were no significant differences among AEA at 3% and the control group (**Table 2**), the overall means were 4.00%, 4.33%, and 1.50% for T1, T2, and T3, respectively. Storage time was differed significantly (P<0.01) on abnormal sperm percentage, as shown in **Table 2**. Throughout all storage intervals, the mean percentages of abnormal sperm varied and declined with longer storage times.. There were no significant differences in mean percentages 72, 96, and 120 hours post-chilling, they were 4.72%, 4.94%, and 4.83%, respectively. The abnormal sperm (%) was significantly (P<0.01) impacted by the interaction between AEA and storage time, except after 48 and 72 hours. after storage, where it showed similar percentages between the control and 3% AEA (**Table 2**).

DEAD SPERM PERCENTAGE

Results show a significant effect of AEA concentrations on dead sperm percentage (%); the highest rate was noticed for T1 (control) and T3 (0.03), compared with the lowest

Table 2: Effect of *aloe Vera* concentration, Cooling Periods (hrs.), and their interaction on the Abnormal sperm percentage (%).

AEAV Con.(%)	Cooling Periods (hrs.) Mean± Stander Error						Mean	Sig.
	0	24	48	72	96	120		
0	2.50±1.80 D b	4.33±1.09 B a	4.00±1.04 B a	3.33±0.16 C c	4.83± 0.16 A b	5.00±1.06 A b	4.00 a	**
0.02	2.66±1.16 D a	2.66±1.16 D b	3.66±1.01 C b	5.00±1.32 B b	6.50±2.17 A a	5.50±1.25 B a	4.33 a	
0.03	1.50±1.25 E c	2.33±0.88 D c	4.00±0.28 B a	5.83±1.20 A a	3.50±0.57 C c	4.00±1.25 B c	1.50 c	
Mean	2.22 b	3.11 ab	3.88 ab	4.72 a	4.94 a	4.83 a		
Sig.	**							

Averages with small letters within one column indicate a comparison between transactions, and averages with capital letters within one row indicate a comparison between different periods within one transaction. **: P≤0.01.

Table 3: Effect of *aloe Vera* concentration, Cooling Periods (hrs.), and their interaction on the Dead sperm percentage (%).

AEAV Con.(%)	Cooling Periods (hrs.) Mean± Stander Error						Mean	Sig.
	0	24	48	72	96	120		
0	2.00±1.15 F b	6.16±0.92 E a	11.66±0.60 D b	21.33±0.72 C a	33.66±0.44 B a	44.00±2.64 A a	19.80 a	**
0.02	1.33±0.88 F c	4.66±0.72 E b	11.16±0.83 D b	18.83±2.18 C b	29.00±1.50 B b	40.83±1.20 A b	17.64 b	
0.03	2.16±1.01 E a	6.16±0.88 D a	12.33±1.16 C a	22.16±1.66 B a	29.00±0.50 B b	40.66±1.20 A b	19.33 a	
Mean	1.83 f	5.66 e	11.72 d	20.77 c	31.72 b	41.83 a		
Sig.	**							

Averages with small letters within one column indicate a comparison between transactions, and averages with capital letters within one row indicate a comparison between different periods within one transaction. **: P≤0.01.

Table 4: Effect of *aloe Vera* concentration, Cooling Periods (hr.), and their interaction on the HOST (%).

AEAV Con.(%)	Cooling Periods (hrs.) Mean± Stander Error						Mean	Sig.
	0	24	48	72	96	120		
0	95.00±0.28 A a	88.00±0.57 B b	79.66±0.88 C b	73.00±1.04 C b	60.66±1.36 D b	49.16±0.72 E b	74.25 b	
0.02	95.50±0.28 A a	89.83±0.16 B ab	84.00±0.28 B a	75.83±1.01 C ab	67.83±1.76 D a	58.83±1.30 E a	78.63 a	
0.03	96.66±0.44 A a	90.00±0.57 B a	84.33±0.92 C a	76.33±1.69 D a	68.16±0.88 E a	57.17±1.58 F a	78.33 a	**
Mean	95.72 a	89.27 b	82.66 c	75.05 d	65.55 e	55.05 f		
Sig.	**							

Averages with small letters within one column indicate a comparison between transactions, and averages with capital letters within one row indicate a comparison between different periods within one transaction. **: P≤0.01.

rate for T2 (0.02) (Table 3). Storage period had a significant effect on dead sperm percentage, least rate was reported in fresh semen (1.83%), compared with the highest rate reported in the 6th period (41.83%). There was a significant effect of interaction between AEA concentrations and storage period, results indicated that the best interaction was reported in fresh semen at T3 (2.16±1.01) compared to the rate reported in semen with T1 concentration and 6th storage period (44.00±2.64). The percentage of dead sperm was significantly (P<0.01) influenced by the interaction between AEA and storage intervals, except after 72 hrs. among T1, and T3, and also after 96 and 120 hrs. among all treatments, where it showed similar percentages (Table 3).

HYPOTHEOTIC SWELLING TEST (HOST)

The HOST percentage results are shown in Table 4. Adding AEA to Tris diluent was significantly increasing (P≤0.01) on the HOST test at T2 and T3 as compared to the control treatment, mean was 78.63%, 78.33%, and 74.25% for

T2, T3, and T1, respectively. Storage time had significant effects (P<0.01) on HOST test percentages in all six periods of storage, with the best and highest rate noticed at zero time (95.72%), and then gradual decreasing up to 120 hrs. (55.05%), as obtained in Table 2. The HOST test was significantly affected (P<0.01) by the interaction between AEA and storage times, except after 48, 72, 96, and 120 hours after storage, where it showed similar percentages between the two concentrations of AEA (Table 2). There were no significant differences in mean percentages 72, 96, and 120 hrs. post- chilling, which were 4.72%, 4.94%, and 4.83%, respectively. Similar results were also observed for all treatments at zero time.

The current investigation assessed the effects of two distinct Aloe vera leaf gel concentrations (0% (control), 2%, and 3%) in Tris-egg yolk extender on local buck semen stored at 5 °C for 120 hours. The results of this study showed a significant difference (P<0.01) in the sperm motility, ab-

normal sperm percentage, dead sperm percentage, and HOST test from 24 throughout the 120 hrs. post cooling process. Many studies provide evidence of the importance of adding plant extracts to semen during different times of storage (Zhao *et al.*, 2009; Neamah and Houbi, 2020; Neamah, 2023), including Aloe vera extract gel for chilling epididymal spermatozoa (Farias *et al.*, 2019; Zareie *et al.*, 2021; Seshoeni, 2023). Another study showed adding 2, 4, and 6% of *Salvia Rosmarinus* extract to the goat semen at cooling conditions enhanced integrity of the plasma membrane and sperm motility, viability, and reduction of the MDA level by 2 % (Zanganeh *et al.*, 2013). In agreement with our findings, Singh *et al.* (2020) observed a significant difference between the Aloe vera and control group in terms of post-thaw% progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities, and HOST test. Also, our findings support the outcomes of Fakhridin and Sudani (2014), who found that Aloe vera positively affects sperm parameters, including a highly significant increase in sperm motility, viability, HOST test, and morphological abnormal sperm. In contrast, our results did not agree with Olugbenga *et al.* (2011) that adding Aloe vera extract as a supplement (3 and 4% Aloe vera extract solution) increases dead sperm percentage abnormalities and decreases sperm motility and live sperm percentage in the buck semen while having no effect of Aloe vera extract on the semen color. The majority of plant species, including Aloe vera, have high levels of antioxidants, which can reduce the negative impacts of oxidative stress on sperm function by acting as ROS scavengers (Santaella and Pintus, 2021). Antioxidants are frequently added to semen extenders and are used for both cooling and cryopreservation storage of sperm (Mona *et al.*, 2023). This could explain the high significant percentages in all parameters obtained in this study. Our research results are in agreement with Zareie *et al.* (2021), which showed that using 20 µg/ml of Aloe vera extract in the extender significantly improved membrane integrity, progressive motility, and total motility when compared to the control treatment. On the other hand, the results conflict with those of (Meena *et al.*, 2019), who found that sperm motility is inhibited by Aloe vera leaf extract. there could be a difference in the experimental circumstances causing this disagreement. According to Melo *et al.* (2014), sperm motility may be considerably increased when cat spermatozoa are incubated with an extender containing AEAV for longer than an hour. Therefore, dilution of spermatozoa with AV. need a longer time for adaptation, which may be attributed to the mucilaginous property found in Aloe vera gel. In the present study, we did not observe this case, and the reason may be attributed to the species of animal, the individual differences in the sperm, or the physiological characteristics of the sperm from one type to another, or the reason may be due to the conditions of planting and irrigation of the plant, which may affect the mucilage property of Aloe vera. Results of the present study do not agree

with Farias *et al.* (2017), who postulated that the use of AV extract at intermediate concentrations (above 10% but less than 20%) might have a better effect on sperm cells due to the plant extract's high viscosity, which can limit sperm movement in viable cells. According to Mona *et al.* (2023), a high IVF rate in Holstein bulls was produced by adding 10 µg/ml of Aloe vera extract to bull spermatozoa, which was found to be the most effective concentration for improving sperm capacitation. High significant ($p \leq 0.01$) differences were found on the sperm abnormality percentage at various cooling periods within each group (Table 2). There were high ($p \leq 0.01$) significant variation between storage periods for all groups. The results indicated a significant decrease ($p \leq 0.01$) in the abnormal and dead sperm percentages, and the reason that may be attributed to using the aqueous extract of the Aloe vera plant is that the leaves do not only contain carbohydrates found in the wall of the ornament, such as cellulose and hemicellulose, but they also contain stored carbohydrates, such as acetyl manna (Ni *et al.*, 2004). The presence of abnormal sperm in this work is consistent with the report of Jones, (2004) that several abnormal sperm are normally in all ejaculates. The decrease in semen properties observed in the present study during storage at 5°C may be due to a combination of factors, such as partially irreversible damage to the epididymal sperm membranes that leads to loss of vital cell components and thus a reduced metabolic rate of sperm. (Holt, 2000 ; De Pauw *et al.*, 2003; Lima *et al.*, 2013). Zareie *et al.*, (2021) suggested adding the extract of Aloe vera improved the goat sperm motility during storage, and the antioxidant activity of Aloe vera led to a decrease in the malondialdehyde level. Yong *et al.* (2017) recorded that fish semen treated with Aloe vera extract enhanced sperm motility post-cryopreservation. Also, Aloe vera as a supplement increases the rat's fertility (Jasem and Nasim, 2011). These studies show that Aloe vera plays a role in stopping sperm oxidation and can save sperm motility during storage. According to the reports, this plant contains zinc and the acid folic, which act as antioxidants and can enhance sperm quality by reducing semen apoptosis (Zareie *et al.*, 2021). A study by Almasry *et al.*, (2023) showed that adding Aloe vera (2 ml) to 1000 ml of drinking water enhances the semen quality. This effect is attributed to the components of Aloe vera, which are rich in antioxidants, as they work to neutralize the free radicals formed, as well as the fact that Aloe vera contains a special polysaccharide compound consisting mainly of mannose sugar, as it interacts with cell surface receptors, stimulating them and activating their faster growth and reproduction (Chandan *et al.*, 2007). Buck spermatozoa are negatively affected by preservation because of the integrity of their cell membrane, which contains highly polyunsaturated fatty acids that quickly oxidize. In comparison to other cells, sperm cells have fewer antioxidants, thus, oxidative stress can more easily damage sperm cells (Khaliq *et al.*, 2023). There were significant differences among concentration of

AEAV and the control group regarding conservation time for the sperm characteristics under study. The impact of various extenders at 4 °C on sperm properties in ejaculated sperm from various species is widely recognized (Azawi *et al.*, 1993; Mara *et al.*, 2007). The composition of the extender has been linked to the differences in this investigation. The current findings clearly show that an extender based on aloe vera provides a better suitable medium to maintain the viability of epididymal sperm storage at 5 °C, as it may preserve sperm properties for up to 120 hours after chilling. The AV composition could explain these best results. Goat semen extenders typically contain egg yolk and AEAV (Campos *et al.*, 2003; 2004). Because of the low density lipoprotein portion (Moussa *et al.*, 2002), egg yolk is well known for protecting sperm membranes against heat shock during storage at 5 °C (Watson and Martin, 1975; De Leeuw *et al.*, 1993). One of the limitations of this study is to determine the effectiveness of using Aloe vera extract in goat epididymal sperm cryopreservation, as well as the ideal viscosity of the extract. We also recommend conducting further studies on the use of Aloe vera extract in in-vitro fertilization and artificial insemination.

CONCLUSIONS AND RECOMMENDATIONS

The current study indicates that adding an aqueous extract of Aloe vera gel with two different concentrations has improved some characteristics of goat epididymal sperm preserved under cooling conditions for different periods. Aloe vera can be an alternative extender of plant origin for the preservation of goat semen, as it protects the sperm membranes, which are necessary for their viability and applicability in artificial insemination programs. According to the experiment's findings, the use of AEAV in the extender of goat epididymal spermatozoa has a positive effect on cell protection during the cooling process. However, new studies need to be carried out in order to evaluate the addition of AEAV during cryopreservation and also after the thawing process.

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NOVELTY STATEMENT

Based on our knowledge, we believe that there are few or no studies that have examined the effect of adding an

aqueous extract of Aloe vera to Tris diluent on some characteristics of local epididymal sperm in goats for different cooling periods (0, 24, 48, 72, 96, and 120 hours.) at low concentrations of AEAV (2 and 3%).

AUTHOR'S CONTRIBUTIONS

All authors contributed equally.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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