Effects of Oregano essential oils on the quality of cooled buck semen

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Abstract

The aim of this study was to analyze the effect of *Origanum onites* and *Origanum vulgare* essential oils on the quality of Beni Arous buck semen during storage at 4°C. Semen samples were collected once a week from six bucks for ten weeks, and extended in skim milk supplemented with various concentrations (0% (control), 0.01%, and 0.05%) of *Origanum onites* and *Origanum vulgare* essential oils. The effects of essential oils on total motility, progressive motility, viability, abnormalities, and lipid peroxidation were assessed at 0, 4, 8, 24, 28, 32 and 48h of storage. The major compounds found in *Origanum onites* were thymol (44.59%), carvacrol (25.94%), and γ-terpinene (13.22%); whereas carvacrol (81,5%) was dominant in *Origanum vulgare*. The findings indicated that the quality of the sperm was influenced by the concentration of *Origanum onites* and *Origanum vulgare* essential oils. After 48 hours of storage, samples supplemented with 0.01% of *Origanum onites* showed significantly higher total and progressive motility, viability, and membrane integrity as well as lower abnormality compared to the control. Similarly, *Origanum vulgare* essential oil showed comparable effects on total motility, viability, and membrane integrity. Additionally, after 48 hours of storage, the control had the highest concentration of malondialdehyde, which is an indicator of oxidative stress ($p < 0.05$). These findings suggest that 0.01% of essential oils have a positive impact on Beni Arous buck sperm during storage at 4°C, by reducing oxidative stress.

Keywords: Beni arous, Buck semen, conservation, essential oil, *Origanum onites*, *Origanum vulgare*.

Impact des huiles essentielles d'*Origanum onites* et d'*Origanum vulgare* sur la qualité du sperme de bouc réfrigéré

Résumé

Le but de cette étude était d'analyser l'effet des huiles essentielles d'*Origanum onites* et d'*Origanum vulgare* sur la qualité du sperme de bouc Beni Arous pendant le stockage à 4°C. Des échantillons de sperme ont été prélevés une fois par semaine auprès de six boucs pendant dix semaines, et conservés dans du lait écrémé additionné de diverses concentrations (0 % (contrôle), 0,01 % et 0,05 %) d'huiles essentielles d'*Origanum onites* et d'*Origanum vulgare*. Les effets des huiles essentielles sur la motilité totale, la motilité progressive, la viabilité, les anomalies et la peroxydation des lipides ont été évalués à 0, 4, 8, 24, 28, 32 et 48 heures de stockage. Les principaux composés trouvés dans Origanum onites étaient le thymol (44,59%), le carvacrol (25,94%) et le γ-terpinène (13,22%), alors que le carvacrol (81,5%) était dominant dans *Origanum vulgare*. Les résultats indiquent que la qualité du sperme est influencée par la concentration des huiles essentielles d'*Origanum onites* et d'*Origanum vulgare*. Après 48 heures de stockage, les échantillons supplémentés avec 0,01% d'*Origanum onites* ont montré une motilité totale et progressive, une viabilité et une intégrité membranaire significativement plus élevées, ainsi qu'une anormalité plus faible par rapport au contrôle. De même, l'huile essentielle d'*Origanum vulgare* a montré des effets comparables sur la motilité totale, la viabilité et l'intégrité de la membrane. En outre, après 48 heures de stockage, le contrôle présentait la plus forte concentration de malondialdéhyde, qui est un indicateur de stress oxydatif ($p < 0,05$). Ces résultats suggèrent que 0,01% d'huiles essentielles ont un impact positif sur les spermatozoïdes du bouc Beni Arous pendant le stockage à 4°C, en réduisant le stress oxydatif.

Mots clés : Beni Arous, Sperme de bouc, conservation, huile essentielle, *Origanum onites*, *Origanum vulgare*.

تأثير الزيوت الأساسية لأوريجانوم أونيت وأوريجانوم فولجار على جودة السائل المنوي للماعز المبرد

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ملخص

كان الهدف من هذه الدراسة هو تحليل تأثير زيوت أوريجانوم أونيت وأوريجانوم فولجار الأساسية على جودة السائل المنوي لماعز بني عروس أثناء التخزين عند 4 درجات مئوية. تم جمع عينات السائل المنوي مرة واحدة في الأسبوع من ستة ذكور لمدة عشرة أسابيع، وتم تخزينها في حليب خالي الدسم مع تركيزات مختلفة (0 ٪ (الشاهد)، 0.01 ٪، و0.05 ٪) من زيوت أوريجانوم أونيت وأوريجانوم فولجار الأساسية .تم تقييم آثار الزيوت الأساسية على الحركة الكلية، والحركة التقدمية، والحيوية، والتشكل غير الطبيعي وسلامة غشاء البلازما، وبيروكسيد الدهون بعد 0 و4 و8 و24 و28 و32 و48 ساعة من التخزين .كانت المركبات الرئيسية الموجودة في أوريجانوم أونيت هي الثيمول (44.59٪)، كارفاكرول (25.94٪)، وبي تيربينين (13.22٪)؛ بينما كان كارفاكرول (81.5٪) هو السائد في أوريجانوم فولجار .أشارت النتائج إلى أن جودة الحيوانات المنوية تأثرت بتركيز أوريجانوم أونيت وزيوت أوريجانوم فولجار الأساسية. بعد 48 ساعة من التخزين، أظهرت العينات المكملة بنسبة 0.01 ٪ من أوريجانوم أونيت أعلى بكثير من الحركة الكلية والتقدمية، والقدرة على البقاء، وسلامة الغشاء بالإضافة إلى انخفاض التشكل غير الطبيعي مقارنةً بالمجموعة الشاهدة. وبالمثل، أظهر زيت أوريجانوم فولجار الأساسي تأثيرات مماثلة على الحركة الكلية، والحيوية، وسلامة الغشاء. بالإضافة إلى ذلك، بعد 48 ساعة من التخزين، كان لعنصر الشاهد أعلى تركيز من المالونديالديهيد، وهو مؤشر على الإجهاد التأكسدي ($p < 0.05$). تشير هذه النتائج إلى أن 0.01٪ من الزيوت العطرية لها تأثير إيجابي على الحيوانات المنوية لماعز بني عروس أثناء التخزين عند 4 درجات مئوية، من خلال تقليل الإجهاد التأكسدي.

الكلمات المفتاحية: بني عروس، نطفة الماعز، الحفظ، الزيوت الأساسية، أوريجانوم أونيت، أوريجانوم فولجار

Introduction

Essential oils are complex mixtures of natural compounds extracted from aromatic plants, they have been widely used in traditional medicine and recently gained interest in reproductive biology due to their antioxidant and antimicrobial properties (Tisserand &Young, 2014). Sperm preservation is crucial step for maintaining genetic diversity in livestock and enhancing breeding programs and can be challenging due to the susceptibility of spermatozoa to oxidative stress (Kowalczyk et al., 2019). Semen quality can deteriorate during storage and transport, leading to reduced fertility and reproductive performance. The use of essential oils as additives to sperm extenders has been proposed as a strategy to improve sperm quality during storage (Ros-Santaella & Pintus, 2012). Essential oils are believed to improve semen quality by reducing oxidative stress and bacterial growth, as well as enhancing sperm motility, viability, and acrosome integrity (Kchikich et al., 2022; Cavalleri et al., 2018). Several studies have investigated the impact of different essential oils on sperm preservation and their protective effects against oxidative damage (Mbaye et al., 2019). This area of research has great potential for improving the efficiency of animal reproduction and may lead to the development of novel sperm preservation techniques.

Origanum onites and *Origanum vulgare* are known for their high content of phenolic compounds such as carvacrol and thymol. These compounds possess antioxidant, antimicrobial, and anti-inflammatory properties (Tisserand & Young, 2014), which make them potential candidates for use in semen preservation. Skim milk has been widely used as a basic diluent for semen preservation due to its rich composition of nutrients, buffering capacity, and osmolality that can help protect sperm cells (Bai et al. 2020). Liquid storage of semen involves cooling to 4°C to reduce metabolic activity and slow down the rate of aging and death of sperm cells. The use of essential oils of *Origanum onites* and *Origanum vulgare* diluted in skim milk may provide a natural and effective approach to preserving buck semen, which can lead to improved reproductive success and genetic progress in goat breeding programs. Therefore, it is important to study the appropriate concentration of essential oils that can improve sperm quality without causing toxicity. The present study aims to investigate the effect of *Origanum onites* and *Origanum vulgare* essential oils on the quality of Beni Arous buck semen during storage at 4°C.

Material and methods

Animals and semen collection

Semen samples were obtained from six male Beni Arous goats, aged between 2 and 3 years old. A total of 60 ejaculates, with ten collections per male, were collected once a week during three months (May–August) using an artificial vagina. The bucks were housed under natural photoperiod at Boukhalef experimental station (35°44′N Latitude, 5°54′ W Longitude) belonging to the National Institute of Agricultural Research (INRA) Center of Tangier. Animals had access to water and fed with a mixture of oat hay and concentrate according to INRA (2007) guidelines. The ejaculate tubes were kept in a 37 °C water bath during the evaluation process, and the semen quality was assessed by determining the volume, concentration, and progressive motility. Only ejaculates with a minimum volume of 0.8 ml, concentration of at least 2.5×10^9 sperm/ml, and progressive motility of 65% or more were selected for the study.

Experimental design

To investigate the effects of essential oils on Beni Arous buck semen quality, semen samples were extended in skim milk supplemented with various concentrations (0%, 0.01%, and 0.05%) of *Origanum onites* and *Origanum vulgare* essential oils and stored at 4°C during 48 hours. The evaluation of sperm motility, viability, membrane integrity, and lipid peroxidation were performed after 0, 4, 8, 24, 28, 32, and 48 hours of storage at 4°C.

Essential oil extraction and chemical composition

The aerial parts of *Origanum onites* and *Origanum vulgare* were collected from the experimental station of Larache of the INRA located in Northern Morocco at 35°8′ N Latitude and 6°8′ W Longitude. The plants were shade-dried for 12 days, and essential oils were extracted from the leaves by hydro-distillation using a Clevenger-type apparatus for 3 hours. The essential oils were then separated by gravity, transferred to dark glass bottles, and stored at 4°C using the Clevenger method. The identification of chemical compounds in the essential oils was performed by using Agilent 6,890 GC® coupled to ITQ 900 MS® with an electron impact scan detector, following the same analytical conditions described by Aitboulahsen et al. (2018).

Total and progressive motility

Sperm total and progressive motility were evaluated using the computer-assisted semen analysis (CASA) system from ISAS® (Proiser R+D SL, Spain), following the method described by El kadili et al. (2019). Briefly, 3.7 µl of semen sample (25×10^6 spz/ml) was placed on a microscope slide, covered with a coverslip, and placed on a heated stage to maintain a temperature of around 37°C. Six random fields were captured using a microscope connected to a camera to determine the percentage of total (%) and progressive motility (%)

Sperm viability and abnormality

Sperm viability and abnormality were evaluated using two different staining methods. Sperm viability was assessed using eosin-nigrosin staining (Minitube®, Germany) to determine the percentage of live and dead sperm according to the method described by Evans and Maxwell (1987). Briefly, 5 µl of semen was mixed with a Nigrosin solution on a microscope slide and allowed to stain for a few minutes. After rinsing, an eosin solution was added to stain non-viable sperm, and the slide was observed under a

microscope at 60x magnification to count the number of viable (unstained) and non-viable (eosin-stained) sperm in several fields. A total of 200 spermatozoa were counted in several fields to calculate the percentage of live and dead spermatozoa. Sperm abnormalities, they were evaluated by preparing a microscope slide with a thin layer of semen and staining the sperm with Diff-Quik solutions as described by El kadili et al. (2019). After staining, 200 sperm were chosen at random from various microscopic fields to establish the proportion of normal versus abnormal sperm.

Membrane integrity

The hypo-osmotic sperm swelling (HOST) test is a technique utilized to evaluate the functional integrity of sperm membranes. To perform this method, semen (400 x 10^6 spz/ml) is diluted in a HOST solution (9g fructose and 4.9g sodium citrate dissolved in 1 liter of distilled water) and incubated at 37°C for 1 hour. Following incubation, the sample is examined under a microscope to identify the presence of swollen sperm with coiled tails, indicating the existence of intact membranes. The percentage of swollen sperm is then calculated (Buckett et al., 1997).

Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) assay was utilized to measure the concentration of malondialdehyde in semen samples. The process involves adding 500 µl of the sample to a thiobarbituric acid solution (which contains 15% w/v trichloroacetic acid, 0.25N hydrochloric acid, 0.375% thiobarbituric acid: w/v in distilled water, and 1% v/v hydroxytoluene butylate at 50 mM). This mixture reacts with per oxidized lipids to form a colored complex that can be quantified spectrophotometrically. The mixture is then heated in a boiling water bath for 10 minutes and subsequently centrifuged at 1500g for 10 minutes to separate the TBARS complex from the mixture. The amount of malondialdehyde is determined by comparing the sample's absorbance to a standard curve generated from the hydrolysis of 1,1,3,3-tetraethoxypropane to produce malondialdehyde, and the results are expressed as nM TBARS/10^8 sperm (Allai et al., 2015).

Statistical analysis

The percentage data were transformed using arcsine normalization transformation (root (p)), and a general linear model of SAS was employed to conduct ANOVA on all data, taking into account the fixed effects of treatment (essential oil x concentration), storage duration. Tukey test was used to compare the different means. The Pearson correlation coefficients between all variables were also analyzed. The data were analyzed using SAS 9.4 software, and a p-value of less than 0.05 was considered statistically significant

Results

Chemical composition

Origanum onites and *Origanum vulgare* essential oil are mainly composed of oxygenated and non-oxygenated monoterpenes. The most predominant component in the *Origanum onites* essential oil was carvacrol, with minor constituents being M-Cymene and γ-Terpinene. *Origanum vulgare* essential oil mainly contains carvacrol, thymol and γ-Terpinene, and with lower quantities of M-Cymene, α-Humulene and α-Phellandrene (Table 1).

Table 1. *Origanum onites* and *Origanum vulgare* essential oils composition

Compounds (%)	*Origanum onites*	*Origanum vulgare*	Retention Index (DIMS5P)
α-Phellandrene	1.02	1.43	1004.1
M-Cymene	5.31	10.1	1022.0
Ocimene	1.84	0.4	1047.7
γ-Terpinene	5.26	13.22	1059.7
Cis-sabinene hydrate	0.96	0.66	1066.5
Thymol	-	44.59	1290.1
Carvacrol	80.15	25.94	1300.4
α-Humulene	0.92	2.78	1453.1

DIMS5P: dimethylsilicone with 5% phenyl groups.

Total and progressive motility

The effects of diluents and storage duration on motility are reported in table 2. Total and progressive motility were significantly impacted by storage duration. Diluents enriched with 0.01% of *Origanum onites* and *Origanum vulgare* essential oils were found to increase total motility at 8 hours and progressive motility at 4 hours, compared to the control group. However, diluents enriched with 0.05% of essential oils showed a decrease in motility parameters at all measured time points. Following 48 hours of storage at 4°C, 0.01% of *Origanum onites* essential oil was more effective than the control in sustaining total and progressive sperm motility, while *Origanum vulgare* essential oil only improved total motility ($p < 0.05$).

Table 2. Impact of different concentrations of *Origanum onites* and *Origanum vulgare* essential oils, and storage duration at 4°C, on the percentage of total and progressive motility in Beni Arouss buck semen

Parameter		Storage duration						
		0h	4h	8h	24h	28h	32h	48h
Total motility	OO1	93.60 ± 1.00 ^{aA}	89.70 ± 1.22 ^{abA}	89.43 ± 4.00 ^{abA}	87.77 ± 3.73 ^{bA}	85.80 ± 1.61 ^{bA}	79.20 ± 1.54 ^{cB}	75.23 ± 2.47 ^{cA}
	OO2	14.07 ± 2.14 ^{aB}	9.23 ± 4.11 ^{bB}	8.17 ± 2.36 ^{bcC}	5.00 ± 1.32 ^{cdC}	3.83 ± 1.53 ^{dC}	1.73 ± 1.29 ^{dC}	1.93 ± 0.59 ^{dD}
	OV1	95.20 ± 2.15 ^{aA}	93.40 ± 2.01 ^{aA}	92.07 ± 2.48 ^{aA}	88.40 ± 1.04 ^{bA}	87.53 ± 2.37 ^{bA}	83.47 ± 1.90 ^{cA}	72.03 ± 1.55 ^{dB}
	OV2	8.53 ± 4.81 ^{aC}	5.93 ± 2.71 ^{abB}	2.87 ± 0.21 ^{bcD}	2.57 ± 0.50 ^{bcC}	1.47 ± 0.72 ^{cC}	1.10 ± 0.98 ^{cC}	1.43 ± 1.32 ^{cD}
	CTR	95.27 ± 1.25 ^{aA}	88.73 ± 3.17 ^{bA}	80.53 ± 2.03 ^{cB}	80.13 ± 1.58 ^{cB}	74.37 ± 5.37 ^{dB}	78.70 ± 0.80 ^{cdB}	66.70 ± 1.55 ^{eC}
Progressive motility	OO1	75.37 ± 2.06 ^{aA}	72.17 ± 3.40 ^{aA}	71.00 ± 3.36 ^{aA}	58.93 ± 1.46 ^{bA}	54.33 ± 1.16 ^{cB}	53.73 ± 0.68 ^{cA}	54.13 ± 4.00 ^{cA}
	OO2	5.80 ± 0.61 ^{aB}	3.83 ± 1.23 ^{abC}	3.33 ± 1.53 ^{bC}	2.00 ± 1.00 ^{bcB}	1.00 ± 1.00 ^{cD}	1.13 ± 1.63 ^{cC}	0.80 ± 0.35 ^{cC}
	OV1	74.50 ± 0.46 ^{aA}	73.17 ± 1.40 ^{aA}	60.50 ± 4.79 ^{bB}	59.13 ± 2.33 ^{bA}	58.03 ± 1.61 ^{bA}	52.10 ± 1.35 ^{cA}	45.33 ± 3.01 ^{dB}
	OV2	3.20 ± 2.49 ^{aB}	1.70 ± 1.15 ^{abC}	0.00 ± 0.00 ^{bC}	0.00 ± 0.00 ^{bB}	0.00 ± 0.00 ^{bD}	0.13 ± 0.23 ^{bC}	0.50 ± 0.46 ^{bC}
	CTR	74.07 ± 1.26 ^{aA}	64.07 ± 1.90 ^{bB}	56.97 ± 3.92 ^{cB}	56.43 ± 6.83 ^{cA}	47.77 ± 3.07 ^{dC}	48.87 ± 0.91 ^{dB}	41.93 ± 0.21 ^{dB}

$^{A, B, C, D, E:}$ Different capital letter indicate a significant effect of essential oil for the same storage duration (p<.05). $^{a, b, c, d, e, f}$ Different lower case indicates a significant effect of storage duration for the same essential oil concentration (p<.05). Data are expressed as mean ± SD. OO1, Origanum onite 0.01%; OO2, Origanum onite 0.05%; OV1, Origanum vulgare 0.01%; OV2, Origanum vulgare 0.05%; CTR, Control.

Viability and morphology

During the storage, there is a significant decrease in the percentage of viability and an increase in abnormalities in all treatments, as shown in Table 3. The addition of both essential oils at 0.01% increased sperm viability after 48 hours of storage, with higher values for *Origanum onites* than *Origanum vulgare* ($p < 0.05$). However, the comparison between the concentrations of essential oils on viability revealed that the addition of *Origanum onites* and *Origanum vulgare* at 0.05% had a strong and significant spermicidal effect after 0 hours of storage.

Table 3. Impact of different concentrations of *Origanum onites* and *Origanum vulgare* essential oils, and storage duration at 4°C, on the percentage of viability and abnormality in Beni Arouss buck semen.

Parameter		Storage duration						
		0h	4h	8h	24h	28h	32h	48h
Viability	OO1	97.83 ± 1.26 ^{aA}	95.50 ± 1.32 ^{abA}	95.17 ± 1.76 ^{abA}	92.83 ± 0.29 ^{bcA}	90.50 ± 0.50 ^{cdA}	89.17 ± 1.04 ^{dA}	83.67 ± 3.33 ^{eA}
	OO2	29.00 ± 8.19 ^{aB}	9.67 ± 2.08 ^{bD}	7.17 ± 2.25 ^{bC}	6.33 ± 3.51 ^{bC}	4.67 ± 3.06 ^{bC}	5.67 ± 1.15 ^{bD}	2.67 ± 1.15 ^{bD}
	OV	97.17 ± 0.29 ^{aA}	96.67 ± 0.76 ^{abA}	94.67 ± 0.58 ^{bcA}	92.83 ± 0.76 ^{cA}	88.33 ± 1.53 ^{dA}	86.33 ± 2.31 ^{dB}	80.00 ± 1.00 ^{eB}
	OV2	35.33 ± 2.57 ^{aB}	12.83 ± 2.36 ^{bC}	6.33 ± 1.15 ^{cC}	5.00 ± 1.32 ^{cC}	3.50 ± 0.50 ^{cdC}	1.33 ± 1.53 ^{dE}	0.67 ± 0.58 ^{dD}
	CTR	97.00 ± 1.00 ^{aA}	88.50 ± 1.32 ^{bB}	85.17 ± 1.04 ^{cB}	82.33 ± 0.58 ^{dB}	80.17 ± 0.76 ^{eB}	79.67 ± 1.15 ^{eC}	71.50 ± 0.87 ^{fC}
Abnormality	OO1	7.83 ± 0.29 ^{bAB}	8.17 ± 0.76 ^{bB}	11.67 ± 1.15 ^{aBC}	12.33 ± 2.08 ^{aAB}	12.67 ± 1.26 ^{aB}	13.17 ± 1.61 ^{aC}	14.00 ± 1.73 ^{aB}
	OO2	8.67 ± 1.15 ^{dAB}	11.00 ± 1.00 ^{dA}	14.67 ± 0.58 ^{cA}	15.67 ± 0.58 ^{bcA}	17.17 ± 2.47 ^{abcA}	17.67 ± 1.53 ^{abA}	19.67 ± 1.53 ^{aA}
	OV	6.83 ± 1.04 ^{cB}	8.67 ± 1.04 ^{cB}	12.33 ± 2.47 ^{bABC}	13.67 ± 1.04 ^{bAB}	13.17 ± 1.76 ^{bB}	17.00 ± 1.00 ^{aAB}	17.50 ± 0.50 ^{aA}
	OV2	9.33 ± 1.15 ^{dA}	12.33 ± 2.08 ^{cdA}	13.83 ± 1.53 ^{bcAB}	13.33 ± 2.47 ^{bcAB}	12.50 ± 2.18 ^{cdB}	16.00 ± 1.00 ^{abAB}	19.00 ± 1.00 ^{aA}
	CTR	5.00 ± 1.00 ^{eC}	7.50 ± 0.50 ^{deB}	10.00 ± 1.00 ^{cdC}	11.83 ± 2.47 ^{cB}	14.67 ± 0.58 ^{bAB}	15.17 ± 0.76 ^{abBC}	17.67 ± 2.31 ^{aA}

$^{A, B, C, D, E:}$ Different capital letter indicate a significant effect of essential oil for the same storage duration (p<.05). $^{a, b, c, d, e, f}$ Different lower case indicates a significant effect of storage duration for the same essential oil concentration (p<.05). Data are expressed as mean ± SD. OO1, Origanum onite 0.01%; OO2, Origanum onite 0.05%; OV1, Origanum vulgare 0.01%; OV2, Origanum vulgare 0.05%; CTR, Control.

Treatments added with essential oils showed a significant increase in abnormal sperm after 0 hours of storage compared to the control group. After 48 hours of storage at 4°C, the 0.01% of *Origanum onites* essential oil showed the lowest percentage of abnormalities compared to the other extenders ($p < 0.05$).

Membrane integrity and lipid peroxidation

Sperm membrane integrity parameters of all treatments decreased gradually over the storage period ($p < 0.05$; Table 4). Compared to the control, sperm diluted in medium containing 0.01% *Origanum Onite* essential oil showed significantly higher membrane integrity after 8, 28, 32 and 48 hours of storage, similarly, those diluted with 0.01% *Origanum Vulgare* essential oil showed significantly higher membrane integrity after 4, 8, 24, 28 and 48 hours of storage. In contrast, the high concentrations of both essential oils (0.05%) showed the lowest values during storage.

Additionally, the results showed an increase in the level of malondialdehyde during storage in all diluents ($p < 0.05$; Table 4). However, the addition of 0.01% *Origanum Onite* essential oils resulted in a significant decrease in malondialdehyde formation at 8, 24, and 48 hours of storage, while the addition of 0.01% *Origanum Vulgare* essential

oils resulted in a similar decrease at 8, 24, 32, and 48 hours of storage. The lowest value of malondialdehyde formation was achieved after 48 hours of storage with 0.05% *Origanum Onite* and *Origanum Vulgare* essential oils compared to the control ($p < 0.05$).

Table 4. Impact of different concentrations of *Origanum onites* and *Origanum vulgare* essential oils, and storage duration at 4°C, on the percentage of membrane integrity and the concentration of malondialdehyde (nM TBARS/10^8 spermatozoa) in Beni Arouss buck semen.

Parameter		Storage duration						
		0h	4h	8h	24h	28h	32h	48h
Membrane integrity	OO1	89.67 ± 1.53 ^{aA}	88.10 ± 1.01 ^{aAB}	85.13 ± 2.01 ^{abB}	79.87 ± 7.71 ^{bcAB}	81.33 ± 1.61 ^{bcA}	76.33 ± 1.61 ^{cA}	69.33 ± 2.31 ^{dA}
	OO2	11.83 ± 1.26 ^{aB}	6.63 ± 2.03 ^{bC}	5.50 ± 0.50 ^{bcD}	3.33 ± 2.25 ^{cdC}	2.50 ± 1.00 ^{deC}	0.77 ± 0.25 ^{eC}	0.67 ± 0.76 ^{eD}
	OV1	93.03 ± 1.50 ^{aA}	90.33 ± 1.26 ^{aA}	89.83 ± 2.36 ^{aA}	84.33 ± 1.61 ^{bA}	80.17 ± 2.36 ^{cA}	73.00 ± 2.29 ^{dB}	66.00 ± 2.18 ^{eB}
	OV2	6.47 ± 2.64 ^{aC}	4.13 ± 1.01 ^{bC}	1.73 ± 0.87 ^{cE}	1.17 ± 1.26 ^{cC}	0.50 ± 0.50 ^{cC}	0.43 ± 0.40 ^{cC}	0.20± 0.26 ^{cD}
	CTR	92.93 ± 2.18 ^{aA}	87.03± 1.75 ^{bB}	77.40 ± 0.85 ^{cC}	76.80 ± 1.47 ^{cdB}	73.63 ± 3.00 ^{deB}	71.67 ± 1.76 ^{eB}	61.83 ± 1.89 ^{fC}
Lipid peroxidation	OO1	0.38 ± 0.08 ^{fA}	0.56 ± 0.10 ^{eA}	0.76 ± 0.07 ^{dBC}	0.98 ± 0.11 ^{cB}	1.26 ± 0.11 ^{bA}	1.30 ± 0.05 ^{bA}	1.58 ± 0.11 ^{aB}
	OO2	0.48 ± 0.09 ^{dA}	0.55 ± 0.06 ^{dA}	0.74 ± 0.03 ^{cBC}	0.85 ± 0.07 ^{cBC}	1.11 ± 0.11 ^{bAB}	1.27 ± 0.08 ^{aA}	1.14 ± 0.12 ^{abC}
	OV1	0.37 ± 0.08 ^{eA}	0.55 ± 0.06 ^{dA}	0.69 ± 0.09 ^{cdC}	0.79 ± 0.09 ^{cC}	1.13 ± 0.12 ^{bAB}	1.13 ± 0.09 ^{bB}	1.52 ± 0.10 ^{aB}
	OV2	0.46 ± 0.08 ^{dA}	0.61 ± 0.02 ^{cA}	0.86 ± 0.08 ^{bB}	0.86 ± 0.03 ^{bBC}	1.05 ± 0.07 ^{aB}	1.12 ± 0.08 ^{aB}	1.02 ± 0.12 ^{aC}
	CTR	0.51 ± 0.03 ^{fA}	0.67 ± 0.04 ^{eA}	1.03 ± 0.10 ^{dA}	1.14 ± 0.10 ^{cA}	1.21 ± 0.02 ^{cAB}	1.37 ± 0.05 ^{bA}	1.79 ± 0.04 ^{aA}

$^{A, B, C, D, E:}$ Different capital letter indicate a significant effect of essential oil for the same storage duration ($p<.05$). $^{a, b, c, d, e, f}$ Different lower case indicates a significant effect of storage duration for the same essential oil concentration ($p<.05$) Data are expressed as mean ± SD. OO1, Origanum onite 0.01%; OO2, Origanum onite 0.05%; OV1, Origanum vulgare 0.01%; OV2, Origanum vulgare 0.05%; CTR, Control.

Correlation

Correlations among semen quality parameters are reported in table 5. The results suggest a significant positive correlation between sperm viability and both total and progressive motility ($r = 0.9$; 0.9; $p < 0.001$). Furthermore, there was a negative correlation between abnormal sperm percentage and total and progressive motility ($r = -0.4$; -0.5; $p < 0.001$), and a negative correlation between abnormality and viability ($r = -0.4$; $p < 0.001$). Membrane integrity was positively correlated with total and progressive motility ($r = 0.9$; 0.9; $p < 0.001$) as well as viability ($r = 0.9$; $p < 0.001$), while it was negatively correlated with abnormality ($r = -0.4$; $p < 0.001$). Lipid peroxidation was only correlated with sperm abnormality ($r = 0.7$; $p < 0.001$)

Table 5. Correlation coefficients among the quality parameters of chilled semen in buck goats.

	Total motility	Progressive motility	Viability	Abnormality	Membrane integrity	Lipid peroxidation
Total motility	-	0.98 ***	0.99 ***	-0.41 ***	0.99 ***	NS
Progressive motility		-	0.98 ***	-0.47 ***	0.99 ***	NS
Viability			-	-0.44 ***	0.99 ***	NS
Abnormality				-	-0.43 ***	0.68 ***
Membrane integrity					-	NS
Lipid peroxidation						-

*Note: The threshold for determining significant correlation coefficients in the analysis was set at ***p < 0.001; NS: non- significant correlation.*

Discussion

The present study aims to examine the effects of *Origanum onites* and *Origanum vulgare* essential oil on the quality of Beni Arouss buck semen during 48 hours of liquid storage at 4°C. Essential oils are natural compounds extracted from plants that have antimicrobial and antioxidant properties (Freires et al., 2015). These properties make them potentially useful in extenders for semen preservation, as they can help to protect sperm from oxidative stress and bacterial contamination during storage. Several studies have shown the positive effects of essential oils on mammalian semen preservation, including *Origanum vulgare* (Liu et al., 2017), *Origanum majorana* (Kchikich et al., 2022), and *Eucalyptus globulus* (Mbaye et al., 2019).

Origanum onites and *Origanum vulgare* essential oils are promising sources of bioactive compounds that have potential therapeutic applications. These essential oils exhibit a range of biological activities, including antioxidant properties due to their phenolic compounds, and other terpenoids and antimicrobial activity against various bacterial, fungal, and viral pathogens (Tisserland & Young, 2014). Such properties make them potentially beneficial in semen storage extenders by safeguarding semen against oxidative stress and bacterial contamination (Kchikich et al., 2022).

The chemical composition analysis of *Origanum onites* and *Origanum vulgare* essential oils revealed the presence of phenolic components, such as thymol and carvacrol, which may contribute to reduce oxidative stress and prevent bacterial growth in Beni Arous buck semen during storage at 4°C. The introduction of essential oils derived from *Origanum onites* and *Origanum vulgare* had a significant impact on all parameters related to semen quality. When administered at a low concentration of

0.01%, these oils demonstrated a protective effect, enhancing motility, viability, membrane integrity, and minimizing abnormality and lipid peroxidation, in comparison to the control. Conversely, when the concentration was increased to 0.05%, the same essential oils demonstrated a spermicidal and immobilizing effect. In similar studies, *Thymus satureioides*, *Origanum majorana* (Kchikich et al., 2021;) and *Zataria multiflora* (Nezhad & Mehr, 2018) essential oils showed a toxic effect on spermatozoa at high concentrations but improved sperm motility, viability, abnormality, membrane integrity, and lipid peroxidation when added in low concentrations, likely due to their antioxidant and antimicrobial properties

During storage, sperm undergo oxidative stress due to the accumulation of reactive oxygen species (ROS) causing lipid peroxidation, protein oxidation, DNA damage, resulting in decreased motility, viability and normal sperm morphology (Revel et al., 2001). However, carvacrol and thymol, found in essential oils derived from *Origanum onites* and *Origanum vulgare,* can scavenge ROS and prevent lipid peroxidation and damage to cellular structures such as the acrosome and flagellum (Tisserland & Young, 2014), thus protecting sperm from oxidative stress. The plasma membrane is an essential component of sperm cells, and its integrity is crucial for maintaining sperm viability and motility. At appropriate concentrations, carvacrol and thymol can bind to sperm plasma membrane proteins to stabilize it and prevent damage or disruption, while reducing its fluidity and increasing its rigidity (Chikhoun et al., 2015; Hyldgaard et al., 2012).

Our study revealed a significant correlation between sperm morphology and lipid peroxidation. We also observed that the abnormal sperm was negatively correlated with the quality parameters, indicating compromised motility and structural integrity. These findings suggest that lipid peroxidation can affect sperm quality by inducing morphological changes and impairing functional properties, leading to reduced fertility potential.

When added at a concentration of 0.05%, these essential oils were harmful to spermatozoa, resulting in a decrease in motility, viability, and integrity, as well as an increase in abnormalities after storage at 4°C ($p < 0.05$). Various studies have found that high concentration of essential oils can have immobilizing, spermicidal, and distorting effects (Chikhoun et al., 2015; Elmi et al., 2017). For instance, *Thymus satureioides* (which contains 28% thymol and 31% carvacrol) was found to reduce immediately total and progressive motility, viability, and membrane integrity, while increasing abnormalities after being stored at 4°C (Kchikich et al., 2021). Similarly, Chikhoun et al., (2015) discovered that exposure to *Thymus munbyanus* essential oil (which contains 52% thymol) resulted in an immediate decrease in motility and viability, as well as an increase in abnormal sperm heads showing dilation of the acrosomal vesicle after a 30 minutes incubation.

These toxic effects may be due to an inactivation of the antioxidant defense mechanisms of sperm, resulting in increased ROS accumulation and oxidative damage (Llana-Ruiz-Cabello et al., 2015). High concentrations of phenolic compounds have been shown to induce apoptosis or programmed cell death in sperm (Chikhoun et al., 2015). In addition, some phenolics can also interfere with intracellular signaling pathways, leading to disruptions in normal cell function (Wang et al., 2020). At high concentrations, carvacrol and thymol can cause thinning and disorganization of the

plasma membrane, resulting in increased permeability and leakage of intracellular contents (Kong et al., 2019), this can lead to decreased sperm motility and integrity.

Conclusion

The use of *Origanum onites* and *Origanum vulgare* essential oil at 0.01% has been shown to have a positive impact on the preservation of sperm quality during storage for up to 48 hours in Beni Arouss goat buck. However, higher concentrations induce spermicidal and immobilizing effects. The potential of *Origanum onites* and *Origanum vulgare* essential oil in preserving sperm quality presents a promising way for improving livestock reproduction and could have important implications in the field of goat breeding.

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Conflict of interest

The authors declare no conflict of interest

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