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Original Paper

Effect of Different Concentrations of White Tea Extract on the Quality of Awassi Ram Semen on Chilled Storage

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The white tea extract (WTE) has compounds that act as antioxidants, which enhance sperm characteristics during storage. The present study was aimed at investigating the effect of adding different concentrations of WTE stored at 4 °C for 24, 48 and 72 h on semen quality. Semen samples were collected from three Awassi rams, and the ejaculates were combined. Semen were divided into four equal parts, and it was diluted with Tris; beside the extender, the control group was (A0, without WTE), the second group A1 (0.05 mg WTE), the third group was A2 (0.10 mg WTE), and the fourth group was A3 (0.15 mg WTE). Results revealed the percentages of individual motility, live, and sperm membrane integrity were enhanced significantly (P <0.05) throughout storage in the A2 group compared to other groups. The A1, A2 and A3 groups decreased significantly (P <0.05) in total sperm abnormality percent at 48 and 72 h. Abnormal head sperm decreased significantly (P <0.05) at 24, 48 and 72 h in the A2 and A3 groups. While the A2 group decreased significantly (P <0.05) in abnormal middle sperm throughout storage. In conclusion, WTE has compounds in it that function as antioxidants. Especially when 0.10 mg of WTE was added to extenders, there was an improvement in individual motility, live sperm, and sperm membrane integrity, as well as a decrease in total sperm abnormality and types of abnormal sperm (head, middle, and tail).

Keywords: white tea, antioxidants, semen, extender, chilling

1 Introduction

Plant teas (*Camellia sinensis*) arise in china for millions of years, so they were famously drunk with different types due to health benefits. Therefore, people in other countries began to recognize it sweet flavor, and tea drinking became widespread throughout the world (Pan et al., 2022). Over the decades, factories began to innovate other ways to export tea, using not only tea leaves, but also infusions and chilled tea, in an attempt to promote their products to consumers for their health benefits (Czarniecka-Skubina et al., 2022).

Generally, the process of harvesting leaves of tea that mature and then takes place in several stages, while, white tea plucked shooters leaves or leaves immature and leaf buds and which just require drying (Durnova et al., 2021) and it does not require fermentation process (Saha et al., 2017). Furthermore, Tea white contained polyphenolic compounds that act as antioxidants, principally catechin derivatives, which play an important role in scavenging free radicals and prohibiting oxidative stress (Somavanshi et al., 2021). Therefore, polyphenol compounds found in tea have attracted the attention of researchers in the medical and biological fields, especially in the field of extraction and purification, due to they play an essential role in decreeing oxidative stress (Qi et al., 2023) which encouraged the treatment of human diseases (Dias et al., 2013; Hinojosa-Nogueira et al., 2021).

Recently, researchers were attracted to interest in the antioxidants in different sources to attempt improvement semen characteristics (Vahedi et al., 2018; Al-Ameri, 2023; Sengul et al., 2024) to overcome challenges affecting semen quality for fruitful artificial insemination (Allai et al., 2023). On the other hand, they used natural antioxidants in different types of teas for enhancing semen quality. Green tea extract (GTE) which it contented polyphenols compound to enhance semen

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parameters during cryopreservation to avoid Reactive oxygen species (ROS) resulting from cellular oxidation due to sperm were especially vulnerable to them (Rahman et al., 2018). Previous studies used different concentrations of GTE add to extender semen in human (Alqawasmeh et al., 2021) bull (Pramesti and Ducha, 2023) ram (Mehdipour et al., 2016) buck (Susilowati et al., 2022). Therefore, results revealed when used low concentrations of GTE add to semen and then the process of cryopreservation, which antioxidants beneficial showed inhibiting ROS and it reduced harmful sperm from oxidative stress as result enhancing semen quality. On the other hand, the phytochemical screening affected the processing of five types of tea, which proves that changes may occur in the compounds of tea (Wong et al., 2022).

Therefore, there was no study conducted to add different concentrations of WTE to semen extenders and investigate the antioxidant potential of WTE to preserve semen quality in Awassi rams for 72 h.

2 Materials and Methods

2.1 Location of Study

The study was carried out at Animal Farm and laboratory owned by Department of Animal Resources, College Agricultural Engineering Science/University of Baghdad, during the period from 10th September to 10th November 2023. Three Awassi rams from farm animals were selected for this study. Their average was age 2–2.5 years. Rams were fed hay, a concentrated diet, and fresh drinking water.

2.2 Extraction of White Tea

The dried white tea leaves (50 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 white tea extract. After obtaining thick white tea, place it over a clean dish for one week until dried, and then, at -20 °C, the extract is stored (Chan et al., 2007).

2.3 Preparation of extender

A basic control extender Tris 2.42 g, citric acid 1.26 g, fructose 1 g, egg yolk 15 ml and gentamycin 0.5 mg·ml^{-1} and distilled water to make the volume 100 ml (Al-Ameri, 2022).

2.4 Experimental design

Ejaculates collected once a week via an artificial vagina were combined to remove any variation in the assessed samples and then kept in a water bath at 37 °C. The replicate of this study eight times. The semen sample was distributed to four parts and diluted 1 : 10 fold with a Tris-based extender. Therefore, the control group (A0, without WTE), the second group is A1 (0.05 mg WTE), the third group is A2 (0.10 mg WTE) and the fourth group is A3 (0.15 mg WTE). Assessment of sperm quality included using a scale from 0 (no motility) to 100 (highest: strong motions) to estimate the percentage of individual motile sperm at 37 °C under a 400x light microscope (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). Eosin-nigrosin staining was used to determine the percentage of living and abnormal sperm. A sperm cell was deemed alive if it appeared uncolored and dead if it stained only partially or entirely. The abnormal sperm were counted under a light microscope at 100x, including the head, midpiece and tail. Semen samples were stored in a refrigerator at 4 °C. At 0, 24, 48, and 72 h, parameters were evaluated.

2.5 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed, and the data were displayed as the mean ±S.E. For comparisons between groups during the storage period, afterwards by the Duncan Multiple Range Test. The SPSS Statistics 24.0 (2016) Applications with a P <0.05 level of significance were used for analyzing the data.

3 Results and Discussion

The result of WTE on the percentages of individual motility sperm in groups were presented in Table 1. The results indicated that individual motility sperm was 74.37 ±1.99 and 63.75 ±2.45 at 48 h and 72 h and was significantly (P <0.05) improved by adding 0.1 mg of group A2 WTE extract to diluted semen compared to groups. A significant improvement (P <0.05) was observed at 48 h of cooling in group A1 and a similar result was observed with group A2. The result decreased 59.37 ±3.19% in group A1 and was similar with group A3 (56.87 ±2.66) at 72 h. However, groups A1 and A3 were non-significant compared to groups A2 and A0 at 72 h (Table 1).

The enhancement of individual sperm motility when adding (0.1 mg) of WTE in A2 group to preserve throughout of storage time in the present study supported the opinion that WTE had antioxidants

Table 1 Impact of adding different WTE concentrations of extender on the percentage of sperm individual motility of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	85.62 ±2.20 a	78.12 ±2.30 a	64.37 ±1.75 b	53.75 ±2.05 b
A1	87.50 ±1.63 a	81.87 ±1.87 a	71.87 ±2.66 a	59.37 ±3.19 ab
A2	90.00 ±1.33 a	83.12 ±1.31 a	74.37 ±1.99 a	63.75 ±2.45 a
A3	86.87 ±1.31 a	80.00 ±1.63 a	70.00 ±1.63 ab	56.87 ±2.66 ab

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

Table 2 Impact of adding different WTE concentrations of extender on the percentage of live sperm of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	83.10 ±0.85 ab	78.23 ±1.71 b	69.98 ±2.68 b	61.89 ±1.60 b
A1	82.51 ±1.19 b	79.51 ±0.90 ab	74.42 ±1.23 ab	63.59 ±3.23 b
A2	86.17 ±1.46 a	82.47 ±1.48 a	75.68 ±0.59 a	70.93 ±1.16 a
A3	84.41 ±0.59 ab	81.20 ±0.68 ab	75.66 ±0.92 a	67.50 ±1.95 ab

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

to scavengers' free radical control (Bernatoniene and Kopustinskiene, 2018). Nevertheless, at 48 h of cooling, the A2 and A1 groups revealed similar results with the lower concentrations (0.05 and 0.10 mg) of WTE added to the extender. This led to a preservation of individual sperm motility for 48 h as compared to the A3 group throughout storage. However, Setumo et al. (2023) provide evidence that the other tea types (*Camellia sinensis*) that based on the levels of oxidation and had antioxidant qualities that could improve the condition of the reproductive. Conversely, reduce the motility of individual sperm in the A1 and A3 groups at 72 h of chilling, which was similar to the A0 group at the same time. Liman et al. (2022) reported that the creation of reactive oxygen species (ROS) from injured sperm be able to impair sperm motility and their capacity to fertilize an oocyte. In addition, Ros-Santaella and Pintus (2021) point out that oxidative damage to proteins, lipids, and nucleic acids, the ability of sperm to fertilize was dramatically reduced by oxidative stress, which frequently increases throughout storage. In addition, Silvestre et al. (2021) who found that reduced sperm quality indicators were the result of the negative balance between antioxidants and ROS during storage periods.

The impact of WTE addition on the live sperm during the storage period was illustrated in Table 2. The results indicated that group A2's addition of 0.10 mg of WTE increased the maintenance live sperm storage duration, and that this difference was significant (P <0.05) when contrast to the other groups. At 48 h, live sperm in group A3 had significantly (P <0.05) improved, and it was similar with group A2 at the same time. Groups A1 and A3 had variance with respect to time of cooling, which was non-significant between groups A1 and A0 at 24 h, while group A1 was between groups A1 and A0. However, groups A1 and A0 were similar at 72 h, while group A3 was non-significant.

When contrasting group A2 with the other groups throughout the preservation period, the percentage of sperm membrane integrity was significantly (P <0.05) higher in group A2. (Table 3). When adding 0.15 mg of WTE, the difference was significant (P <0.05) sperm membrane integrity improved 77.59 ±0.65 at 24 h and 72.13 ±1.25 at 48 h of cooling, while group A1 71.05 ±1.38 was significant at 48 h. However, the percentage of sperm membrane integrity was significantly (P <0.05) improved in groups A2, A1, and A3 compared to group A0 at 48 h. In comparison, groups A1 and A3 were non-significant compared to groups A2 and A0 at 72 h (Table 3).

The percentage of live sperm and sperm membrane integrity in A2 group that indicate provide evidence that the concentration (0.10) mg of WTE which was best to maintain the sperm and their ingredients compared to other groups. Bresm and Habeeb. (2023) pointed

Table 3 Impact of adding different WTE concentrations of extender on the percentage of sperm membrane integrity of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	77.93 ±1.47 b	72.98 ±2.00 b	62.56 ±3.47 b	55.44 ±3.35 b
A1	78.85 ±1.08 ab	75.20 ±0.67 ab	71.05 ±1.38 a	59.47 ±3.48 ab
A2	82.48 ±1.66 a	78.21 ±1.85 a	71.13 ±1.24 a	66.37 ±1.78 a
A3	79.49 ±0.65 ab	77.59 ±0.65 a	72.13 ±1.25 a	63.05 ±2.14 ab

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

Table 4 Impact of adding different WTE concentrations of extender on total sperm abnormality percentage of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	12.17 ±1.46 a	19.52 ±1.73 a	25.41 ±1.29 a	34.89 ±2.26 a
A1	12.85 ±0.51 a	16.47 ±0.65 a	20.55 ±1.92 b	26.09 ±1.74 b
A2	11.71 ±1.16 a	15.86 ±1.84 a	21.22 ±0.67 b	26.24 ±0.83 b
A3	11.99 ±1.05 a	15.14 ±1.27 a	19.44 ±0.75 b	26.64 ±0.83 b

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

out that compounds or substances that function as antioxidants are important because they enable the sperm to be stored. It has been reported that (Jumintono et al., 2021) the benefits of antioxidants, which help preserve semen when used in extenders at the ideal concentrations to lessen factors that could harm semen while it's being stored. On the other hand, the concentrations of WTE play a role to conserve live sperm in A3 group at 48h, and it was significant effect on sperm membrane integrity in A1 and A3 groups at the same time. These findings revealed that WTE has the ability to preserve sperm and their constituents after 48 h of chilling due to a balance between oxidative stress and antioxidant-containing WTE. Almansa-Ordonez et al. (2020) indicate that the adaptive benefit of preserving low ROS levels to avoid harm to cells and apoptosis was proposed as the explanation for the minimized metabolic rates. In addition, significant amounts of biochemical components found in white tea, particularly tannins, catcehins, total polyphenols, and flavonoids, have antioxidant properties (Saha et al., 2017). It has been reported that (Mueed et al., 2023) polyphenols have abundant to function stability, bioavailability, and bioactivity to protect cell from oxidative. However, the percentage of live sperm and sperm membrane integrity in the A1 and A3 groups was non-significant with the A0 group after 48 h, and it was reduced contrast with the A2 group. Results in the A2 group provide evidence that the appropriate WTE concentration (0.05 mg) could enhance live sperm and sperm membrane integrity throughout the period of storage.

In the current study, the impact of different concentrations of WTE on the percentage of total sperm abnormalities is shown in Table 4. The results indicate a significant decrease in total sperm abnormalities (P <0.05) in groups A1, A2, and A3 contrasted to group A0 at 48 h and 72 h of cooling in Table 4.

In this study, the results of total sperm abnormalities showed a significant reduction in the A1, A2, and A3 groups compared to the A0 group after 24 h of storage. These results agreed with the finding Al-Amery (2023) who found decreased total sperm abnormalities when used difference concentrations of honey. On the other hand, the increase in total sperm abnormalities coincides with decreased in the percentage of individual motility and plasma membrane integrity, which agrees with current results. Furthermore, difference of concentrations of WTE when added, to extender were in role important to preserve sperm and their ingredients throughout storage. However, Al-Ameri (2023) reported that sperm abnormalities increased gradually with aspect storage time. However, Wang et al. (2024) point out that some factors cause harm brought on by oxidative, osmotic, and sperm metabolic decreases. In contrast, the mechanism of sperm protection from abnormalities during storage may describe difference of concentrations of WTE when

Table 5 Impact of adding different WTE concentrations of extender on the percentage of abnormal head sperm of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	6.44 ±0.98 a	9.02 ±0.62 a	11.82 ±0.51 a	14.99 ±1.03 a
A1	5.89 ±0.54 a	7.48 ±0.47 ab	9.40 ±0.64 b	11.94 ±1.05 b
A2	5.16 ±0.52 a	7.17 ±0.77 b	9.05 ±0.41 b	11.61 ±0.64 b
A3	5.15 ±0.46 a	6.26 ±0.49 b	8.40 ±0.28 b	11.81 ±0.74 b

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

Table 6 Impact of adding different WTE concentrations of extender on the percentage of abnormal middle sperm of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	0.98 ±0.18 a	0.97 ±0.17 a	1.14 ±0.15 a	1.00 ±0.21 a
A1	0.44 ±0.22 ab	0.62 ±0.15 ab	0.43 ±0.21 b	0.74 ±0.23 a
A2	0.30 ±0.12 b	0.38 ±0.12 b	0.66 ±0.19 ab	0.96 ±0.20 a
A3	0.54 ±0.16 ab	0.93 ±0.21 a	0.50 ±0.19 b	0.93 ±0.21 a

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

Table 7 Impact of adding different WTE concentrations of extender on the percentage of abnormal tail sperm of Awassi rams during different times (mean ±SE)

Groups	Storage period (h)			
	0 h	24 h	48 h	72 h
A0	4.74 ±0.77 a	9.51 ±1.28 a	12.43 ±0.80 a	18.89 ±1.96 a
A1	6.51 ±0.68 a	8.36 ±0.74 a	10.84 ±1.37 a	13.41 ±1.17 b
A2	6.25 ±0.83 a	8.30 ±1.07 a	11.50 ±0.66 a	13.28 ±0.53 b
A3	6.30 ±0.83 a	7.94 ±0.67 a	10.54 ±0.55 a	13.89 ±0.68 b

For every column containing different small letters (a, b, c), there is a significant difference in the means between the groups; A0 – control; A1 – WTE 0.05 mg; A2 – WTE 0.10 mg; A3 – WTE 0.15 mg

added, to extender which in role important to preserve sperm and their ingredients from oxidative stress compare to control throughout storage.

When compared to group A0, WTE addition to the extender led to significant (P <0.05) changes in the time to the abnormal head sperm of cooling, which reduced group treatments (Table 5). The results showed variables on the percentage of middle sperm abnormalities during the storage time (Table 6). The percentage of middle sperm abnormalities was significantly (P <0.05) decreased in group A2 (0.30 ±0 12) and (0.38 ±0.12) at 0 h and 24 h, respectively. While groups A1 and A3 decreased at 48 h. Meanwhile, the percentage of middle sperm abnormalities was non-significant at 72 h in all groups.

In the current study, the percentage of tail sperm abnormalities when using different concentrations of WTE showed a significant (P <0.05) decrease in groups A1, A2, and A3 compared to group A0. Although, the percentage of tail sperm abnormalities was non-significant with group A0 until 48 h.

In the current study, the difference in concentrations of WTE added to extenders indicated a decrease in abnormal head, middle piece, and tail sperm compared to control. Therefore, compounds in WTE serve as antioxidants to safeguard sperm and their ingredients they contain while being stored. These findings, however, supported those of Azawi et al. (1993), who found that sperm and its constituents might be preserved by a certain kind of extender during 4 °C storage. Furthermore, Al-Ameri (2023) reported that compounds

added to extenders served as antioxidants, protecting sperm and their constituent parts during storage. On the other hand, these current results were contrary to previous studies (Gundogan, 2009; Al-Subaihawi et al., 2017 and Mirajuddin et al., 2021) that found that cold shock causes a progressive increase in abnormal head, middle piece, and tail sperm.

4 Conclusion

The current study provided strong evidence that WTE has compounds in it that function as antioxidants, especially when 0.10 mg of WTE was added to extenders. Improvements in individual motility, live sperm, and sperm membrane integrity were shown. Moreover, WTE preserves sperm by decreasing total sperm abnormality and types of abnormal sperm (head, middle, and tail).

Authors contribution

The study concept, design, and acquisition of data in the laboratory: Maad Hasani Al-Ameri. Drafting of the manuscript and critical revision of the manuscript for intellectual content, statistical analysis, technical and material support: Maad Hasani Al-Ameri.

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