

ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2026): 4.69
IJAN 2026; 8(10): 32-35
www.agriculturejournal.net
Received: 13-07-2026
Accepted: 01-09-2026
Published: 13-10-2026

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Effect of adding oat seed aqueous extract in Tri's diluent on some chilled semen characteristics of Awassi rams

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DOI: https://www.doi.org/10.33545/26646064.2026.v8.i10a.766

Abstract
The objective is to determine the impact of various concentrations of oat seed aqueous extract (*Avena sativa*) with Tris on subsequent cold storage properties of spermatozoa from four Awassi ram breeds. The semen collection was done at a rate of one ejaculation per week from each ram. The experiment was conducted in four treatments. T_1 was the control; T_2 was Tris diluted with 1% oat seed aqueous extract added; T_3 was Tris diluted with 2% oat seed aqueous extract added; T_4 was Tris diluted with 3% oat seed aqueous extract added. Based on the results, it can be observed that the T_2 treatment significantly induced the highest percentage of individual movement compared to the other treatments. This occurred after 48 and 72 hours of cooling. The results also showed that T_2 led to a significant increase ($P \leq 0.05$) in the integrity percentage of acrosomes and plasma membranes of sperm compared to other treatments after cooling for 48 and 72 hours, while the results showed a significant decrease ($P \leq 0.05$) in the percentage of total abnormalities in T_1 compared to other treatments. Based on the findings of this study, it was determined that the utilization of 1% aqueous extract of oat seeds resulted in an enhancement of certain indicators of semen in cooling Awassi rams, including individual movement, acrosome integrity, and plasma membrane integrity of sperm, as well as a reduction in the percentage of total sperm abnormalities.

Keywords: Awassi rams, Oat seed, Tri's diluent, Semen

Introduction

Enhancing the reproductive efficiency of artificial insemination programs for livestock herds and widening the use of sires with different genetic traits is one of the main priorities of breeders worldwide, as is achieving significant progress in the reproductive performance of females (Mallory *et al.*, 2010; Ayantoye *et al.*, 2025) [1, 2] and achieving an increase in female fertility (Banana *et al.*, 2021) [3], but the greatest increase here depends on the performance of reproductive management of sires (Petrunkina *et al.*, 2011) [4]. The field of agricultural research has achieved great success with the need for cryopreservation of reproductive cells and tissues. The quality of semen utilized in the studies was based on preparing a suitable diluent and the amount of added materials (El-Harairy *et al.*, 2016) [5]. The dilution of sperm with antioxidants and adding them to diluents of agricultural animals during cooling and freezing protects the sperm from the harmful effects of increased level (ROS-Reactive oxygen species) and improves sperm motility, vitality, and fertility after cooling and freezing (Eidan, 2016) [6]. Use of antioxidants from natural sources of plants that produce more than 4000 polyphenolic compounds and phenolic compounds that are good antioxidant sources (Al-Mufarji, 2018) [7] has been on the rise recently. Due to their high nutritional value, oats are considered as one of the major feedstuffs in humans and animals (Riely, 2003) [8]. Oats contain the good content of polyphenolic compounds that are antioxidant by scavenging reactive nitrogen species and oxygen species (Alemayehu *et al.*, 2023) [9]. Consequently, this research was designed to determine the effect of enriching diluent Tri with oat seeds on ew semen traits using the Awassi ram (5 °C) and the storage period (48 and 72 hours).

Materials & methods

Sample collection

Four Awassi rams were sampled on a weekly basis and one ram twice a week for semen. To reduce the variation between the rams, semen from four rams was pooled together to make the semen sample for each replicate. The tests of the semen evaluate and then diluted using Tri's diluent which was prepared in advance according using (Salamon *et al.*, 2000) [10] method. The experiment was conducted in four treatments, the first treatment (T_1) was without addition, the second treatment (T_2) was with addition of 1% of oat seed aqueous extract, the third treatment (T_3) was with addition of 2% of oat seed aqueous extract and the fourth treatment

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(T_4) was with addition of 3% of oat seed aqueous extract. The analyses tests were done after 48 and 72 hours of cooling at 5 °C and the following traits studied.

Motility individual percentage

The motility of individual sperm was estimated using (Chen *et al.*, 2007) [11].

The percentage of sperm plasma membrane integrity

The integrity of the sperm plasma membrane was assessed using the swelling assay under reduced osmotic pressure (Jeyendran *et al.*, 1984) [12].

The percentage of sperm integrity acrosome

The percentage of integrity acrosome was estimated, and the sperm of abnormal sperm was estimated based on the method of (Hancock) [13].

Statistical analysis

The effect of different treatments on the studied traits was analyzed using a statistical analysis program (SAS, 2018) [14] in accordance with a completely randomized design (CRD), with the mean separation of the significant differences being performed using the Duncan multiple range test [15].

Results & discussion

The results of Table 1 showed significant differences in the individual motility trait, where treatment T_2 was excelled when adding 1% of oat seed aqueous extract to Tri's diluent after cooling for 48 hours, as it recorded (49.00 ± 1.00%) compared to treatments (T_1, T_3 and T_4) as it recorded (43.00 ± 1.22, 44.00 ± 1.00, and 38.00 ± 1.22) respectively. The results also showed the superiority of treatment T_2 in the individual motility trait after cooling for 72 hours, as it recorded (48.00 ± 1.22) compared to treatments (T_1, T_3, and T_4), as it recorded (38.00 ± 1.22, 44.00 ± 1.00, and 33.00 ± 1.22) respectively. As a result of the addition of oat seed aqueous extract in treatment T_2, it is highly possible that a significant increase in individual motility occurred. The occurrence of this is because of the content of oats of active compounds that have antioxidant properties (Agarwal *et al.*, 2021; Khdhr *et al.*, 2024) [16, 17]. The antioxidant activity of flavonoids that are present in many plants has been shown in a number of previous studies. In the present study, a number of these compounds were investigated for their abilities to attack free radicals derived from reactive oxygen species (Chen *et al.*, 2007) [11]. (a) The aqueous extract of oat seeds is an antioxidant which effectively mops up free radicals and reduces any damage to sperm when these free radicals attack its plasma membrane, which in turn leads to decreased sperm motility and reduced fertilizing ability (Majeed *et al.*, 2024) [18].

Table 1. Impact of multiple oat seed extract aqueous incorporation levels on the proportion of individual sperm motility following cooling for 48 hours and after 72 hours

Treatments	Mean ± Standard Error %	
	After 72 hours of cooling	After 48 hours of cooling
T_1	43.00 ± 1.22^b	38.00 ± 1.22^c
T_2	49.00 ± 1.00^a	48.00 ± 1.22^a
T_3	44.00 ± 1.00^b	44.00 ± 1.00^b
T_4	38.00 ± 1.22^c	33.00 ± 1.22^d
Level of Significance	*	*

T_1 = control treatment with *Tris dilutes* without antioxidant addition, T_2 = Tris dilute + 1% oat seed extract, T_3 = Tris dilute + 2% oat seed extract, T_4 = Tris dilute + 3% oat seed extract. Significant differences exist between means with the same letters within the same column. * (Taking values less than or equal to 0.05 results in a P value from NULL to 0.05.)

Table 2 revealed the performance of treatments in percentage plasma membrane of sperm integrity, where the treatment T_2 by adding 1% oat seed aqueous extract after 48 hours cooling showed significant difference as it recorded (79.00 ± 1.00) whereas the T_1, T_3 and T_4 treatments recorded (77.00 ± 1.22, 78.00 ± 1.00 and 77.00 ± 1.22) respectively. The results also revealed that the percentage of sperm plasma membrane integrity was superior in T_2 (79.00 ± 1.22) than in T_3, T_4 and T_1 (77.00 ± 1.00, 76.00 ± 1.22 and 75.00 ± 1.55%) respectively after cooling for 72 hours. This higher percentage of integrity of the sperm plasma membrane in treatment T_2 could be explained by the use of the oat seed aqueous extract. The lipids are large molecule, easy to be peroxidated, and present in the sperm plasma membrane as polyunsaturated fatty acids (PUFA) of which the number of carbon-carbon double bonds is > 2. The components of the sperm plasma membrane that contribute to membrane flexibility are the fatty acids (PUFA) (Leahy *et al.*, 2011; Essa *et al.*, 2025) [19, 20]. Unsaturated fatty acids (PUFA) are known to trigger a chain of Chemical Reactions, termed as lipid peroxidation, which ultimately leads to a defect in the membrane mechanics resulting in a reduction of membrane enzymes and channel ions activity and consequently a loss of membrane elasticity, which further hinders the membrane mechanics required for the process of fertilization (Agarwal *et al.*, 2006) [21]. Lipid peroxidation (LPO) occurs in sperm which is self-replicating and is stopped by antioxidants: antioxidants in oats would work to reduce and minimise the impact of harmful free radicals (Li *et al.*, 2017) [22].

Table 2. shows the impact of various quantities of oat seed aqueous extract on the percentage of integrity of the plasma membrane of the sperm cooled for 48 hours and 72 hours.

Treatments	Mean ± Standard Error %	
	After 72 hours of cooling	After 48 hours of cooling
T_1	75.00 ± 1.55^d	77.00 ± 1.22^b
T_2	79.00 ± 1.22^a	79.00 ± 1.00^a
T_3	77.00 ± 1.00^b	78.00 ± 1.00^b
T_4	76.00 ± 1.22^c	77.00 ± 1.22^b
Level of Significance	*	*

T_1 = control treatment with *Tris dilutes* without antioxidant addition, T_2 = Tris dilute + 1% oat seed extract, T_3 = Tris dilute + 2% oat seed extract, T_4 = Tris dilute + 3% oat seed extract. Means with letters different within the same column are significantly different from each other. * ($P \leq 0.05$).

The results of Table 3 showed significant differences in the percentage sperm of acrosome integrity, as treatment T_2 was excelled when adding 1% of oat seed aqueous extract to Tri's diluent after 48 hours of cooling, as it recorded (89.00 ± 1.20%) compared to treatments (T_3, T_4 and T_1), as it recorded (88.00 ± 1.10, 87.00 ± 1.20, and 86.00 ± 1.19%) respectively. The results also showed superiority of treatment T_2 in the percentage of sperm acrosome integrity after 72 hours of cooling, as it recorded (89.00 ± 1.30%) compared to treatments (T_1, T_3, and T_4), as it recorded (87.00 ± 1.15, 87.00 ± 1.05, and 86.00 ± 1.11%) respectively. The significant increase in the percentage of acrosome integrity of sperm in treatment T_2 may be attributed to the addition of oat seed aqueous extract. The result of the current study can be interpreted that the integrity of the acrosome is related to the integrity of the plasma membrane sperm, as the sperm plasma membrane in turn protects the healthy cells in the acrosome vesicle at the tip in the head so that the acrosome vesicle remains intact and increases the protection of the acrosome protective cover (Rizal *et al.*, 2003; Al-ani *et al.*, 2023) [23, 24]. Oat extract acts as an antioxidant by activating the role of glutathione, a strong antioxidant in seminal

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plasma, which works to reduce the damage of oxidative factors and improve the qualities of semen (Rushworth *et al.*, 2013) [25].

The results of Table 3 indicated that there was significant difference between the percentage of sperm with intact acrosome after 48 hours of cooling in treatment T_2 (89.00 ± 1.20) as it excelled over treatments (T_3, T_4 and T_1) with (88.00 ± 1.10, 87.00 ± 1.20, and 86.00 ± 1.19) respectively. It was also observed that the percentage of sperm acrosome integrity after 72 hours of cooling indicated that the treatment T_2 recorded the higher percentage (89.00 ± 1.30%) compared to T_1, T_3 and T_4 (87.00 ± 1.15, 87.00 ± 1.05 and 86.00 ± 1.11%) respectively. The percentage of the sperm that were acrosome intact in treatment T_2 can be concluded due to the addition of oat seed aqueous extract. The result of the current study can be interpreted that the integrity of the acrosome is related to the integrity of the plasma membrane sperm, as the sperm plasma membrane in turn protects the healthy cells in the acrosome vesicle at the tip in the head so that the acrosome vesicle remains intact and increases the protection of the acrosome protective cover (Rizal *et al.*, 2003; Al-ani *et al.*, 2023) [23, 24]. It has antioxidant activity as it increases the activity of glutathione, a potent antioxidant in seminal plasma, which will help to reduce the damage caused by oxidative factors and improve the characteristics of semen (Rushworth *et al.*, 2013) [25].

Table 3. Effect of different concentrations of oat seed extract aqueous addition (ppm) to the percentage of acrosome integrity of sperm after 48 and 72 hours cooling period

Treatments	Mean ± Standard Error %	
	After 72 hours of cooling	After 48 hours of cooling
T_1	^{b} 1.15 ± 87.00	^{c}1.19 ± 86.00
T_2	^{a}1.30 ± 89.00	^{a}1.20 ± 89.00
T_3	^{b}1.05 ± 87.00	^{b}1.10 ± 88.00
T_4	^{d}1.11 ± 86.00	^{b}1.20 ± 87.00
Level of Significance	*	*

T_1 = control treatment with *Tris dilutes* without antioxidant addition, T_2 = Tris dilute + 1% oat seed extract, T_3 = Tris dilute + 2% oat seed extract, T_4 = Tris dilute + 3% oat seed extract. Means with letters different within the same column are significantly different from each other. * ($P \leq 0.05$).

Table 4 results indicated that the percentage of total sperm abnormalities decreased significantly after addition of 1% of oat seed aqueous extract to Tri's diluent after 48 hours cooling in the treatment T_2 as it recorded 0.1 ± 9.01 while the T_1, T_3 and T_4 recorded 11.50 ± 0.2, 10.10 ± 0.7 and 10.01 ± 0.6%, respectively. The results also indicated that after 72 hours cooling treatment T_2 had reduced the percentage of total sperm abnormality (11.90 ± 0.1%) compared to treatments (T_1, T_3, and T_4), as it recorded (11.80 ± 0.5, 10.3 ± 0.9, and 10.2 ± 0.8%) respectively. This marked downfall in the percentage of total abnormality in the sperm in treatment T_2 may be due to the inclusion of oat seed aqueous extract. Oxidative stress damaging the membranes during the cryopreservation and freezing process is responsible for the damage to the membranes in sperm. Raised levels of lipid oxidation can cause abnormalities in sperm. Sperms can become abnormal as a result of lipid oxidation. Semen trait improvement during cryopreservation can be obtained by using OAE in the semen diluent, while OAE as an antioxidant can protect the sperm from damage caused by the oxidative stress during cryopreservation as evidenced by semen trait improvement such as reduction in the incidence of sperm abnormalities (Alemayehu *et al.*, 2023; Duncan, 1955) [9, 15].

Table 4. Effect different levels of adding of oat seed extract aqueous on the percentage of total sperm abnormalities after refrigeration for 48 hours and after 72 hours.

Treatments	Mean ± Standard Error %	
	After 72 hours of cooling	After 48 hours of cooling
T_1	^{b}0.5 ± 11.80	^{a}0.2 ± 11.50
T_2	11. 90 ± 0.1^{a}	^{d}0.1 ± 9.01
T_3	^{c}0.7 ± 10.10	^{b}0.7 ± 10.10
T_4	^{c}0.7 ± 10.00	^{c}0.6 ± 10.01
Level of Significance	*	*

T_1 = control treatment with *Tris dilutes* without antioxidant addition, T_2 = Tris dilute + 1% oat seed extract, T_3 = Tris dilute + 2% oat seed extract, T_4 = Tris dilute + 3% oat seed extract. Means with letters different within the same column are significantly different from each other. * ($P \leq 0.05$).

Conclusions

The results obtained in this study showed that the addition of 1% of aqueous extract of oat seeds improved some traits of the semen of Awassi rams at the post-cooled phase including individual movement, acrosome integrity, and plasma membrane integrity of sperm, along with a decrease in a total sperm abnormality percentage.

Supplementary materials

There are no supplementary materials.

Author contributions

M. K. H. AL-dulaimi: methodology, writing original draft preparation; M. K. A Al-juhaishi writing; H. R. Alnajm review and editing; P.R Tapaloaga; A. Javanmard and D. Tapaloaga statistical analysis. The manuscript was reviewed by all authors.

Funding

This research did not receive any external funding.

Institutional review board statement

The study was carried out based on the protocol approved by the Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Department of Animal Production Techniques.

Data availability statement

Data is available upon request.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

Thanks to the Dean of the College Head of Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Iraq, Department of Animal Production Techniques and all who assisted in this study.

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How to cite this article: Al-Dulaimi MKH, Al-Juhaishi MKA. Effect of adding oat seed aqueous extract in Tri's diluent on some chilled semen characteristics of Awassi rams. *International Journal of Agriculture and Nutrition*. 2026;8(10):32-35. doi: https://www.doi.org/10.33545/26646064.2026.v8.i10a.766

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