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Research Article

Effect of adding different concentrations of chamomile (*Matricaria chamomilla L*) extract to preserve the quality of chilled Awassi ram semen

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Abstract
Chamomile extract has numerous compounds that act as antioxidants, enhancing semen characteristics during cooling for 72 h. The present study aimed to evaluate the effects of adding chamomile (*Matricaria chamomilla L*) extract to sperm extenders during cooling storage on semen quality parameters. Three rams were taken, semen was pooled and distributed into four parts and diluted with a Tris-based extender. Therefore, the control group was C0, without chamomile extract; the second group was C1 (0.30 mg chamomile extract); the third group was C2 (0.60 mg chamomile extract); and the fourth group was C3 (0.90 mg chamomile extract). Samples were stored at 4 °C for 72 h. The individual motility, live sperm, membrane integrity, total, and types of abnormalities (head, middle, and tail) of sperm were evaluated. Results showed that individual motility, live sperm, and sperm membrane integrity in the C1 group were significantly increased (P<0.05) throughout storage. The C2 group showed significant improvements (P<0.05) in individual motility and sperm membrane integrity for 72 h. Sperm abnormalities decreased significantly (P<0.05) in groups C1 and C2 at 48 and 72 h compared to the C0 and C3 groups. Abnormal tail sperm decreased significantly (P<0.05) in the C1, C2, and C3 groups compared to the C0 group at 72 h. The study indicated that adding 0.30 mg and then 0.60 mg of chamomile extract to semen extender enhances sperm motility, sperm membrane integrity, and live sperm, while also reducing total abnormal sperm and abnormal sperm (head, middle, and tail) compared to 0.90 mg.

Keywords: Antioxidants, Chamomile, Chilling, Extender, Ram semen

INTRODUCTION

Globally distributed, Matricaria chamomilla L. is a well-known medicinal herb (El Mihyaoui *et al*., 2022). Among a group of species belonging to the Asteraceae family (Bahmani *et al.,* 2015; Yang *et al.,* 2024). Roman chamomile, or Chamaemelum nobile L., and German chamomile, or Matricaria chamomilla L., are the two types of chamomile that are most frequently used and have medicinal uses (Sah *et al*., 2022; Dai *et al*., 2023).Therefore, Plant chamomile- content substances that have a positive effect in human health are thus used in the pharmaceutical industry (Abd Noor, 2020). Nowadays researchers use chamomile, which is the beneficial biological effect of agonist diseases in humans (Akram *et al*., 2024).

Moreover, German chamomile is unquestionably safe, high-quality, and free of harmful substances (Ahmed *et al*., 2022). Furthermore, previous studies indicated the effect of chamomile as an antimicrobial, antioxidant, and anti-inflammatory (Qasem *et al*., 2022; Sarma *et al*., 2023).Therefore, researchers were interested in performing an analysis of the phytochemicals of chamomile, which revealed that the plant contains more than 120 components (El Mihyaoui *et al*., 2022). Most bioactive substances acting as antioxidants were present in flowers, followed by the root, stem, and leaf (El Mihyaoui *et al*., 2021). Considering this, its pharmacological characteristics and active ingredients have been investigated for their significance in numerous therapeutic compositions (Chauhan *et al*., 2022).

Sperm are more sensitive to lipid peroxide during storage, which requires reducing their damage through the addition of antioxidants that act to scavenge free radicals (Akhter *et al*., 2023). Therefore, antioxidants play a role in decreasing sperm harm and improving semen quality during storage (Berean *et al*., 2024) and give a chance to successful artificial insemination and increased fertility in sheep (Chiselita *et al*., 2023; El Amiri and Rahim, 2024).

Nowadays, researchers tend to use natural antioxidants in an attempt to improve semen characteristics (Diab *et al*., 2021). Moreover, previous studies added plant extract to extenders to preserve sperm during short and/or long storage. Therefore, plants extracts such as Thyme (Vahedi *et al*., 2018) eggplant peel (Khodadadi *et al*., 2023) *Opuntia ficus-indica* (Allai *et al*., 2023) lemon, onion and garlic (Bassuony *et al*., 2023) black maca (Levano *et al*., 2023) green tea (Susilowati *et al*., 2022) *Moringa oleifera* leaves (Shokry *et al*.,2021) . These studies provided evidence that plant extracts added as extenders protected sperm during storage and improved semen qualities because these plants contain numerous components that function as antioxidants. Therefore, no study has been conducted to add different concentrations of chamomile extract to semen extenders in sheep. The aim of this study was to evaluate the effect of adding different concentrations of chamomile extract to semen extenders to preserve semen quality in Awassi rams during storage at 4°C.

MATERIALS AND METHODS

Location and animals

This study was carried out at an animal farm and laboratory belonging to the Department of Animal Resources, College of Agricultural Engineering Science / University of Baghdad, from November 15, 2023, to January 15, 2024. Three Awassi rams were used for this study. Their average age was 2–2.5 years. Rams were fed hay, a concentrated diet, and fresh drinking water.

Extraction of chamomile

The dried chamomile leaves (100 g) were purchased from the market in Baghdad and then ground into powder and macerated with 1 L of 96% ethanol in a foil-covered container to prevent the evaporation process for 72 h at room temperature. A rotary evaporator running at 50 °C and 45 RPM was used to evaporate the filtrate to make chamomile extract. After obtaining thick chamomile, it was placed over a clean dish for one week until dried, and then, at -20 °C, the extract was stored (Chan *et al*., 2007).

Preparation of extender

A basic control extender was prepared with the following components: Tris 2.42 g, citric acid 1.26 g, fructose 1 g, egg yolk 15 ml, gentamycin 0.5 mg/ml, and distilled water, resulting in a final volume of 100 ml (Al-Ameri, 2022).

Experimental design

Using an artificial vagina, a total of 24 semen samples from three rams were collected. The experiment involved the regular collection of ejaculates once a week and eight replications of this study. Semen was stored at 37 °C in a water bath. To eliminate individual differences, the ejaculates were combined. The semen sample was distributed to four parts and diluted with a Tris-based extender. Therefore, the control group was C0, without chamomile extract; the second group was C1 (0.30 mg chamomile extract); the third group was C2 (0.60 mg chamomile extract); and the fourth group was C3 (0.90 mg chamomile extract). Semen characteristics to be evaluated included the percentage of individual motility sperm at 37 °C under a 400x microscope (Soltanpour and Moghaddam, 2013; Al-Ameri, 2022). Sperm membrane integrity: Hypo-osmotic solution test HOST (0.735 g of sodium citrate and 1.351 g of fructose) dissolved in 100 mL of distilled water and 1 mL plus 0.1 mL of sperm were incubated for 45 min at 37 ° C. To determine the percentage of sperm membrane integrity, swollen sperm showed tail-curling and were determined to have intact plasma membranes (Jeyendran *et al*., 1984). To estimate the percentage of living and abnormal sperm using eosin-nigrosin staining. A microscope at 100x was used to counter the abnormal sperm (head, middle, and tail), and semen samples were kept in a refrigerator at 4 °C. at 0, 24, 48, and 72 h, parameters were assessed, including individual motility sperm, live sperm, total abnormal sperm, abnormal sperm (head, middle, and tail), and sperm membrane integrity.

Data analysis

The data were calculated as the mean ± S.E. One-way analysis of variance (ANOVA) was used to compare groups during the storage period, followed by the Duncan Multiple Range Test. The SPSS Statistics 24.0 (2016) program was used to analyze the data, with the significance level set at $P<0.05$.

Animal ethical approval

The research ethics committee at the College of Scientific Engineering Agriculture/University of Baghdad examined and approved the study protocol and accepted the document according to session number (8) on the date 10/3/2024.

RESULTS AND DISCUSSION

Percentage of sperm individual motility

The results on the effect of chamomile extract addition at several concentrations on the percentage of individual motility in Awaasi rams during storage are shown in Table 1. The C1 and C2 groups increased significantly ($P<0.05$) the percentage of individual motility throughout storage compared to the control group when using 0.30 mg and 0.60 mg of chamomile extract. Moreover, the C3 group showed a significantly improved ($P<0.05$) percentage of individual motility of 77.50±2.11 at 24 h

compared to the C0 group of 63.75±1.56. However, after 24 hours of cooling, there was no significant difference among groups simultaneously.

Chamomile extract has antioxidants that act to scavenge free radicals (El Mihyaoui *et al*., 2021). For this reason, different concentrations of chamomile extract were evaluated when extender semen was added during storage. The percentage of sperm individual motility in the C1 and C2 groups was preserved throughout storage compared to the C0 group. These findings concurred with when added antioxidant substances - such as vitamin E, L-arginine and luteolin to semen in sheep and goats (Soltanpour *et al*., 2014; Susilowat *et al*., 2019; Khozein *et al*., 2024) who found that low concentrations induce sperm metabolism, increasing individual motility. Additionally, volatile fatty acids and organic acids are among the chemicals found in chamomile (Chiș *et al*., 2019) phenolics, and flavonoids (Saeedi *et al*., 2024). Moreover, the antioxidant properties of these chamomile chemicals operate to scavenge or reduce the activity of free radicals (El Mihyaoui *et al*., 2021; El Mihyaoui *et al*., 2022; Lee *et al*., 2022). Antioxidant effects of chamomile- such as reducing oxidative stress, were shown in these studies, which resulted in improved progressive sperm motility during storage for the C1 and C2 groups as compared to the C0 group. Moreover, the motility of individual sperm was enhanced by these low concentrations. Furthermore, to support these results, it has been reported that the oxidation stress activity is late, beginning with antioxidants (Loizzo and Silva, 2021). On the other hand, in the present study, the C3 group showed similar results to the C1, C2 and C0 groups after 24 hours of cooling. Neila-Montero *et al*. (2024) reported that ram sperm have been conserved and seem to need time to adjust to the extender. Sohail *et al*. (2024) point out that when conserved at 4 ºC, sheep sperm are extremely vulnerable to oxidative and per-oxidative damage caused by free radicals, which lowers the semen quality. Free radicals are produced during metabolic processes, and antioxidants act to counteract them (Ifeanyi, 2018). However, the concentration level (0.90 mg) did not preserve sperm individual motility from oxidative stress activity after 24 h.

Live sperm

Table 2 shows the results of different concentrations of chamomile extract added to live sperm. The C1 group was significant (P<0.05) and preserved live sperm throughout storage compared to other groups. However, the C2 and C3 groups were non-significant with the C1 and C0 groups at 24h. On the other hand, after 24h, these C2 and C3 groups significantly (P<0.05) enhanced live sperm compared to the C0 group. After 48 h of cooling change occurred, the C3 group maintained live sperm while the C2 group non-significant between C1 and C3 groups. However, these C2 and C3 groups were significant (P<0.05) and maintained live sperm compared to the C0 group at 72h (Table 2).

In the present study, the percentage of live sperm in the C1 group revealed a concentration of 0.30 mg of chamomile extract, which was suitable for sustaining live sperm during storage. This helps to protect both the sperm and their constituents. Therefore, the current

Table 1. Impact of adding different chamomile extract concentrations of extender on the percentage of sperm individual motility of Awassi rams during different times (Mean± SE)

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	88.12±1.31 a	63.75±1.56 b	63.75±1.56 b	53.75±1.56 b
C1	85.00±1.88 a	78.75±2.45 a	72.50±3.13 a	63.75±3.62 a
C2	86.25±1.56 a	80.00±2.31 a	75.00±2.31 a	66.25±3.37 a
C3	86.25± 1.56 a	77.50±2.11 a	70.00±2.98 ab	60.00±3.53 ab

Means of each group within each column that have different small letters (a, b, c) differ significantly; - C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract.

Table 2. Impact of adding different chamomile extract concentrations of extender on the percentage of live sperm of Awassi rams during different times (Mean± SE)

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	84.31±0.52 a	80.87±0.66 b	74.67±0.58 c	67.53±1.47 c
C1	86.14±0.81 a	83.40±0.81 a	81.93±0.83 a	76.74±0.75 a
C2	85.31±0.65 a	81.89±0.37 ab	78.60±0.24 b	73.90±0.39 ab
C3	84.65±1.51 a	81.21±1.13 ab	78.42±1.23 b	72.20±1.34 b

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

study agreed with other studies (Soltanpour *et al*., 2014; Susilowati *et al*., 2019; Triyaningrum *et al*., 2024) that found that added antioxidant substances (vitamin E, L-arginine and melatonin) to semen extenders in sheep and goats may protect live sperm during storage time. In addition, Dias *et al*. (2022) observed that high concentrations of chamomile led to improved 39% live sperm indicating that chamomile has antioxidant potential to preserve viability from oxidative stress. These studies provide evidence that low concentrations of chamomile extract used in the present study are appropriate to improve live sperm during storage time.

Percentage of sperm membrane integrity

Furthermore, an improved percentage of sperm membrane integrity (P<0.05)was also noted in the C1 and C2 groups when adding 0.30 mg and 0.60 mg of chamomile extract to the extender compared to the C0 group during cooling 72h (Table 3). However, the C3 group enhanced significantly (P<0.05) after 24h (72.80±0.91) compared to the C0 group. On the other hand, the concentration of chamomile extract 0.90 mg supplemented to extender maintained sperm membrane integrity in the C3 group at 72 h and was smaller in the C1 and C2 groups compared to the C0 group (Table 3).

The concentrations of chamomile extract, on the other hand, play a role in improving sperm membrane integrity in the C1, C2, and C3 groups compared to the C0 group throughout storage. Khozein *et al*. (2024) found that decreasing lipid peroxidation by flavonoid promotes the protection of ram sperm and their ingredients. Moreover, Susilowati *et al.* (2019) pointed out that reduced malondialdehyde levels during storage time conserved goat sperm and their ingredients. It has been reported that there were ingredients, such as antioxidants, that protect sperm and preserve sperm against harmful factors during chilling storage (Jumintono *et al*., 2021).

Total percentage of sperm abnormality

In the present study, the C1, C2, and C3 groups recorded a lower (P<0.05)total percentage of sperm abnormality after 24 h (18.22±0.83; 20.04±0.33 and 20.24±0.96%, respectively) of the cooling period compared to the C0 group (24.18±1.02) (Table 4). Moreover, the total percentage of sperm abnormality decreased significantly (P<0.05) in the C1 and C2 groups (22.05±0.70 and 23.52±0.72%, respectively) and then in the C3 group compared to the C0 group (29.33±0.40 %) at 72 h of storage.

The present study indicated a decreased total percentage of sperm abnormality in the C1, C2 and C3 compared to the C0 throughout storage. These results align with the findings (Al-Ameri, 2023), which observed a decrease in sperm abnormality in the treatment groups compared to the control group in sheep. This decrease was attributed to the addition of substances to the extender, which helped maintain sperm and its components during storage. In contrast, Mahdy *et al*. (2024) noted that ram sperm abnormally increased gradually during storage time. However, Ros-Santaella and Pintus. (2021) provide evidence that the substances in the plant extracts are mostly appropriate to promote the conservation of livestock sperm and allow sperm and

Table 3. Impact of adding different chamomile extract concentrations of extender on the percentage of sperm membrane integrity of Awassi rams during different times (Mean± SE)

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	78.98±0.68 a	75.56±0.76 a	70.11±0.34 c	62.48±1.41 b
C1	80.98±1.02 a	77.87±1.29 a	75.82±0.76 a	70.45±1.29 a
C2	80.91±0.82 a	77.46±0.51 a	74.74±0.26 a	69.83±0.39 a
C3	80.34±1.19 a	75.75±1.03 a	72.80±0.91 b	66.93±1.30 a

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

Table 4. Impact of adding different chamomile extract concentrations of extender on the percentage of total sperm abnormality of Awassi rams during different times (Mean± SE)

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	13.52±0.32 a	18.50±0.84 a	24.18±1.02 a	29.33±0.40 a
C1	13.89±0.84 a	16.39±0.71 a	18.22±0.83 b	22.05±0.70 c
C2	13.11±0.26 a	17.02±0.08 a	20.04±0.33 b	23.52±0.72 c
C3	13.16±1.24 a	17.48±1.04 a	20.24±0.96 b	26.33±0.85 b

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

their ingredients to remain steady during storage time. According to the results of the present study, the C1 and C2 groups, followed by the C3 group, in comparison to the C0 group during storage, preserved individual motility, live sperm, sperm membrane integrity, and sperm abnormality at concentrations of chamomile extract added to extenders. Al-Snafi and Hasham (2023) reported that chamomile, including more substances such as phenols and flavonoids, has the potential for biological antioxidants. Moreover, Bisht *et al*. (2012) observed that the biological activity of chamomile included the main ingredients chamazulene and á-bisabolol oxides, which belong to essential oils and have antioxidant potential. In addition, Fırat *et al*. (2018) reported that these essential oil constituents could inhibit free radicals. On the other hand, the results of the current study indicated that the concentrations of chamomile extract protected sperm from reactive oxygen species (ROS) during storage time. However, Berakdar and Alahmad. (2022) pointed out that cells' metabolism creates ROS related to biomolecules (lipids, proteins, and DNA), harming cells. Furthermore, Thuwaini (2023) reported that cells' metabolism process creates free radicals that affect them if they reach abundance; therefore, antioxidants act by scavenging free radicals.

Percentage of abnormal head sperm

Table 5 illustrates that the percentage of abnormal head sperm was higher and significant (P<0.05). The C0 group increased the percentage of abnormal head sperm throughout the storage period. However, the C2 group resulted in mostly constant storage time. Furthermore, the C1 and C3 groups (8.30±0.47 and 9.63±0.54%, respectively) were non-significant compared to the C2 and C0 groups (7.87±0.42 and 10.19±0.99%) at 24 h of cooling. However, the percentage of abnormal head sperm decreased (P<0.05) in the C1 group (9.15±0.49%) compared to other groups at 48 h of cooling. It was similar to the C2 group at 72 h, while the C3 group was non-significant, with the C0 group at 72 h of cooling.

Percentage of abnormal middle sperm

The C2 group exhibited a lesser (P<0.05) percentage of abnormal middle sperm (0.36±0.07%) at 48 h compared to other groups (Table 6). However, the C1 and C2 groups showed non-significant differences (0.82±0.17 and 0.82±0.19%, respectively) and were similar among the C0 and C3 groups at 72 h.

Percentage of abnormal tail sperm

When adding different concentrations of chamomile extract to the extender, there was a significant decrease (P< 0.05) in the abnormal tail sperm in the C1, C2, and C3 groups (10.29±0.45, 10.92±0.35, and 11.05±0.23%, respectively) compared to the C0 group (13.05±0.60%) at 72 h of cooling (Table 7).

In the present study, when different concentrations of chamomile extract were added to extenders, a decrease in abnormal head, middle, and tail sperm was observed compared to that of the control group during storage time. Therefore, the substances in chamomile act as antioxidants, protecting sperm and its ingredients during storage. However, these results agreed with Azawi *et al*. (1993) who found that the type of ex-

Table 5. Impact of adding different chamomile extract concentrations of extender on the percentage of abnormal head sperm of Awassi rams during different times (Mean± SE .

Groups	Storage period (h)			
	0h	**24h**	**48h**	**72h**
C0	8.40±0.88 a	10.19±0.99 a	13.02±1.05 a	15.83±0.69 a
C1	6.70±0.45 ab	8.30±0.47 ab	9.15±0.49 c	10.93±0.31 b
C2	5.96±0.26 b	7.87±0.42 b	10.02±0.49 bc	11.78±0.73 b
C3	7.93±0.77 a	9.63±0.54 ab	11.23±0.43 ab	14.30±0.75 a

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

Table 6. Impact of adding different chamomile extract concentrations of extender on the percentage of abnormal middle sperm of Awassi rams during different times (Mean± SE)

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	0.62±0.22 a	0.95±0.31 a	1.16±0.16 a	0.44±0.12 b
C1	0.83±0.22 a	0.86±0.26 a	0.97±0.24 a	0.82±0.17 ab
C2	1.02±0.18 a	0.48±0.18 a	0.36±0.07 b	0.82±0.19 ab
C3	0.49±0.16 a	0.90±0.19 a	0.93±0.11 a	0.98±0.09 a

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

Table 7. Impact of adding different chamomile extract concentrations of extender on the percentage of abnormal tail sperm of Awassi rams during different times (Mean± SE).

Groups	Storage period (h)			
	0h	24h	48h	72h
C0	4.50±0.77 a	7.35±0.94 a	9.99±0.78 a	13.05±0.60 a
C1	6.35±0.78 a	7.22±0.70 a	10.60±2.13 a	10.29±0.45 b
C2	6.12±0.23 a	8.66±0.43 a	9.66±0.24 a	10.92±0.35 b
C3	4.73±0.63 a	6.94±0.49 a	8.20±0.57 a	11.05±0.23 b

Means of each group within each column that have different small letters (a, b, c) differ significantly; C0= control; C1= 0.30 mg chamomile extract; C2= 0.60 mg chamomile extract; C3= 0.90 mg chamomile extract

tender might preserve sperm and their ingredients during storage time at 4 ºC. Moreover, Al-Ameri. (2023) indicated that substances added to extenders acted as antioxidant to protect sperm and their ingredients during storage time. In contrast, the results in the present study disagreed with those of previous studies, Gundogan. (2009) reported that the abnormal sperm progressively increased throughout the time of storage in ram semen when diluted with Tris extender. Al-Subaihawi *et al*., (2017); Al-Subaihawi and Al-Saab. (2017) point out that increased abnormalities in sperm when added LDL lyophilized to the ram semen extender during storage time might be due to cold shock.

Conclusion

The present study revealed evidence that chamomile extract enhanced semen quality in Awaasi rams during 72 h. Moreover, the best concentrations of chamomile extract, when added in low concentrations (0.30 mg and then 0.60 mg) to semen extender were that it improved sperm motility, sperm membrane integrity, and live sperm and reduced total abnormal sperm and abnormal sperm (head, middle, and tail) compared to high concentration (0.90 mg). The next step requires using low concentrations of chamomile extract to understand the effect of freezing and thawing.

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Conflict of interest

The authors declare that they have no conflict of interest.

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