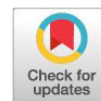


## Research Article



# Effect of Different Levels of Hops (*Humulus lupulus* L.) Alcoholic Extract on Short-Term Chilled Storage of Local Iraqi Goat Semen With and Without Seminal Plasma Removal

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**Abstract** | Chilled semen storage lowers the temperature of sperm cells, potentially inducing oxidative stress that leads to lipid peroxidation. Additionally, bulbourethral secretions in goat semen may interact negatively with components of egg yolk-based extenders, resulting in sperm toxicity. This study aimed to evaluate the effects of different levels of hops (*Humulus lupulus* L.) alcoholic extract and seminal plasma removal (washing) on post-chilling semen quality and oxidative stress parameters in local Iraqi goats. Semen was collected from four healthy black local Iraqi bucks, pooled, and divided into two portions: the first was directly diluted with Tris-based extender, while the second was washed with phosphate-buffered saline (PBS) before dilution. Both portions were further subdivided into three treatment groups: C (control, no extract), T1 (30  $\mu$ L/mL of 1% hops alcoholic extract), and T2 (50  $\mu$ L/mL of 1% hops alcoholic extract). All samples were stored at 4 °C and evaluated at 0 and 4 hours post-treatment. Parameters assessed included individual sperm motility, viability, membrane integrity, total abnormalities, malondialdehyde (MDA) levels, catalase (CAT), reactive oxygen species (ROS), and superoxide dismutase (SOD) activity. The results indicated that the T1 treatment (30  $\mu$ L/mL) significantly improved ( $p < 0.05$ ) individual motility, sperm viability, and membrane integrity compared to the control. Washing of semen showed no significant difference compared to unwashed samples. In conclusion, hops extract at 30  $\mu$ L/mL enhanced the quality of chilled goat semen during short-term storage. Further research is recommended to explore its potential in cryopreservation protocols.

**Keywords** | Hops, Seminal plasma removal, Prolonged semen storage, Antioxidant

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## INTRODUCTION

Chilled semen is commonly used in the artificial insemination of goats to support food security (Ali *et al.*, 2024). This method is technically simpler than freezing, more cost-effective, and allows easier transportation of samples (Liu *et al.*, 2016). While cooling is essential for prolonging sperm viability, it also helps minimize temperature shock and facilitates equilibration between

sperm cells and the extender before freezing or storage (Zhao *et al.*, 2009). However, maintaining goat semen viability during chilling remains challenging due to bioactive factors in seminal plasma that can significantly reduce sperm viability. Additionally, the formation of reactive oxygen species (ROS) contributes to oxidative stress, further compromising sperm quality (Aboagla and Terada, 2004; Bresm and Habeeb, 2023).

Goat semen diluents contain either egg yolk or skimmed milk, but the seminal plasma contains several factors and enzymes that are secreted by the accessory sex glands of the male reproductive system, which might interact with some components of egg yolk or skimmed milk, causing a significant decrease or toxicity to the goat male sperm (Liu *et al.*, 2016). To avoid this problem, the idea of washing the semen (seminal plasma removal) before cooling to remove these harmful components has been proposed. Some studies have shown that washing before cooling or freezing may improve sperm quality after thawing in goats (Hobi *et al.*, 2006; Naing *et al.*, 2011). On the other hand, several studies did not find a significant decrease in buck goat spermatozoa when goat seminal plasma was removed, either in chilled or frozen preservation (Azerêdo *et al.*, 2001; Dorado *et al.*, 2007; Purdy, 2006; Roof *et al.*, 2012; Sariözkan *et al.*, 2010). This controversial subject suggests that another investigation is warranted due to the potential risks associated with prolonged semen preservation, including the production of reactive oxygen species (Sun *et al.*, 2020).

Several substances have been used as additives to diluents in the process of semen cryopreservation, such as medicinal herbs. Extracts from herbs and plants with medicinal properties have been added to semen diluents in ruminants for improving semen preservation, whether through cooling or freezing. This is because they contain bioactive and effective substances that act as natural antioxidants (Wang *et al.*, 2014). Hops (*Humulus lupulus* L.) is a group of polyphenols, bioactive materials which has been used in the brewing industry (Almaguer *et al.*, 2014). However, its uses expand over time due to its benefits to human health as an antioxidant (Yang *et al.*, 2020; Zugravu *et al.*, 2022). Environmentally friendly antioxidants from natural sources play an essential role in inhibiting lipid peroxidation, protecting the cell membrane from free radicals, DNA damage, and thus preventing cell damage (Zugravu *et al.*, 2022). Different alcoholic extracts used have found that the antioxidant and antimutagenic activity of the alcoholic extract may vary depending on the concentration used (Wang *et al.*, 2022). In addition to that, hops prevent DNA damage in mammalian cells and prevent apoptosis (Ferracchiato *et al.*, 2021). Furthermore, feeding the male Sprague-Dawley rats with the hydroalcoholic extract of hops significantly increased the testis weight and seminal fluid, but not sperm motility (Karbalaei *et al.*, 2019).

To our knowledge, the novelty of the current research lies in the fact that, despite the numerous benefits of hops, including its antioxidant properties, there are no studies examining the effect of hops' alcoholic extract on the semen characteristics of farm animals worldwide. Therefore, this study aimed to verify the impact of different levels of hops' alcoholic extract and washing on post-cooling ability and

oxidative stress parameters in local Iraqi goat.

## MATERIALS AND METHODS

### ANIMAL AND ETHICAL STATEMENT

This study was conducted in the animal field of the Faculty of Agriculture at Al-Qasim Green University in Babil Governorate (ethical approval No. 3155, 2024) to demonstrate the effects of different levels of hops' alcoholic extract and washing on post-cooling ability and oxidative stress parameters in local Black Iraqi goats. Four buck goats, with an average age of 2-3 years and an average of 35-45 kg, were purchased and housed in semi-open pens. "The goats were fed a concentrated diet at 2% of their live body weight. In addition, they were allowed to graze twice daily, once in the morning and once in the evening. Clean drinking water was available *ad libitum*, and the animals received continuous monitoring and veterinary care throughout the study period.

### SEMEN COLLECTION AND TREATMENT WITH HOP'S ALCOHOLIC EXTRACT

Hop's alcoholic extract (1%) was prepared at the Ministry of Higher Education and Scientific Research/ Department of Environment and Water. Semen was collected from buck goats using an artificial vagina. Male goats were trained for the semen collection process using an artificial vagina for three weeks before the start of the experiment. Following training, semen samples were collected in graded plastic tubes, transferred to the adjacent lab, and placed in a water bath at 37°C until processing. Semen samples were pooled and then divided into two parts. The first part was diluted with a Tris extender, and the other part was washed with PBS (9.869 g/L) twice (Leboeuf *et al.*, 2000). Briefly, semen samples were diluted with 4 mL PBS and then centrifuged for 15 minutes at 600-1000 × g at 30 °C. Following centrifugation, the supernatant was discarded, and the pellet was resuspended in PBS solution. The sample was then centrifuged again. Following the second centrifugation, Tris extender (Tris, 3.63 g; citric acid monohydrate, 1.99 g; glucose, 1 g; egg yolk, 10 ml; pH 7.0; antibiotic, 0.050 mg; double-distilled water, 100 ml) (Salamon and Maxwell, 2000) was used at a 1:10 dilution. Then, the treatment was added for both the first and second parts: C (control group), T1 (30 µL), and T2 (50 µL). Tubes were placed in a water bath at 37°C for estimation of semen parameters. Following this step, all samples were stored in a refrigerator at 4 °C. Samples were evaluated at 0 and 4 hours following the cooling process. For the antioxidant parameters, the treatment and control groups were centrifuged at 1000 g for 15 minutes at 0 and 4 hours following the cooling process. Seminal plasma was kept at 20 °C for antioxidant evaluations. Semen samples were collected weekly for five weeks for both physical and

antioxidant parameters.

### INDIVIDUAL SPERM MOTILITY

Individual motility was estimated by measuring the progressive motility. Briefly, 10  $\mu$ L of the diluted sample was placed on warmed glass slides covered with a cover slide and then examined under 40X magnification by using a microscope. The estimation was based on a 0-100 scale depending on the forward progressive motility (Ali *et al.*, 2024).

### PERCENTAGE OF LIVE SPERM

The live and dead sperm method was used to estimate the live spermatozoa. Briefly, a 1:2 sample was placed in an Eosin-Nigrosin (5% eosin and 10% nigrosin) solution, placed on a warmed glass slide, smeared, and air-dried at room temperature. Two hundred spermatozoa were counted in different fields from the same slide, and the white head sperm were counted as live sperm, while the pinkish-headed sperm were counted as dead sperm. The examination was performed using a microscope with a magnification of x400 (Sacko *et al.*, 2022).

### TOTAL ABNORMAL SPERM

The percentage of total sperm was evaluated using the same slide as that used for live sperm estimation. Two hundred sperm were tested in different slide fields, and the percentage was then recorded (Ali *et al.*, 2024).

### PLASMA MEMBRANE INTEGRITY

Regarding the integrity of the plasma membrane, the hypo-osmotic swelling test (HOST) was used in the current study. Briefly, 500 microliters of HOST solution (fructose, 8.72 g/L, and sodium citrate, 4.74 g/L) were mixed with 10 microliters of the sample, and then incubated at 37 °C for 30 minutes. Ten microliters of the mixture were placed on a clean, warm glass slide, smeared, and then air dried. Swollen head and tail, and curved tail were counted as an intact plasma membrane. However, a straight tail was considered abnormal. Slides were tested under 40x magnification by using a light optical microscope (MEIJI MT4200L, Meiji Techno Co., Ltd., Japan) for a total of 200 sperm, and then the percentage of intact plasma membrane was estimated (Jeyendran *et al.*, 1984).

### MALONDIALDEHYDE (MDA) PRODUCTION

The activity of malondialdehyde (MDA) was estimated in all samples. Briefly, 0.6 mL of TCA-TBA-HCl reagent was added to 0.4 mL of seminal plasma, and the mixture was boiled at 100 °C for 30 minutes. After heating, the samples were allowed to cool at room temperature for 20 minutes. Subsequently, 1N sodium hydroxide (NaOH) was added to the cooled solution. A blank solution was prepared in the

same manner as the sample solutions, except that distilled water was used instead of seminal plasma. Samples and blank absorbance were read at 535 nm (Buege and Aust, 1978).

### ESTIMATION OF REACTIVE OXYGEN SPECIES (ROS)

The estimation of ROS in seminal plasma was used to estimate oxidative stress. The basis of this method is the Fe (III) ion- $\alpha$ -dianisidine complex. Briefly, 900 microliters of reagent one and 44 microliters of reagent two were added to 140 microliters of seminal plasma. The solution was vortexed and incubated at 37°C for 30 minutes. After incubation, samples were measured by using a spectrophotometer (Jasco V-550 UV-vis) at an absorbance of 650 nm. (Erel, 2005).

### SUPEROXIDE OXIDE DISMUTASE (SOD) ACTIVITY

SOD (U/ml) activity was determined according to the method described by Marklund and Marklund (Marklund and Marklund, 1974). Accordingly, seminal plasma (50 microliters), Pyrogallol (1 mL), and Tris buffer (1 mL) for each tube (sample and control). Distilled water (1 mL) was added to the control tube only. Absorption was measured at a wavelength of 420 nm against Tris-EDTA buffer at zero time and after 1 minute of pyrogallol addition.

### CATALASE (CAT) ACTIVITY

CAT activity was determined according to the method described by Goth (1991). Accordingly, substrate buffer (1 mL) and seminal plasma (0.2  $\mu$ L) were mixed and incubated at 37°C for 1 minute. Following incubation, ammonium molybdate (1 mL of 32.4 mM) was added, and the absorbance was then measured using a spectrophotometer at 405 nm.

### STATISTICAL ANALYSIS

A three-way analysis of variance (ANOVA) was conducted to evaluate the effects of washing status, hops extract treatment, time, and their interactions in a completely randomized design (CRD) using the Statistical Analysis System (SAS, 2012). Significant differences between treatment means were assessed at a significance level of  $P \leq 0.05$ . The experiment was replicated five times.

## RESULTS

### EFFECT OF SEMEN WASHING STATUS ON PHYSICAL AND ANTIOXIDANT PARAMETERS IN LOCAL IRAQI GOATS

The statistical analysis revealed there was no significant difference between the washed and unwashed semen for numerous parameters including individual motility, live sperm, total abnormal sperm, membrane integrity, ROS, MDA, CAT, and SOD activity (Table 1).



**Table 1:** Effect of semen washing on physical and antioxidative parameters in local Iraqi goats.

| Parameter                | Washed semen (Mean ± SE) | Unwashed semen (Mean ± SE) |
|--------------------------|--------------------------|----------------------------|
| Individual motility (%)  | 64.00 ± 1.81 a           | 66.4 ± 2.09 a              |
| Live sperm (%)           | 86.08 ± 1.02 a           | 86.33 ± 1.06 a             |
| Total abnormal sperm (%) | 0.81 ± 0.07 a            | 1.00 ± 0.09 a              |
| Membrane integrity (%)   | 88.93 ± 0.97 a           | 88.88 ± 1.21 a             |
| MDA (nmol/ml)            | 19.33 ± 1.90 a           | 18.22 ± 1.81 a             |
| CAT (U/ml)               | 57.83 ± 2.93 a           | 59.29 ± 2.64 a             |
| ROS (U/ml)               | 28.37 ± 1.04 a           | 29.67 ± 0.61 a             |
| SOD (U/ml)               | 37.40 ± 1.60 a           | 35.45 ± 1.44 a             |

All results were presented as Means ± SEM. Means with different letters in the same row differ significantly ( $P \leq 0.05$ ).

### EFFECT OF TIME ON PHYSICAL AND ANTIOXIDANT PARAMETERS IN LOCAL IRAQI GOATS

The data showed a significant effect of cooling time on individual motility, live sperm, and membrane integrity (Table 2). Individual motility decreased significantly ( $p \leq 0.001$ ) at 4 h ( $54.27 \pm 1.96$ ) following the cooling process compared to time 0 h ( $73.67 \pm 1.42$ ) (Table 2). Additionally, the live sperm count was significantly lower ( $p \leq 0.0001$ ) at 4 h ( $82.07 \pm 0.78$ ) following the cooling process compared to time 0 h ( $90.34 \pm 0.62$ ) (Table 2). Additionally, sperm membrane integrity decreased significantly ( $p \leq 0.05$ ) at 4 h ( $86.73 \pm 1.09$ ) following the cooling process, compared to time 0 ( $91.08 \pm 0.94$ ) (Table 2).

### EFFECT OF HOPS' ALCOHOLIC EXTRACT ON PHYSICAL AND ANTIOXIDANT PARAMETERS IN LOCAL IRAQI GOATS

The results revealed that the effect of hops extract significantly affects individual motility, live sperm, and membrane integrity (Table 3). The individual motility and live sperm increased significantly ( $P \leq 0.0001$  and  $P \leq 0.001$ ) in T1 ( $68.73 \pm 2.16$  and  $88.09 \pm 1.09$ ) and T2 ( $66.87 \pm 2.16$  and  $86.73 \pm 1.14$ ), respectively, compared to the control group ( $60.00 \pm 2.58$  and  $60.00 \pm 2.58$ ). Additionally,

membrane integrity increased significantly ( $P \leq 0.001$ ) in T1 ( $91.45 \pm 0.93$ ) compared to control group ( $86.33 \pm 1.60$ ) but did not differ from T2 ( $88.94 \pm 1.19$ ) (Table 3).

**Table 2:** Effect of time on physical and antioxidant parameters in local Iraqi goats.

| Parameter                | 0 h            | 4 h            |
|--------------------------|----------------|----------------|
| Individual motility (%)  | 73.67 ± 1.42 a | 54.27 ± 1.96 b |
| Live sperm (%)           | 90.34 ± 0.62 a | 82.07 ± 0.78 b |
| Total abnormal sperm (%) | 0.87 ± 0.08 a  | 0.95 ± 0.086 a |
| Membrane integrity (%)   | 91.08 ± 0.94 a | 86.73 ± 1.09 b |
| MDA (nmol/ml)            | 18.22 ± 1.84 a | 19.33 ± 1.88 a |
| CAT (U/ml)               | 59.04 ± 2.94 a | 58.07 ± 2.63 a |
| ROS (U/ml)               | 28.12 ± 0.85 a | 29.92 ± 0.85 a |
| SOD (U/ml)               | 36.68 ± 1.43 a | 36.17 ± 1.63 a |

All results were presented as Means ± SEM. Means with different letters in the same row differ significantly ( $P \leq 0.05$ ).

### INTERACTION EFFECTS OF WASHING STATUS, TIME, AND HOPS ALCOHOLIC EXTRACT ON PHYSICAL AND ANTIOXIDANT PARAMETERS OF SEMEN IN LOCAL IRAQI GOATS

The results showed a significant interaction effect between washing, time, and hops alcoholic extract on individual motility, live sperm, total abnormal sperm, and membrane integrity (Table 4). The individual motility and live sperm were significantly increased ( $p \leq 0.01$ ) in T1 at 4 hours ( $61.00 \pm 5.34$  and  $84.75 \pm 1.39$ ) compared to the control at 4 hours ( $46.00 \pm 5.01$  and  $78.35 \pm 1.26$ ) in unwashed semen (Table 4). In the same manner, live sperm showed a significant increase ( $p \leq 0.01$ ) in T2 time at 4 hours ( $84.25 \pm 2.80$ ) compared to the control time at 4 hours ( $79.05 \pm 1.72$ ) in washed semen (Table 4). Also, the total abnormal sperm was significantly decreased ( $P \leq 0.05$ ) in the T2 at 0h ( $0.58 \pm 0.15$ ) in washed semen compared to T1 at 4h ( $1.28 \pm 0.35$ ) in unwashed semen (Table 4). Additionally, membrane integrity was significantly decreased ( $p \leq 0.01$ ) in the control group at time 0 hours ( $83.60 \pm 3.35$ )

**Table 3:** Effect of hops alcoholic extract on physical and antioxidant parameters in local Iraqi goats.

| Parameter                | C              | T1             | T2              |
|--------------------------|----------------|----------------|-----------------|
| Individual motility (%)  | 60.00 ± 2.58 b | 68.73 ± 2.16 a | 66.87 ± 2.16 a  |
| Live sperm (%)           | 83.80 ± 1.40 b | 88.09 ± 1.09 a | 86.73 ± 1.14 a  |
| Total abnormal sperm (%) | 0.97 ± 0.10 a  | 0.94 ± 0.11 a  | 0.82 ± 0.09 a   |
| Membrane integrity (%)   | 86.33 ± 1.60 b | 91.45 ± 0.93 a | 88.94 ± 1.19 ab |
| MDA (nmol/ml)            | 17.97 ± 1.72 a | 18.41 ± 2.64 a | 19.96 ± 2.42 a  |
| CAT (U/ml)               | 59.19 ± 3.48 a | 55.14 ± 3.43 a | 61.35 ± 3.28 a  |
| ROS (U/ml)               | 29.08 ± 0.86 a | 28.95 ± 1.14 a | 29.04 ± 1.17 a  |
| SOD (U/ml)               | 37.10 ± 2.07 a | 35.83 ± 1.27 a | 36.35 ± 2.21 a  |

All results were presented as Means ± SEM. C= control (no treatment), T1=30 µL hops alcoholic extract, T2=50 µL hops alcoholic extract. Means with different letters in the same row differ significantly ( $P \leq 0.05$ ).

**Table 4:** Interaction effects of washing status, storage time, and hops alcoholic extract on physical and antioxidant parameters in chilled semen of local Iraqi goats.

| Washing   | Hops extract (μL) | Time (hrs) | Individual motility (%) | Live sperm (%) | Total abnormal sperm (%) | Membrane integrity (%) | MDA         | CAT         | ROS         | SOD         |
|-----------|-------------------|------------|-------------------------|----------------|--------------------------|------------------------|-------------|-------------|-------------|-------------|
| Un-washed | C                 | 0          | 71.00±3.32abc           | 89.45±1.78ab   | 1.04±0.14ab              | 89.20±4.00ab           | 18.47±4.36a | 54.48±6.89a | 30.3±0.4a   | 33.86±1.72a |
|           |                   | 4          | 46.00±5.01d             | 78.35±1.26f    | 0.96±0.24ab              | 83.60±3.35b            | 18.74±2.15a | 65.11±6.94a | 28.48±1.29a | 42.10±6.5a  |
|           | T1                | 0          | 77.00±4.63a             | 92.55±1.60a    | 0.80±0.03ab              | 93.45±1.39a            | 18.97±4.61a | 53.16±7.52a | 29.87±0.65a | 40.3±3.29a  |
|           |                   | 4          | 61.00±5.3cb             | 84.75±1.39bcd  | 1.28±0.35a               | 89.80±2.67ab           | 13.94±1.88a | 59.39±5.63a | 29.86±1.98a | 31.50±0.94a |
|           | T2                | 0          | 74.60±4.26ab            | 90.50±1.02a    | 0.94±0.18ab              | 91.15±2.26ab           | 22.28±8.79a | 64.65±4.52a | 29.38±2.57a | 33.88±1.33a |
|           |                   | 4          | 58.00±3.74cd            | 82.35±1.37def  | 1.00±0.26ab              | 86.10±2.67ab           | 16.94±2.88a | 58.93±8.20a | 30.13±1.77a | 31.08±2.18a |
| Washed    | C                 | 0          | 72.00±2.55abc           | 88.35±1.85abc  | 1.08±0.31ab              | 88.80±2.53ab           | 17.51±3.80a | 60.54±8.99a | 27.44±2.99a | 35.71±1.96a |
|           |                   | 4          | 45.99±2.24d             | 79.05±1.72ef   | 0.80±0.08ab              | 83.70±2.78b            | 17.14±4.25a | 56.61±6.14a | 30.09±1.40a | 36.72±4.75a |
|           | T1                | 0          | 77.4±3.36a              | 91.40±1.41a    | 0.76±0.20ab              | 92.65±1.23a            | 15.09±1.44a | 60.37±7.01a | 24.99±2.67a | 34.95±1.51a |
|           |                   | 4          | 58.00±2.00cd            | 83.65±0.77cde  | 0.90±0.12ab              | 89.90±1.78ab           | 25.63±9.21a | 47.66±7.71a | 31.05±2.80a | 36.55±2.54a |
|           | T2                | 0          | 70.0±2.74abc            | 89.80±1.30a    | 0.58±0.15b               | 91.20±1.98ab           | 17.03±2.51a | 61.04±9.96a | 26.74±1.60a | 41.36±7.35a |
|           |                   | 4          | 57.60±5.46cd            | 84.25±2.80cd   | 0.76±0.10ab              | 87.30±2.40ab           | 23.59±3.17a | 60.77±3.72a | 29.92±3.44a | 39.08±3.94a |

All results were presented as Means ± SEM. C= control (no treatment), T1= 30 μL hops alcoholic extract, T2= 50 μL hops alcoholic extract. Means with different letters in the same row differ significantly (P≤0.05).

compared to T1 time at 4 hours (93.45 ± 1.39) in unwashed semen. Membrane sperm integrity decreased significantly (p ≤ 0.01) in the control group (83.70 ± 2.78) compared to T1 time 0 (92.65 ± 1.23) in washed semen (Table 4). However, there was no significant interaction effect on MDA, SOD, ROS, and CAT.

DISCUSSION

In the current study, the washing did not differ significantly from that of the unwashed seminal plasma. This result was in agreement with (Bhat *et al.*, 2025; Sariözkan *et al.*, 2010; Shah *et al.*, 2023), who reported that the removal of seminal plasma from buck semen was not beneficial to the viability and cryopreservation ability of sperm. However, this result disagreed with (Anand *et al.*, 2017; Santiago-Moreno *et al.*, 2017; Silva *et al.*, 2019). The goat seminal plasma is routinely removed from the buck goat when prepared for cryopreservation. This idea aims to prevent interaction between the bulbourethral secretion and the egg yolk phospholipids of the extender. The bulbourethral phospholipase and lipase enzymes hydrolyze the phospholipids in both the semen environment and the sperm membrane (Sias *et al.*, 2005). This interaction results in the production of toxic material, which then reduces viability and prevents goat semen from being cryopreserved (Liu *et al.* 2016). In our case, the local Iraqi goat was not affected by the removal of the seminal plasma, indicating the beneficial effect of seminal plasma, or not toxic materials were released on the local buck semen prepared for cryopreservation (Bhat *et al.*, 2025; Roof *et al.*, 2012).

Our data indicated a beneficial effect of hops' alcoholic extract on the individual motility, live sperm, and membrane integrity of buck semen. The active ingredients of hops are phenols and flavonoids, which have an antioxidant effect (Yang *et al.*, 2020; Zugravu *et al.*, 2022), on semen cooling and cryopreservation (Silvestre *et al.*, 2021). Goat semen is susceptible to oxidative stress due to the unsaturated free fatty acid component of the sperm cell membrane (Li *et al.*, 2024). The cooling process consists of reducing the environmental temperature, which increases oxidative stress, causing damage to spermatozoa's double-layer membrane, motility, viability, and then fertility (Bucak *et al.*, 2010). Many medical plants have strong antioxidants according to their contents, such as phenols and flavonoids, which may decrease the adverse effects of free radicals initiated from oxidative stress (Urbańska *et al.*, 2025).

In the current study, individual motility, live, and membrane integrity were increased in the 30 and 50 μL/mL of hops alcoholic extract compared to the control. These data agree with Purdy *et al.* (2004), who reported that flavonoid supplementation to buck goat chilled semen enhances sperm motility after 96 hours of storage (Purdy *et al.*, 2004). Also, Caamaño *et al.* (2023) found that using 10 μg/ml of taxifolin in chilled goat semen enhances sperm motility (Caamaño *et al.*, 2023). Additionally, Atiyah *et al.* (2024) concluded that the progressive motility and membrane integrity, in addition to live sperm, were protected in buck goat following the cooling process (Atiyah *et al.*, 2024). On the other hand, these data did not agree with those of Karbalaie *et al.* (2019), who concluded that feeding male Sprague-Dawley rats with the hydroalcoholic extract of hops significantly increased testis weight and seminal

fluid, but not sperm motility (Karbalaie *et al.*, 2019). To sum up, environmentally friendly flavonoids and phenolic antioxidants from hops play an essential role in inhibiting lipid peroxidation, protecting the cell membrane from free radicals, and preventing cell damage, which may be a promising source of antioxidant activity for goat semen cryopreservation (Zugravu *et al.*, 2022).

## CONCLUSIONS

In conclusion, the washing process is not necessary when adding strong 1% hops alcoholic extracts as antioxidants to the goat semen extender. 30  $\mu$ L or 50  $\mu$ L of hop alcoholic extract increased individual motility, live sperm, and sperm membrane integrity parameters for chilled goat semen preserved for a short period. A new investigation is warranted to use the hops alcoholic extract for cryopreservation.

## ACKNOWLEDGMENT

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## NOVELTY STATEMENT

The novelty of the current research lies in the fact that, despite the numerous benefits of hops, including their antioxidant properties, there are no studies on farm animals worldwide examining the beneficial effect of hops' alcoholic extract on the semen characteristics of local Iraqi bucks.

## AUTHOR'S CONTRIBUTION

Diyar L. Al-Rubaie: Conceptualization, data curation, investigation, methodology, project administration, resources, software, writing original draft, writing review and editing.

Hayder MH. Habeeb and Rahman H.H. Al-Qasimi: Conceptualization, formal analysis, resources, investigation, software, supervision, writing original draft, writing review and editing.

## GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

We did not use AI technology for the current research, except Grammarly for Grammar and spell check.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## REFERENCES

- Aboagla EME, Terada T (2004). Effects of egg yolk during the freezing step of cryopreservation on the viability of goat spermatozoa. *Theriogenology*, 62(6): 1160–1172. <https://doi.org/10.1016/j.theriogenology.2004.01.013>
- Ali RA, Hamza RH, Habeeb HMH, Al-Nuaimi AJ (2024). Evaluation of semen characteristics, testicular measurements, and blood parameters for three genetic groups of goat bucks in Iraq. *J. Anim. Health Prod.*, 12(2): 233–239. <https://doi.org/10.17582/journal.jahp/2024/12.2.233.239>
- Almaguer C, Schönberger C, Gastl M, Arendt EK, Becker T (2014). *Humulus lupulus* a story that begs to be told. A review. *J. Inst. Brew.*, 120(4): 289–314. <https://doi.org/10.1002/jib.160>
- Anand M, Baghel G, Yadav S (2017). Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen. *J. Appl. Anim. Res.*, 45(1): 560–565. <https://doi.org/10.1080/09712119.2016.1232265>
- Atiyah SS, Al-Sadoon AAZ, Jieish SK (2024). Effect of adding an aqueous extract of aloe vera (aeav) to tris extender on some characteristics of goat epididymal spermatozoa at different cooling times. *Adv. Anim. Vet. Sci.*, 12(9): 1810–1817. <https://doi.org/10.17582/journal.aavs/2024/12.9.1810.1817>
- Azerêdo GA, Esper CR, Resende KT (2001). Evaluation of plasma membrane integrity of frozen-thawed goat spermatozoa with or without seminal plasma. *Small Rumin. Res.*, 41(3): 257–263. [https://doi.org/10.1016/S0921-4488\(01\)00189-4](https://doi.org/10.1016/S0921-4488(01)00189-4)
- Bhat GR, Lone FA, Khatun A, Anand M, Dhariya R, Rana S, Shah RA, Ahmad HA, Hussain I (2025). Lowering the level of egg yolk in diluent or removal of seminal plasma with standard egg yolk level do not help in the efficient freezing of buck semen. *Indian J. Anim. Reprod.*, 46(1): 39–48. <https://doi.org/10.48165/ijar.2025.46.01.7>
- Bresm HAM, Habeeb MHH (2023). Effect of vitamin D3 on some antioxidant parameters in chilled semen in Awassi ram. *Arch. Razi. Inst.*, 78(2): 681–687.
- Bucak MN, Sariözkan S, Tuncer PB, Sakin F, Ateşşahin A, Kulaksız R, Çevik M (2010). The effect of antioxidants on post-thawed Angora goat (*Capra hircus ancyrensis*) sperm parameters, lipid peroxidation and antioxidant activities. *Small Rumin. Res.*, 89(1): 24–30. <https://doi.org/10.1016/j.smallrumres.2009.11.015>
- Buege JA, Aust SD (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, 52(C): 302–310. [https://doi.org/10.1016/S0076-6879\(78\)52032-6](https://doi.org/10.1016/S0076-6879(78)52032-6)
- Caamaño JN, Santiago-Moreno J, Martínez-Pastor F, Tamargo C, Salman A, Fernández A, Merino MJ, Lacalle E, Toledano-Díaz A, and Hidalgo CO (2023). Use of the flavonoid taxifolin for sperm cryopreservation from the threatened Bermeya goat breed. *Theriogenology*, 206: 18–27. <https://doi.org/10.1016/j.theriogenology.2023.05.004>
- Dorado J, Rodríguez I, Hidalgo M (2007). Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. *Theriogenology*, 68(2): 168–177. <https://doi.org/10.1016/j.theriogenology.2007.04.048>
- Erel O (2005). A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, 38(12): 1103–1111. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>



- Ferracchiato S, Di-iacovo N, Scopetti D, Piobbico D, Castelli M, Pieroni S, Gargaro M, Manni G, Brancorsini S, Della-Fazia MA, Servillo G (2021). Hops/Tmub1 heterozygous mouse shows haploinsufficiency effect in influencing p53-mediated apoptosis. *Int. J. Mol. Sci.*, 22(13): 7186. <https://doi.org/10.3390/ijms22137186>
- Góth L (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clin. Chim. Acta*, 196(2–3): 143–151. [https://doi.org/10.1016/0009-8981\(91\)90067-M](https://doi.org/10.1016/0009-8981(91)90067-M)
- Hobi AKA, Asofi MK, Hamra Ah (2006). Deep freezing of the shami bucks semen. *Jordan J. Agric. Sci.*, 2(3): 302–314.
- Jeyendran R, Van der Ven HH, Perez-Peleaz M, Crabo BG, Zaneveld LJ (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fert.*, 70(1): 219–228. <https://doi.org/10.1530/jrf.0.0700219>
- Karbalaie N, Sadeghi N, Nekoeian A, Malekzadeh A (2019). Impact of hydroalcoholic extract of *Humulus lupulus* L. on sperm quality, reproductive organs and hormones in male rats. *Chin. J. Integr. Med.*, 25(7): 529–535. <https://doi.org/10.1007/s11655-019-3025-7>
- Leboeuf B, Restall B, Salamon S (2000). Production and storage of goat semen for artificial insemination. *Anim. Reprod. Sci.*, 62(1–3): 113–141. [https://doi.org/10.1016/S0378-4320\(00\)00156-1](https://doi.org/10.1016/S0378-4320(00)00156-1)
- Li C, Lv C, Larbi A, Liang J, Yang Q, Wu G, Quan G (2024). Revisiting the injury mechanism of goat sperm caused by the cryopreservation process from a perspective of sperm metabolite profiles. *Int. J. Mol. Sci.*, 25(16): 9112. <https://doi.org/10.3390/ijms25169112>
- Liu CH, Dong HB, Ma DL, Li YW, Han D, Luo MJ, Chang ZL, Tan JH (2016). Effects of pH during liquid storage of goat semen on sperm viability and fertilizing potential. *Anim. Reprod. Sci.*, 164: 47–56. <https://doi.org/10.1016/j.anireprosci.2015.11.011>
- Marklund S, and Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.*, 47(3): 469–474. <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>
- Naing WS, Haron WA, Goriman MAK, Yusoff R, Bakar MZA, Sarsaifi K, Bakar MM, Thein M, Kyaw T, San MM (2011). Effect of seminal plasma removal, washing solutions, and centrifugation regimes on Boer goat semen cryopreservation. *Pertanika J. Trop. Agric. Sci.*, 34(2): 271–279.
- Purdy PH (2006). A review on goat sperm cryopreservation. *Small Rumin. Res.*, 63(3): 215–225. <https://doi.org/10.1016/j.smallrumres.2005.02.015>
- Purdy PH, Ericsson SA, Dodson RE, Sternes KL, Garner DL (2004). Effects of the flavonoids, silibinin and catechin, on the motility of extended cooled caprine sperm. *Small Rumin. Res.*, 55(1–3): 239–243. <https://doi.org/10.1016/j.smallrumres.2004.02.005>
- Roof DJ, Bowley S, Price LL, Matsas DJ (2012). Comparison of two commercial extenders for cryopreservation of goat semen without sperm washing. *Theriogenology*, 77(2): 412–420. <https://doi.org/10.1016/j.theriogenology.2011.08.015>
- Sacko I, Dao M, Sanogo S, Orounladi BM, Diakité K, Traore D, Coulibaly M, Cisse AB (2022). Semen characteristics of crossbred bucks Anglo-Nubian × Sahelian goats in Mali. *Adv. Anim. Vet. Sci.*, 10(9): 1900–1906. <https://doi.org/10.17582/journal.aavs/2022/10.9.1900.1906>
- Salamon S, Maxwell WMC (2000). Storage of ram semen. *Anim. Reprod. Sci.*, 62(1–3): 77–111. [https://doi.org/10.1016/S0378-4320\(00\)00155-X](https://doi.org/10.1016/S0378-4320(00)00155-X)
- Santiago-Moreno J, Estes MC, Castaño C, Toledano-Díaz A, Delgadillo JA, and López-Sebastián A (2017). Seminal plasma removal by density-gradient centrifugation is superior for goat sperm preservation compared with classical sperm washing. *Anim. Reprod. Sci.*, 181: 141–150. <https://doi.org/10.1016/j.anireprosci.2017.04.002>
- Sariözkán S, Bucak MN, Tuncer PB, Taşdemir U, Kinet H, and Ulutaş PA (2010). Effects of different extenders and centrifugation/washing on postthaw microscopic-oxidative stress parameters and fertilizing ability of Angora buck sperm. *Theriogenology*, 73(3): 316–323. <https://doi.org/10.1016/j.theriogenology.2009.09.015>
- SAS (2012). Statistical analysis system, user's guide. Statistical. Version 9.1<sup>th</sup> ed. SAS. Inst. Inc. Cary. N.C. USA.
- Shah SAH, Haider MS, Ahmed H, Fayyaz MH, Andrabi SMH (2023). Cryopreservation protocol resolving the “temperature” challenges of long-distance transportation of Beetal buck (*Capra hircus*) sperm. *Small Rumin. Res.*, 226: 107030. <https://doi.org/10.1016/j.smallrumres.2023.107030>
- Sias B, Ferrato F, Pellicer-Rubio MT, Forgerit Y, Guillouet P, Leboeuf B, Carrière F (2005). Cloning and seasonal secretion of the pancreatic lipase-related protein 2 present in goat seminal plasma. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids.*, 1686(3): 169–180. <https://doi.org/10.1016/j.bbalip.2004.09.008>
- Silva RAJA, Batista AM, Arruda LCP, de Souza HM, Nery IH de AV, Gomes WA, Soares PC, Silva SV, Guerra MMP (2019). Concentration of soybean lecithin affects short-term storage success of goat semen related with seminal plasma removal. *Anim. Reprod.*, 16(4): 895–901. <https://doi.org/10.21451/1984-3143-AR2019-0012>
- Silvestre, MA, Yáñez, JL, Peña, FJ, Santolaria, P, Castelló-Ruiz, M (2021). Role of antioxidants in cooled liquid storage of mammal spermatozoa. *Antioxidants*, 10(7): 1096. <https://doi.org/10.3390/antiox10071096>
- Sun L, Fan W, Wu C, Zhang S, Dai J, Zhang D (2020). Effect of substituting different concentrations of soybean lecithin and egg yolk in tris-based extender on goat semen cryopreservation. *Cryobiology*, 92: 146–150. <https://doi.org/10.1016/j.cryobiol.2019.12.004>
- Urbańska DM, Kurhaluk N, Tkaczenko H, Rutkowska K, Kawecka-Grochocka E, Brzozowska P, Czopowicz M, Mickiewicz M, Kaba J, Bagnicka E (2025). Effects of turmeric and rosemary extract on oxidative stress markers in goats. *Animals*, 15(3): 369. <https://doi.org/10.3390/ani15030369>
- Wang F, Cho BO, Shin JY, Hao S, Jang SII (2022). Humulus japonicus extract alleviates oxidative stress and apoptosis in 6-hydroxydopamine-induced PC12 cells. *Asian Pac. J. Trop. Biomed.*, 12(5): 197–206. <https://doi.org/10.4103/2221-1691.343387>
- Wang X, Yang L, Yang X, Tian Y (2014). *In vitro* and *in vivo* antioxidant and antimutagenic activities of polyphenols extracted from hops (*Humulus lupulus* L.). *J. Sci. Food Agric.*, 94(8): 1693–1700. <https://doi.org/10.1002/jsfa.6534>
- Yang J, Liu Z, Chen P, Du W, Fan X, Shi M, Liu Y (2020). Antioxidant and antibacterial activities of β-acid homologue mixtures with different ratios from hops. *Shipin Kexue/ Food Sci.*, 41(23): 83–90.
- Zhao BT, Han D, Xu CL, Luo MJ, Chang ZL, Tan JH (2009).

Protocol optimization for long-term liquid storage of goat semen in a chemically defined extender. *Reprod. Domest. Anim.*, 44(6): 865–872. <https://doi.org/10.1111/j.1439-0531.2008.01101.x>

Zugravu CA, Bohiltea RE, Salmen T, Pogurschi E, Otelea MR (2022). Antioxidants in Hops: Bioavailability, Health Effects and Perspectives for New Products. *Antioxidants (Basel)*, 11(2): 241. <https://doi.org/10.3390/antiox11020241>