Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Effect of Bromhexine Supplementation on Reproductive Performance and its Implications for Meat and Milk Production in Damascus Goat Bucks during the Non-Breeding Season

Layla Abd-Alsattar Sadiq Laylani[1] , Ansam Hussein Ali[2] , Yahya Waheeb Ibrahim

1. Community Health Department , Technical Medical Institute Kirkuk, Nortern Technical University
2. Northern Technical University , Al-dor Technical Institute , Department of pharmacy techniques
3. Department of Biotechnology , College of Applied Sciences , University of Samarra

ARTICLE INFO

Article History:

Received: 2025/12/28
Accepted: 2026/2/6

Keywords:

Bromhexine,

Damascus goat,

semen quality,

meat production,

milk supply,

food security,

animal husbandry

DOI: 10.48311/fsct.2026.118564.83009.

*Corresponding Author E-Mail:

hiba.alwaan@uokerbala.edu.iq,
hibaalsalame@g.alzahu.edu.iq

ABSTRACT

Seasonal fluctuations in reproductive performance of Damascus goats pose significant challenges to year-round milk and meat production, impacting the sustainability of supply chains. Bromhexine, a mucolytic agent, has shown potential in improving semen quality by reducing viscosity, which could indirectly enhance breeding efficiency and, consequently, animal-derived food production. This study investigated the effect of bromhexine supplementation on semen quality parameters in Damascus goat bucks during the non-breeding season, with a focus on implications for reproductive management and its downstream effects on milk and meat output. Twelve sexually mature Damascus bucks (2-3 years old, 45-55 kg) were randomly assigned to a treatment group receiving oral bromhexine (1 mg/kg day) or a control group receiving a placebo for 60 days during the non-breeding season. Semen was collected and evaluated for volume, liquefaction time, viscosity, sperm concentration, motility, viability, and morphology. Serum reproductive hormones and seminal plasma biochemical components were also analyzed. Bromhexine significantly reduced liquefaction time (8.2±1.1 min vs. 15.7±1.8 min, $p<0.001$) and seminal plasma viscosity (2.8±0.3 cP vs. 4.6±0.5 cP, $P<0.01$). Progressive sperm motility improved (66.3±3.4% vs. 52.8±4.2%, $p<0.05$). No significant differences were observed in sperm concentration, viability, morphology, hormonal profiles, or seminal plasma biochemistry. Bromhexine improves key semen physical properties, potentially enhancing breeding efficiency during the non-breeding season. This improvement can support more consistent year-round breeding, leading to stabilized milk and meat production. The absence of hormonal alterations suggests that bromhexine does not interfere with the natural physiology of food-producing animals, making it a viable option for integration into sustainable animal husbandry practices aimed at optimizing food supply chain resilience.

1.Introduction

The stability and sustainability of animal-derived food supply chains represent a cornerstone of global food security and economic viability within the agricultural sector. Small ruminants, particularly goats, play an indispensable role in this context, serving as a primary source of high-quality protein, essential fats, and micronutrients for a significant portion of the world's population, especially in arid and semi-arid regions [1]. Among the most esteemed breeds, the Damascus goat (Capra hircus), also known as the Shami goat, stands out for its exceptional dairy yield, superior meat quality, and remarkable adaptability to challenging environmental conditions [2]. This breed constitutes a critical agricultural asset across the Middle East and Mediterranean basins, forming the backbone of many local economies and subsistence farming systems. However, the productivity of this valuable genetic resource is severely constrained by a fundamental biological limitation: pronounced seasonal reproductive patterns [3]. Like many photoperiod-sensitive species inhabiting subtropical and temperate zones, Damascus goats exhibit robust seasonal variations in reproductive activity, governed by the complex interplay between daylight length (photoperiod) and the neuroendocrine axis [4]. This seasonality manifests most acutely during the long-day photoperiods of spring and early summer, a period universally recognized as the non-breeding season for this breed in the Northern Hemisphere [5]. During this phase, a significant decline in reproductive performance is observed, characterized by diminished libido, reduced testicular volume, lowered testosterone secretion, and a marked deterioration in semen quality parameters [6]. The seminal characteristics most adversely affected include ejaculate volume, sperm concentration, progressive motility, and, most notably, the physical properties of the semen itself, such as increased viscosity and prolonged liquefaction time [7]. These physiological changes are not merely academic observations; they translate directly into tangible impediments for breeding programs. The elevated viscosity and delayed liquefaction physically entrap spermatozoa within a mucoid network, restricting their liberation and subsequent motility, even when the sperm cells themselves possess intrinsic functional competence [8]. Consequently, the fertilizing capacity of ejaculates collected during the non-breeding season is drastically reduced, leading to lower conception rates from both natural mating and artificial insemination (AI) [9]. From the perspective of food systems and industrial agriculture, this reproductive seasonality poses a formidable challenge to the consistent and efficient production of goat meat (chevon) and milk. The direct consequence is a highly seasonal and pulsed supply of kids and milk, creating a "feast or famine" cycle in the market [10]. This irregularity disrupts the steady flow of raw materials to processing plants, complicates inventory management for retailers, and leads to price volatility that disadvantages both producers and consumers [11]. For dairy operations, the inability to maintain year-round breeding results in seasonal lactation curves, forcing processors to deal with fluctuating milk intake and creating inefficiencies in capacity utilization [12]. In meat production systems, the concentration of kidding within a short window leads to a glut of animals for slaughter at specific times, depressing market prices, followed by periods of scarcity [13]. Therefore, mitigating seasonal infertility is not merely a goal of reproductive physiology but a critical imperative for optimizing the resilience, profitability, and sustainability of the entire small ruminant food industry.

Traditional strategies to overcome seasonal reproductive suppression have primarily focused on manipulating the endocrine axis. These include the administration of exogenous hormones such as melatonin, gonadotropin-releasing hormone (GnRH) analogs, and prostaglandins, or the implementation of

controlled photoperiod regimens using light-proof housing [14]. While these methods can be effective, they are often fraught with practical and economic limitations. Hormonal treatments raise concerns regarding residue accumulation in animal products, a significant issue in an increasingly health-conscious consumer market, and may require prolonged withdrawal periods, complicating management schedules [15]. Furthermore, the use of hormonal interventions can face regulatory hurdles and consumer skepticism, potentially limiting market access [16]. Photoperiod manipulation, on the other hand, necessitates substantial capital investment in specialized infrastructure and increases operational energy costs, making it unfeasible for many small to medium-scale producers [17]. This creates a compelling need for alternative, non-hormonal, and economically viable interventions that can enhance reproductive performance during the non-breeding season without these associated drawbacks. It is within this context that the potential of bromhexine hydrochloride demands exploration. Bromhexine (2-amino-3,5-dibromo-N-cyclohexyl-N-methylbenzylamine) is a well-established mucolytic agent, originally developed and widely used in human and veterinary medicine for the treatment of respiratory conditions characterized by excessive and viscous mucus [18]. Its primary mechanism of action involves the depolymerization of mucopolysaccharide fibers, specifically by breaking down disulfide bonds and reducing the molecular weight of complex glycoproteins, thereby decreasing the viscosity of respiratory secretions and facilitating their clearance [19]. The chemical structure of bromhexine enables it to interact with glycoprotein complexes beyond the respiratory tract, including those present in various biological fluids [20]. Given that the seminal plasma of goats and other mammals contains substantial quantities of mucoproteins, glycosaminoglycans, and semenogelin proteins that contribute to the formation of the post-ejaculatory coagulum and overall semen viscosity [21], it is biologically plausible that bromhexine could exert a similar mucolytic effect within the seminal plasma. By depolymerizing these mucoid components, bromhexine could potentially accelerate semen liquefaction, reduce residual viscosity, and physically liberate spermatozoa, thereby enhancing their observable motility without necessarily altering their intrinsic biological quality or the underlying hormonal milieu [22]. This proposed peripheral mechanism of action is particularly attractive from a food industry standpoint, as it circumvents the need for direct hormonal intervention, thereby alleviating concerns related to hormonal residues in meat and milk and aligning with consumer preferences for "cleaner" production practices. Evidence from other species provides supportive, albeit preliminary, justification for this approach. Studies in rams have demonstrated that bromhexine administration can reduce semen coagulation time and improve progressive motility, with effects being more pronounced during the non-breeding season [23]. In bulls, treatment with bromhexine was associated with improved semen flowability and processing characteristics, which are critical for AI and semen cryopreservation [24]. Similarly, research in roosters has shown that bromhexine supplementation can ameliorate heat stress-induced semen quality decline, including reductions in viscosity and improvements in motility [25].

These cross-species findings suggest a conserved mechanism that could be effectively harnessed in Damascus goats. However, a critical research gap persists. While the effects of bromhexine on semen physical properties are promising, a comprehensive investigation within the specific context of Damascus goat bucks during the non-breeding season, with explicit consideration of the implications for meat and milk production systems, is lacking. It remains to be conclusively determined whether the improvements in semen parameters translate into a practical tool for stabilizing annual production cycles. Furthermore, it is essential to verify that bromhexine's action is indeed peripheral and does not perturb the hypothalamic-pituitary-gonadal

(HPG) axis, as this would reinforce its safety profile for use in food-producing animals. Confirming the absence of effects on key seminal plasma biomarkers of accessory sex gland function (e.g., fructose from seminal vesicles, citric acid from the prostate) would further solidify the hypothesis that its benefits are purely physical and not secretory [26]. Therefore, this study was designed to bridge this gap by conducting a rigorous evaluation of the effects of oral bromhexine supplementation on a comprehensive panel of semen quality parameters in Damascus goat bucks during the non-breeding season. We employed advanced analytical techniques, including computer-assisted sperm analysis (CASA) for precise motility kinematics and standardized rheological measurements for viscosity, to obtain a detailed understanding of the treatment effects [28,29]. Concurrently, we monitored systemic reproductive hormone concentrations and seminal plasma biochemistry to delineate the mechanism of action. Our central hypothesis is that bromhexine will act primarily as a mucolytic agent within the seminal plasma, significantly reducing liquefaction time and viscosity, which will in turn enhance sperm motility parameters by alleviating physical constraints, all without inducing significant changes in spermatogenesis, sperm viability, endocrine function, or the biochemical composition of seminal plasma. The findings of this research are anticipated to provide a scientifically sound, practical, and safe strategy to enhance reproductive efficiency in a key dairy and meat breed, thereby contributing directly to the stabilization of animal protein supplies, the reduction of seasonal production fluctuations, and the promotion of greater sustainability within the goat farming sector.

2. Materials and Methods

2.1 Ethical Considerations

This study was conducted in accordance with institutional guidelines for animal care and use, following the principles outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (Approval No. IACUC-2024-037). Animals were handled humanely throughout the experimental period, and procedures were designed to minimize stress and discomfort.

2.2 Experimental Animals and Management

Twelve sexually mature Damascus bucks, aged 2-3 years with body weights ranging from 45 to 55 kg, were selected from a commercial goat farm located at 35°N latitude. Bucks were chosen based on previous breeding soundness examinations confirming normal reproductive capacity and semen quality during the breeding season. Animals were housed in individual pens (2.5 × 3 m) under natural photoperiod conditions, with free access to fresh water. The diet consisted of a concentrate mixture formulated to meet nutritional requirements for breeding males (16% crude protein, 2.8 Mcal/kg metabolizable energy), alfalfa hay, and seasonal green fodder. All animals were vaccinated according to regional protocols and dewormed with ivermectin two weeks before the experiment commenced. Bucks were free from clinical diseases throughout the experimental period, as confirmed by weekly veterinary examinations.

2.3 Experimental Design and Treatment

The experiment was conducted during the non-breeding season (April through June), corresponding to the period of long-day photoperiods (average 14.2 hours daylight) and naturally reduced reproductive activity in this breed. Following a two-week adaptation period during which all animals were trained for semen collection, bucks were randomly allocated to two equal groups (n=6 per group) using a completely randomized design:

Treatment Group (BRX): Bucks received oral bromhexine hydrochloride (Bisolvon®, Boehringer Ingelheim, pharmaceutical grade purity >98%) at a dose of 1 mg/kg body weight daily. The drug was

dissolved in 10 ml normal saline and administered orally using a dosing syringe each morning at 0800 h before feeding. Body weights were recorded weekly and doses adjusted accordingly.

Control Group (CON): Bucks received an equivalent volume of oral placebo (normal saline, 10 ml) using the same administration protocol to control for handling effects.

The treatment period extended for 60 days, covering approximately two complete spermatogenic cycles in goats (approximately 49 days from spermatogonia to mature spermatozoa), ensuring that all spermatozoa evaluated post-treatment had been exposed to bromhexine throughout their development and epididymal maturation.

2.4 Semen Collection and Initial Evaluation

Semen samples were collected twice weekly (Tuesday and Friday mornings between 0900-1100 h) using an artificial vagina maintained at 42-45°C, with an estrous doe used as teaser. Collection frequency and timing were standardized to minimize variation due to sexual rest periods. For each collection, bucks were allowed two false mounts followed by semen collection on the third mount to ensure adequate sexual stimulation. Immediately after collection, samples were protected from light and temperature shock by placing collection tubes in a portable water bath maintained at 37°C.

Initial macroscopic evaluation was performed within 2 minutes of collection and included:

Ejaculate Volume: Measured directly from the graduated collection tube to the nearest 0.05 ml, excluding the gel fraction when present.

Color and Consistency: Recorded using standardized descriptors (color: white, creamy-white, yellowish; consistency: watery, normal, thick, very thick).

pH: Measured using a calibrated digital pH meter (Hanna Instruments, HI98103) at 37°C immediately after collection.

Gross Motility (Wave Motion): Assessed subjectively by placing a drop of undiluted semen on a pre-warmed slide (37°C) and observing wave patterns under 40× magnification, scored from 0 (no movement) to 5 (rapid, vigorous waves).

2.5 Liquefaction Time Assessment

Liquefaction time, defined as the interval from ejaculation until semen could flow freely as individual droplets, was determined using the standardized tilt method [31]. Immediately after collection, the sample tube was tilted at 45° every minute. Complete liquefaction was recorded when semen flowed as separate drops rather than forming viscous threads. Liquefaction time was recorded in minutes from the moment of ejaculation. This parameter is critical for understanding bromhexine's primary mechanism of action.

2.6 Seminal Plasma Viscosity Measurement

Seminal plasma viscosity was measured using a calibrated Ostwald viscometer maintained at 37°C in a water bath [32]. After liquefaction, 2 ml of semen was centrifuged at 3000 × g for 10 minutes at room temperature to separate seminal plasma from spermatozoa. The supernatant was carefully collected without disturbing the sperm pellet. Viscosity was determined by measuring the flow time of seminal plasma through the viscometer capillary and comparing it to distilled water (reference standard). Dynamic viscosity was calculated using the formula:

$$\eta = (t \times \rho \times K)$$

where η = dynamic viscosity (centipoise, cP), t = flow time (seconds), ρ = density (assumed 1.0 g/ml for seminal plasma), and K = viscometer constant (determined by calibration with standard fluids). All measurements were performed in duplicate, and the mean value was recorded.

2.7 Sperm Motility Analysis

Progressive sperm motility was evaluated using both subjective and objective computerized methods:

Subjective Assessment: After complete liquefaction, a 10 µl aliquot of semen was diluted 1:20 with pre-warmed (37°C) phosphate-buffered saline (PBS, pH 7.2). A 5 µl drop was placed on a pre-warmed microscope slide and covered with a 22×22 mm coverslip. Progressive motility was assessed at 400× magnification using phase-contrast microscopy (Olympus CX43) equipped with a heated stage maintaining 37°C. A single experienced technician, blinded to treatment groups, evaluated at least 200 spermatozoa per sample across five random fields. Spermatozoa were classified as progressively motile (forward movement, regardless of speed), non-progressively motile (all other movement patterns), or immotile. Results were expressed as percentage of progressively motile spermatozoa.

Computer-Assisted Sperm Analysis (CASA): Sperm kinematic parameters were objectively analyzed using a CASA system (AndroVision®, Minitube GmbH, Germany) with settings optimized for caprine spermatozoa: frame rate 60 Hz, minimum 25 frames captured per field, detection parameters set for goat sperm head size (30-50 µm²). Samples were diluted 1:20 in pre-warmed Tris-citrate extender (pH 6.8) and loaded into a Leja 4-chamber slide with 20 µm depth. At least seven microscopic fields and 500 spermatozoa per sample were analyzed at 37°C. Parameters evaluated included:

- Total motility (TM, %): percentage of spermatozoa showing any movement
- Progressive motility (PM, %): percentage showing forward progression (VAP >25 µm/s and STR >80%)
- Curvilinear velocity (VCL, µm/s): total distance traveled divided by time
- Straight-line velocity (VSL, µm/s): straight-line distance from start to end point divided by time
- Average path velocity (VAP, µm/s): velocity along the smoothed average path
- Linearity (LIN, %): (VSL/VCL) × 100, measuring deviation from straight line
- Straightness (STR, %): (VSL/VAP) × 100, measuring departure from average path
- Wobble (WOB, %): (VAP/VCL) × 100, measuring oscillation of actual path
- Amplitude of lateral head displacement (ALH, µm): magnitude of lateral head movement
- Beat cross frequency (BCF, Hz): frequency of flagellar beating

2.8 Sperm Concentration Determination

Sperm concentration was determined using a hemocytometer method with confirmatory spectrophotometric analysis:

Hemocytometer Method: Semen was diluted 1:100 in formol-saline solution (containing 0.9% NaCl and 4% formaldehyde to immobilize spermatozoa) and thoroughly mixed. After a 5-minute equilibration period, the diluted sample was loaded into both chambers of an improved Neubauer hemocytometer. Spermatozoa were counted in five large squares (1 mm² each) in both chambers under 400× magnification. The concentration was calculated using the standard formula and expressed as $\times 10^9$ spermatozoa/ml. Samples with coefficient of variation >15% between chambers were recounted.

Spectrophotometric Verification: Sperm concentration was also estimated using a photometer (Accucell®, IMV Technologies, France) calibrated specifically for caprine semen according to manufacturer protocols. This method provided rapid verification of hemocytometer results.

2.9 Sperm Viability Assessment

Sperm viability was assessed using the eosin-nigrosin staining technique [33]. A 10 µl aliquot of liquefied semen was mixed with 20 µl of eosin-nigrosin stain (1% eosin Y and 10% nigrosin in distilled water) on a pre-warmed glass slide at 37°C. After 30 seconds of gentle mixing, a thin smear was prepared using the edge of another slide and allowed to air-dry at room temperature. Slides were examined within 2 hours at 1000× magnification under oil immersion using bright-field microscopy. At least 200 spermatozoa per sample were evaluated in random fields. Spermatozoa with intact plasma membranes exclude the eosin dye and appear white (live), while those with compromised membranes take up the stain and appear

pink to red (dead). Results were expressed as percentage of viable (unstained) spermatozoa.

2.10 Hypo-osmotic Swelling Test (HOST)

Plasma membrane functional integrity was assessed using the hypo-osmotic swelling test [34]. A 100 µl aliquot of liquefied semen was gently mixed with 1 ml of pre-warmed (37°C) hypo-osmotic solution (100 mOsm/kg, consisting of 0.735 g sodium citrate dihydrate and 1.351 g D-fructose dissolved in 100 ml distilled water, osmolality verified using a freezing-point osmometer). Samples were incubated at 37°C for 60 minutes in a water bath. After incubation, a 10 µl drop was placed on a microscope slide and examined at 400× magnification using phase-contrast microscopy. At least 200 spermatozoa were evaluated in random fields. Spermatozoa with functional plasma membranes respond to hypo-osmotic stress by allowing water influx, resulting in characteristic tail swelling or coiling patterns (classified as g-type curling). Non-responsive spermatozoa with compromised membrane function maintain normal straight tail morphology. Results were expressed as percentage of HOST-positive spermatozoa.

2.11 Sperm Morphological Evaluation

Sperm morphology was evaluated on eosin-nigrosin stained smears prepared for viability assessment. At least 200 spermatozoa per sample were examined at 1000× magnification under oil immersion using bright-field microscopy. Morphological abnormalities were classified according to standard veterinary andrological protocols into three main categories:

Head abnormalities: Pyriform (pear-shaped), narrow at the base, small or large heads, double heads, detached heads, abnormal acrosome

Midpiece abnormalities: Bent or folded midpiece, thickened midpiece (suggesting mitochondrial sheath defects), proximal cytoplasmic droplets (located at head-midpiece junction), distal cytoplasmic droplets (located along midpiece or at midpiece-principal piece junction)

Tail abnormalities: Coiled or bent tail, short tail, detached tail, double tail

Normal spermatozoa were defined as those with normal oval head shape, intact acrosome, straight midpiece and principal piece without droplets, and no detached components. Total abnormal spermatozoa percentage was calculated by summing all abnormality categories. Individual abnormality types were also recorded separately.

2.12 Seminal Plasma Biochemical Analysis

For biochemical analyses, semen samples from each buck were centrifuged at 3000 × g for 15 minutes at 4°C within 30 minutes of collection. The seminal plasma supernatant was carefully separated without disturbing the sperm pellet, aliquoted into 1.5 ml cryovials, and immediately frozen at -80°C until batch analysis. All samples from a given parameter were analyzed in the same assay run to eliminate inter-assay variation.

Total Protein Concentration: Measured using the Bradford protein assay [35] with reagent from Sigma-Aldrich. Bovine serum albumin was used as standard (concentration range 0.1-1.0 mg/ml). Absorbance was read at 595 nm using a spectrophotometer. Results were expressed as mg/ml.

Fructose Concentration: Determined using the resorcinol colorimetric method [36]. Fructose, primarily secreted by seminal vesicles in response to androgens, serves as an indicator of seminal vesicle secretory function and provides the principal energy substrate for spermatozoa. A standard curve was prepared using D-fructose solutions (0-5 mg/ml). Results were expressed as mg/ml seminal plasma.

Citric Acid Concentration: Measured enzymatically using a commercial kit (Sigma-Aldrich, MAK057). Citric acid is primarily secreted by the prostate gland and serves as a marker of prostatic function. Results were expressed as mg/ml seminal plasma.

2.13 Hormone Assays

Blood samples (10 ml) were collected via jugular venipuncture from all bucks at three time points: immediately before treatment initiation (Day 0), at the mid-point of treatment (Day 30), and at the end of the experimental period (Day 60). Samples were collected in plain vacuum tubes between 0800-0900 h to minimize circadian variation in hormone concentrations. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 × g for 15 minutes. Serum was separated, aliquoted, and stored at -20°C until analysis. All samples were analyzed in duplicate within the same assay batch.

Testosterone Concentration: Measured using enzyme-linked immunosorbent assay (ELISA) with commercial kits (DRG International Inc., USA, Catalog No. EIA-1559) previously validated for caprine serum. The assay is based on competitive binding between testosterone in samples and horseradish peroxidase-conjugated testosterone for a fixed number of antibody binding sites. Intra-assay coefficient of variation was 4.2%, inter-assay coefficient of variation was 7.8%, and analytical sensitivity was 0.083 ng/ml. Results were expressed as ng/ml.

Follicle-Stimulating Hormone (FSH): Determined using a competitive ELISA kit (MyBioSource Inc., USA, Catalog No. MBS269090) validated for ruminant species. Intra-assay CV was 5.1%, inter-assay CV was 9.2%, and sensitivity was 0.5 mIU/ml. Results were expressed as mIU/ml.

Luteinizing Hormone (LH): Measured using ELISA with commercial kits (Cusabio Technology LLC, USA, Catalog No. CSB-E06869Cp) validated for caprine serum following manufacturer's protocols. Intra-assay CV was 4.8%, inter-assay CV was 8.5%, and sensitivity was 0.39 mIU/ml. Results were expressed as mIU/ml.

2.14 Statistical Analysis

All data were initially screened for outliers using Grubbs' test (α=0.05). Data were then tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Data meeting parametric assumptions were analyzed using repeated measures analysis of variance (ANOVA) with treatment as the between-subject factor and collection time (weeks) as the within-subject factor, using the mixed procedure in SAS (SAS Institute Inc., version 9.4, Cary, NC, USA). The statistical model included treatment, time, and treatment × time interaction as fixed effects, with individual animal as a random effect.

For semen parameters collected multiple times per week, data were averaged by week for each animal before statistical analysis to avoid pseudoreplication. When significant main effects or interactions were detected ($P<0.05$), pairwise comparisons of least squares means were performed using Tukey's honestly significant difference (HSD) adjustment for multiple comparisons.

Data not meeting parametric assumptions after transformation attempts (logarithmic, square root, or arcsine transformations) were analyzed using non-parametric alternatives. The Mann-Whitney U test was used for comparing two independent groups, and the Kruskal-Wallis test followed by Dunn's post-hoc test was used when comparing more than two groups.

Hormone concentration data were analyzed using repeated measures ANOVA with three time points (Day 0, 30, 60). When sphericity assumption was violated (Mauchly's test, $P<0.05$), Greenhouse-Geisser correction was applied.

Pearson correlation coefficients were calculated to examine relationships among semen quality parameters, with particular emphasis on relationships between viscosity, liquefaction time, and motility parameters. Correlation matrices were constructed and visualized using GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA).

All statistical analyses were two-tailed, and statistical significance was declared at $P<0.05$. Trends were discussed at $0.05<P<0.10$. Results are presented as least squares mean ± standard error of the mean (SEM) unless otherwise stated.

Sample size determination was based on previous studies examining bromhexine effects on ram semen

quality [22], with power analysis indicating that n=6 per group would provide 80% power to detect a 15% difference in progressive motility with α=0.05.

3. Results

3.1 Macroscopic Semen Characteristics

Macroscopic semen characteristics of Damascus bucks during the non-breeding season are presented in Table 1. Ejaculate volume did not differ significantly between groups (1.08±0.09 ml in BRX vs. 0.95±0.08 ml in CON, P=0.31). All samples exhibited creamy-white coloration typical of caprine semen, with no differences in color between groups. Semen pH was statistically similar (6.85±0.12 in BRX vs. 6.82±0.11 in CON, P=0.86). However, subjective assessment of consistency revealed that bromhexine-treated samples were easier to handle and showed improved flowability during laboratory processing, though this was not formally quantified.

Table 1. Effect of Bromhexine on Macroscopic Semen Characteristics

Parameter	Control	Bromhexine	P-value
Ejaculate volume (ml)	0.95±0.08	1.08±0.09	0.31
pH	6.82±0.11	6.85±0.12	0.86
Color	Creamy-white	Creamy-white	-
Gross motility (0-5)	3.2±0.3	3.7±0.3	0.24

Values are least squares means ± SEM

3.2 Liquefaction Time and Seminal Plasma Viscosity

Bromhexine supplementation dramatically reduced semen liquefaction time compared to controls (Table 2). The mean liquefaction time in the BRX group was 8.2±1.1 minutes, significantly shorter than 15.7±1.8 minutes in the CON group (P<0.001). This represents a 48% reduction in liquefaction time, indicating substantial enhancement of the liquefaction process (Figure 1).

Seminal plasma viscosity, measured after complete liquefaction, was significantly lower in bromhexine-treated bucks (2.8±0.3 cp vs. 4.6±0.5 cp, p<0.01). This 39% reduction in viscosity confirms the mucolytic action of bromhexine on seminal plasma components. The temporal pattern showed that these effects became evident after approximately 14-21 days of treatment and remained stable throughout the remainder of the experimental period.

Table 2. Effect of Bromhexine on Liquefaction Time and Seminal Plasma Viscosity

Parameter	Control	Bromhexine	P-value
Liquefaction time (min)	15.7 ± 1.8^{a}	8.2 ± 1.1^{b}	<0.001
Seminal plasma viscosity (cP)	4.6 ± 0.5^{a}	2.8 ± 0.3^{b}	<0.01

Values are least squares means ± SEM. $^{a'b}$ Different superscripts within rows indicate significant differences (p<0.05).

3.3 Sperm Concentration and Total Sperm Output

Sperm concentration showed no significant difference between treatment groups (Table 3). The mean concentration in the BRX group was $3.2\pm0.4\times10^{9}$/ml compared to $2.9\pm0.3\times10^{9}$/ml in the CON group (p=0.58). Consequently, total sperm output per

ejaculate, calculated as volume × concentration, also did not differ significantly (3.46±0.52×10⁹ in BRX vs. 2.76±0.38×10⁹ in CON, p=0.29). These findings indicate that bromhexine's effects did not extend to spermatogenic processes or testicular sperm production.

Table 3. Effect of Bromhexine on Sperm Concentration

Parameter	Control	Bromhexine	P-value
Sperm concentration (×10⁹/ml)	2.9±0.3	3.2±0.4	0.58
Total sperm per ejaculate (×10⁹)	2.76±0.38	3.46±0.52	0.29

Values are least squares means ± SEM

3.4 Sperm Motility Parameters

Progressive sperm motility, assessed by subjective evaluation, was significantly improved in the bromhexine treatment group (66.3±3.4% vs. 52.8±4.2% in controls, p<0.05) (Table 4). This 26% relative improvement represents the most functionally important finding of the study, as progressive motility is a critical predictor of fertility.

Computer-assisted sperm analysis revealed that bromhexine treatment significantly enhanced straight-line velocity (VSL: 52.4±3.8 μm/s vs. 41.7±3.2 μm/s, p<0.05), reflecting improved linear progressive movement. Average path velocity (VAP) showed a similar trend (68.5±4.1 μm/s vs. 59.2±3.7 μm/s, p=0.09). However, curvilinear velocity (VCL), which represents total distance traveled, did not differ significantly (112.5±7.2 μm/s in BRX vs. 105.8±6.8 μm/s in CON, p=0.50).

Linearity (LIN) and straightness (STR) indices showed improvement in the BRX group, though only STR reached statistical significance (75.8±2.4% vs. 70.2±2.8%, p<0.05). Importantly, wobble (WOB) and beat cross frequency (BCF), which reflect intrinsic flagellar beat patterns, showed no significant differences between groups (WOB: 60.9±2.1% vs. 59.6±2.3%, P=0.68; BCF: 7.8±0.6 Hz vs. 7.5±0.5 Hz, P=0.72), indicating that bromhexine did not alter the fundamental motility apparatus of spermatozoa.

Table 4. Effect of Bromhexine on Sperm Motility Parameters

Parameter	Control	Bromhexine	P-value
Progressive motility (%)	52.8±4.2[b]	66.3±3.4[a]	<0.05
Total motility (%)	64.5±4.8	74.2±4.1	0.15
VCL (μm/s)	105.8±6.8	112.5±7.2	0.50
VSL (μm/s)	41.7±3.2[b]	52.4±3.8[a]	<0.05
VAP (μm/s)	59.2±3.7	68.5±4.1	0.09
LIN (%)	39.4±2.6	46.6±2.9	0.07
STR (%)	70.2±2.8[b]	75.8±2.4[a]	<0.05
WOB (%)	59.6±2.3	60.9±2.1	0.68
ALH (μm)	3.8±0.4	3.6±0.3	0.72
BCF (Hz)	7.5±0.5	7.8±0.6	0.72

VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; LIN: linearity; STR: straightness; WOB: wobble; ALH: amplitude of lateral head displacement; BCF: beat cross frequency. Values are least squares means ± SEM. [a'b] Different superscripts within rows indicate significant differences (p<0.05)

3.5 Sperm Viability and Membrane Integrity

Sperm viability, assessed by eosin-nigrosin staining, did not differ significantly between groups (Table 5). The percentage of viable spermatozoa was 77.4±3.2% in the BRX group compared to 73.8±3.8% in controls (p=0.48). Similarly, the hypo-osmotic swelling test revealed no significant difference in plasma membrane functional integrity (71.2±3.5% HOST-positive in BRX vs. 67.5±4.1% in CON, p=0.51). These findings indicate that bromhexine treatment did not substantially affect sperm membrane integrity or viability status.

Table 5. Sperm Viability and Membrane IntegritySperm

Parameter	Control	Bromhexine	P-value
Sperm viability (%)	73.8±3.8	77.4±3.2	0.48
HOST-positive (%)	67.5±4.1	71.2±3.5	0.51

HOST: hypo-osmotic swelling test. Values are least squares means ± SEM

3.6 Sperm Morphology

Total sperm morphological abnormalities were statistically similar between groups (Table 6). The BRX group exhibited 11.8±1.4% total abnormalities compared to 13.7±1.6% in the CON group (p=0.40). When categorized by abnormality type, no significant differences were observed for head abnormalities (3.2±0.6% vs. 3.8±0.7%, p=0.54), midpiece abnormalities (4.5±0.8% vs. 5.2±0.9%, p=0.58), or tail abnormalities (4.1±0.7% vs. 4.7±0.8%, p=0.60).

Cytoplasmic droplet retention, including both proximal and distal droplets, also showed no significant difference between groups (2.3±0.4% in BRX vs. 2.8±0.5% in CON, P=0.47). These results indicate that bromhexine treatment did not affect sperm morphogenesis or epididymal maturation processes during the non-breeding season.

Table 6. Effect of Bromhexine on Sperm Morphology

Abnormality Type	Control (%)	Bromhexine (%)	P-value
Head abnormalities	3.8±0.7	3.2±0.6	0.54
Midpiece abnormalities	5.2±0.9	4.5±0.8	0.58
Cytoplasmic droplets	2.8±0.5	2.3±0.4	0.47
Tail abnormalities	4.7±0.8	4.1±0.7	0.60
Total abnormalities	13.7±1.6	11.8±1.4	0.40

Values are least squares means ± SEM

3.7 Seminal Plasma Biochemical Composition

Seminal plasma total protein concentration showed no significant difference between groups (Table 7). The BRX group had 58.7±4.5 mg/ml compared to 55.3±4.1 mg/ml in controls (p=0.59). Fructose concentration, an indicator of seminal vesicle secretory function, was also statistically similar (9.8±0.9 mg/ml in BRX vs. 9.2±0.8 mg/ml in CON, p=0.64). Citric acid concentration, reflecting prostatic function, did not differ significantly (12.9±1.2 mg/ml vs. 12.1±1.1 mg/ml, p=0.63).

These biochemical findings indicate that bromhexine did not substantially alter the secretory activity of accessory sex glands or change the gross biochemical composition of seminal plasma, supporting the

hypothesis that its effects are primarily physical (mucolytic) rather than metabolic or secretory.

Table 7. Effect of Bromhexine on Seminal Plasma Biochemical Composition

Parameter	Control	Bromhexine	P-value
Total protein (mg/ml)	55.3±4.1	58.7±4.5	0.59
Fructose (mg/ml)	9.2±0.8	9.8±0.9	0.64
Citric acid (mg/ml)	12.1±1.1	12.9±1.2	0.63

Values are least squares means ± SEM

3.8 Serum Hormone Concentrations

Serum reproductive hormone concentrations are presented in Table 8. Testosterone concentrations did not differ significantly between groups at any time point. Mean testosterone levels across all time points were 2.7±0.3 ng/ml in the CON group and 2.9±0.4 ng/ml in the BRX group (treatment effect: P=0.67; time effect: p=0.82; treatment × time interaction: p=0.91).

Similarly, FSH concentrations showed no treatment effect (3.5±0.4 mIU/ml in CON vs. 3.7±0.5 mIU/ml in BRX, p=0.76) or time-related changes (P=0.68). LH concentrations were also unaffected by bromhexine treatment (2.3±0.3 mIU/ml vs. 2.5±0.4 mIU/ml, p=0.70).

These hormonal findings confirm that bromhexine's effects on semen quality parameters occurred independently of alterations in the hypothalamic-pituitary-gonadal axis, supporting a peripheral mechanism of action localized to seminal plasma rather than systemic endocrine effects.

Table 8. Effect of Bromhexine on Serum Hormone Concentrations

Hormone	Control	Bromhexine	P-value
Testosterone (ng/ml)	2.7±0.3	2.9±0.4	0.67
FSH (mIU/ml)	3.5±0.4	3.7±0.5	0.76
LH (mIU/ml)	2.3±0.3	2.5±0.4	0.70

FSH: follicle-stimulating hormone; LH: luteinizing hormone. Values are least squares means ± SEM averaged across three time points (Days 0, 30, 60)

3.9 Correlation Analysis

Correlation analysis revealed significant relationships among key semen quality parameters (Table 9). Liquefaction time was strongly negatively correlated with progressive motility (r=-0.76, p<0.001), indicating that longer liquefaction times were associated with reduced motility. Similarly, seminal plasma viscosity showed a strong negative correlation with progressive motility (r=-0.71, p<0.001).

Straight-line velocity (VSL) was negatively correlated with both liquefaction time (r=-0.68, p<0.01) and viscosity (r=-0.64, P<0.01). Interestingly, liquefaction time and viscosity were strongly positively correlated with each other (r=0.82, P<0.001), suggesting that these two parameters reflect related aspects of seminal plasma physical properties.

No significant correlations were found between sperm concentration and any motility parameters (p>0.05 for all comparisons), nor between hormone concentrations and semen quality parameters (p>0.05). These correlation patterns support the interpretation that bromhexine's beneficial effects on motility are mediated through its effects on seminal

plasma physical properties rather than through alterations in sperm production or hormonal status.

Table 9. Correlation Coefficients Among Selected Semen Quality Parameters

Variables	Correlation coefficient (r)	P-value
Liquefaction time vs. Progressive motility	-0.76	<0.001
Viscosity vs. Progressive motility	-0.71	<0.001
Liquefaction time vs. VSL	-0.68	<0.01
Viscosity vs. VSL	-0.64	<0.01
Liquefaction time vs. Viscosity	0.82	<0.001
Sperm concentration vs. Progressive motility	0.12	0.58
Testosterone vs. Progressive motility	0.08	0.71

VSL: straight-line velocity

4. Discussion

The present study demonstrates that oral bromhexine supplementation significantly improves specific semen quality parameters in Damascus bucks during the non-breeding season through a clearly defined mucolytic mechanism. The selective nature of these improvements—dramatic reductions in liquefaction time and viscosity coupled with enhanced progressive motility, while leaving sperm production, viability, morphology, and hormonal status unaffected—provides compelling evidence that bromhexine acts primarily on the physical properties of seminal plasma rather than through biological effects on spermatogenesis or endocrine function.

4.1 Mucolytic Mechanism and Liquefaction Enhancement

The most striking finding of this study is the 48% reduction in semen liquefaction time in bromhexine-treated bucks (8.2 vs. 15.7 minutes). This dramatic acceleration of the liquefaction process directly reflects bromhexine's primary pharmacological action: depolymerization of mucopolysaccharide complexes through disruption of disulfide bonds and reduction of glycoprotein molecular weight [16, 17].

Freshly ejaculated mammalian semen forms a coagulum or gel-like structure primarily composed of high-molecular-weight glycoproteins (particularly semenogelins) secreted by the seminal vesicles, cross-linked through disulfide bonds and non-covalent interactions [7]. This transient coagulation serves evolutionary functions, including maintaining sperm concentration at the site of deposition and potentially protecting spermatozoa from the hostile environment of the female reproductive tract [8]. However, for spermatozoa to achieve their full fertilizing potential, they must be liberated from this matrix through liquefaction.

Physiological liquefaction occurs through the action of proteolytic enzymes, particularly prostate-specific antigen (PSA) and other serine proteases secreted by the prostate gland, which cleave semenogelin proteins [9]. In seasonal breeders during the non-breeding period, reduced androgen-dependent secretory activity of accessory sex glands may result in suboptimal ratios of coagulating proteins to lytic enzymes, leading to prolonged liquefaction times and elevated viscosity [10, 11].

Bromhexine's mucolytic action complements and accelerates physiological liquefaction by chemically disrupting the mucopolysaccharide networks that contribute to semen viscosity. By breaking disulfide bonds and reducing polymer chain length, bromhexine facilitates more rapid dissolution of the coagulum structure [18,37]. The observed liquefaction time reduction from 15.7 to 8.2 minutes represents a clinically significant improvement, bringing non-

breeding season samples closer to the liquefaction times typically observed during peak breeding season (usually 5-10 minutes in healthy bucks) [38].

The parallel 39% reduction in seminal plasma viscosity (from 4.6 to 2.8 cP) provides independent confirmation of bromhexine's mucolytic effects. Viscosity measurements were performed after complete liquefaction, indicating that bromhexine not only accelerates the liquefaction process but also produces a final seminal plasma with fundamentally lower viscosity [32]. This residual effect suggests that bromhexine's action extends beyond merely speeding up enzymatic liquefaction—it appears to alter the final physical state of seminal plasma by reducing the molecular weight and complexity of mucopolysaccharide networks that persist even after enzymatic cleavage of semenogelin proteins [39].

4.2 Physical Liberation and Enhanced Sperm Motility

The significant improvement in progressive motility (52.8% to 66.3%, a 26% relative increase) represents the most functionally important outcome of this study. This enhancement can be mechanistically attributed to physical liberation of spermatozoa from viscous constraints rather than to improvement of intrinsic sperm function.

Several lines of evidence support this interpretation. First, the CASA analysis revealed that while straight-line velocity (VSL) increased significantly (from 41.7 to 52.4 μm/s), curvilinear velocity (VCL) did not change significantly (105.8 vs. 112.5 μm/s). VCL represents the total distance traveled by a spermatozoon regardless of direction and reflects the inherent capacity of the flagellar apparatus to generate movement [23]. The unchanged VCL indicates that bromhexine did not enhance the fundamental motility-generating machinery of spermatozoa.

Second, wobble (WOB) and beat cross frequency (BCF), which reflect intrinsic flagellar beat patterns and amplitude [29], showed no significant differences between groups. If bromhexine had direct effects on sperm metabolism, energy production, or flagellar function, changes in these parameters would be expected [40]. Their absence strongly suggests that the motility improvements result from environmental factors rather than cellular modifications.

Third, the improvements in linearity (LIN) and especially straightness (STR) indices indicate that spermatozoa in less viscous medium achieve more efficient, directional swimming patterns [30]. When spermatozoa swim through highly viscous medium, their forward progression is impeded by increased drag forces, resulting in more circular, less productive swimming trajectories [41]. By reducing viscosity, bromhexine decreased medium resistance, allowing spermatozoa to convert their flagellar beating into more efficient forward progression.

The strong negative correlations between viscosity and progressive motility ($r=-0.71$) and between viscosity and VSL ($r=-0.64$) provide statistical support for this mechanistic model. These correlations demonstrate that seminal plasma physical properties significantly influence observed sperm motility, independent of intrinsic sperm quality.

From a biophysical perspective, sperm swimming is governed by the Reynolds number (Re), which describes the ratio of inertial to viscous forces [42]. At the microscopic scale of spermatozoa, re is extremely low ($<10^{-4}$), meaning that viscous forces dominate and sperm propulsion is highly sensitive to medium viscosity [43]. Even modest reductions in viscosity can substantially improve swimming efficiency and velocity, as demonstrated in this study.

The practical implications are significant. During the non-breeding season, Damascus bucks naturally experience elevated semen viscosity that physically restricts sperm motility even when the spermatozoa themselves are functionally competent [4, 5]. By pharmacologically reducing viscosity, bromhexine unmasks the true motility potential of these cells, potentially improving their fertility despite the photoperiod-induced suppression of reproductive function.

4.3 Absence of Effects on Sperm Production and Concentration

The finding that sperm concentration remained statistically similar between groups (3.2×10^9/ml vs. 2.9×10^9/ml, P=0.58) is equally important as the positive findings, as it helps define the boundaries of bromhexine's action. Sperm concentration reflects testicular spermatogenic efficiency and epididymal sperm reserves [34]. The absence of treatment effects indicates that bromhexine does not influence spermatogenesis, which is consistent with the observed lack of hormonal changes and with bromhexine's known mechanism of action [16].

This finding contrasts with some previous reports suggesting increased sperm concentration following bromhexine treatment [21, 22]. These apparent discrepancies likely reflect methodological differences in sperm counting. In highly viscous semen, incomplete sample homogenization during dilution can result in uneven sperm distribution and artificially low concentration estimates [35, 36]. Additionally, viscous semen may retain aggregated sperm clumps that are counted as single units, further underestimating true concentration [37].

In the present study, concentration measurements were performed after complete liquefaction and with meticulous homogenization procedures, likely providing more accurate estimates that were less susceptible to viscosity-related artifacts. The similarity in concentrations between groups therefore likely reflects true biological equivalence rather than technical limitations.

The unchanged ejaculate volume (1.08 ml vs. 0.95 ml, P=0.31) deserves comment. While some previous studies reported increased ejaculate volume with bromhexine treatment [12, 20], these apparent increases may partly reflect improved recovery of viscous samples from collection vessels rather than true increases in secretory volume [33]. Highly viscous semen tends to adhere to vessel walls and may not flow completely into graduated portions of collection tubes, potentially underestimating true volume [44].

However, even acknowledging potential collection artifacts, the present study's results suggest that bromhexine does not substantially enhance accessory sex gland fluid secretion. This interpretation is supported by the finding that seminal plasma protein, fructose, and citric acid concentrations—biochemical markers of accessory gland secretory activity [15]—remained statistically similar between groups. If bromhexine had stimulated secretory epithelial cells, increases in these parameters would be expected [45].

4.4 Viability, Membrane Integrity, and Morphology: Biological Equivalence

The absence of significant differences in sperm viability (77.4% vs. 73.8%, P=0.48), membrane functional integrity assessed by HOST (71.2% vs. 67.5%, P=0.51), and morphological abnormalities (11.8% vs. 13.7%, P=0.40) provides important insights into bromhexine's mechanism of action and practical utility.

These findings indicate that bromhexine treatment during the non-breeding season does not fundamentally alter sperm biology, cellular integrity, or structural quality. The spermatozoa remain biologically equivalent between groups; what differs is the physical environment in which they swim [46]. This biological equivalence is reassuring from a safety perspective and reinforces the interpretation that bromhexine's benefits derive from physical rather than cellular mechanisms.

The unchanged viability percentages are particularly informative. If bromhexine had antioxidant effects or protected spermatozoa from oxidative stress—mechanisms proposed in some previous studies [26]—improvements in viability would be expected, as oxidative damage is a primary cause of sperm membrane permeabilization and cell death [25], [47]. The absence of viability differences suggests that bromhexine does not possess substantial antioxidant activity in this model, or that oxidative stress was not a limiting factor in sperm viability during this particular experimental period.

Similarly, the lack of morphological improvements, particularly in cytoplasmic droplet retention (2.3% vs. 2.8%, P=0.47), indicates that bromhexine did not enhance epididymal maturation processes [36]. Cytoplasmic droplets are normally shed during

epididymal transit as part of sperm maturation, and their retention reflects incomplete maturation or epididymal dysfunction [48]. Some previous studies suggested that bromhexine might improve epididymal secretory function and enhance maturation [22], but the present findings do not support this hypothesis in Damascus bucks during the non-breeding season.

An alternative explanation for some previous reports of reduced morphological abnormalities with bromhexine treatment relates to assessment artifacts in viscous samples. Mucoid material adhering to spermatozoa can create false impressions of morphological defects [49]. By reducing viscosity and mucoid content, bromhexine may enable more accurate morphological assessment rather than actually reducing true abnormality rates. The present study's rigorous morphological evaluation on properly liquefied samples likely provides a more accurate representation of true morphological status.

4.5 Hormonal Independence and Peripheral Action

The absence of significant effects on testosterone (2.9 vs. 2.7 ng/ml, P=0.67), FSH (3.7 vs. 3.5 mIU/ml, P=0.76), and LH (2.5 vs. 2.3 mIU/ml, P=0.70) concentrations provides definitive evidence that bromhexine's effects occur independently of the hypothalamic-pituitary-gonadal axis. These hormonal findings are consistent with bromhexine's known pharmacology and with previous reports in other species [13, 14, 22].

This hormonal independence has important practical and theoretical implications. First, it distinguishes bromhexine from hormonal interventions used to manage seasonal infertility, such as melatonin, gonadotropin-releasing hormone (GnRH) analogs, or exogenous gonadotropins [13]. These hormonal approaches aim to override photoperiod-mediated suppression of the reproductive axis but carry risks of receptor desensitization, feedback inhibition, and disruption of normal endocrine homeostasis [50].

Second, the peripheral site of action means that bromhexine is unlikely to cause systemic endocrine side effects or interfere with normal reproductive physiology. This safety profile is advantageous for practical application in commercial breeding operations [51]. The drug can potentially be used long-term without concern for hypothalamic-pituitary desensitization or testicular dysfunction that can occur with prolonged hormonal treatments [52].

Third, the maintained low testosterone concentrations during the non-breeding season in both groups (approximately 2.7-2.9 ng/ml, well below breeding season values of 5-8 ng/ml in Damascus bucks [3]) confirm that the experimental period corresponded to genuine seasonal reproductive suppression. This validation is important because it demonstrates that bromhexine's benefits occurred despite persistent photoperiod-mediated hormonal suppression, suggesting practical utility precisely when it is most needed.

The lack of hormonal effects also helps explain why some parameters—particularly those dependent on androgen-regulated secretory processes, such as seminal vesicle fructose production [53]—remained unchanged. Bromhexine does not substitute for or enhance androgenic stimulation of accessory glands; rather, it works within the constraints of existing hormonal status to optimize the physical properties of whatever secretions are produced [54].

4.6 Biochemical Composition: Physical vs. Secretory Effects

The finding that seminal plasma total protein (58.7 vs. 55.3 mg/ml, P=0.59), fructose (9.8 vs. 9.2 mg/ml, P=0.64), and citric acid (12.9 vs. 12.1 mg/ml, P=0.63) concentrations remained statistically similar between groups provides further evidence that bromhexine's effects are primarily physical rather than secretory or metabolic.

Fructose, secreted by seminal vesicles under androgenic control, serves as the primary energy substrate for ejaculated spermatozoa and is a widely used marker of seminal vesicle function [36, 53]. Citric acid, secreted predominantly by the prostate gland, serves both as a buffer and as a substrate for energy metabolism, and reflects prostatic secretory activity [55]. Total protein concentration provides a

general index of overall accessory gland secretory output [45].

If bromhexine functioned as a secretagogue—stimulating epithelial cell secretion as proposed in some studies [10]—increases in these biochemical markers would be expected. Their absence indicates that bromhexine did not substantially enhance the secretory activity of accessory sex glands in this study. This interpretation is consistent with the unchanged ejaculate volume and supports a mechanism localized to modification of existing secretions rather than stimulation of additional secretion.

It is worth noting that bromhexine could theoretically alter the qualitative composition of secretions without changing total quantities of major components. For example, it might modify the glycosylation patterns of mucoproteins or alter the relative proportions of different protein species [56]. Such subtle changes would not be detected by the gross biochemical measurements employed in this study but could contribute to altered physical properties. More sophisticated proteomic or glycomic analyses would be required to detect such alterations [57].

The maintained biochemical composition is reassuring from a physiological perspective, as it suggests that the treated semen retains normal metabolic support systems for spermatozoa. The improved motility observed in bromhexine-treated samples cannot be attributed to enhanced energy substrate availability (unchanged fructose) or altered ionic environment (unchanged citric acid), further supporting the interpretation that improvements derive from reduced physical constraints rather than metabolic enhancement [58].

4.7 Correlation Patterns and Mechanistic Insights

The strong correlations observed between physical parameters (liquefaction time, viscosity) and motility outcomes provide compelling statistical evidence for the proposed mechanistic model. The negative correlation between liquefaction time and progressive motility ($r=-0.76$, $P<0.001$) demonstrates that delayed liquefaction substantially impairs observed sperm motility, likely by prolonging the period during which spermatozoa are physically entrapped in the coagulum [59].

Similarly, the strong negative correlation between viscosity and progressive motility ($r=-0.71$, $P<0.001$) directly implicates medium viscosity as a determinant of sperm swimming efficiency. This relationship is predicted by fundamental principles of low-Reynolds-number hydrodynamics, which govern sperm locomotion at microscopic scales [42, 43]. The consistency between observed correlations and theoretical predictions strengthens confidence in the mechanistic interpretation. The positive correlation between liquefaction time and viscosity ($r=0.82$, $P<0.001$) suggests that these parameters reflect related aspects of seminal plasma physical chemistry—likely the quantity and molecular weight distribution of mucopolysaccharides and glycoproteins that resist enzymatic breakdown and contribute to viscosity [60]. Samples with high mucopolysaccharide content would be expected to exhibit both prolonged liquefaction times (due to more substrate requiring enzymatic cleavage) and elevated residual viscosity (due to incompletely degraded polymers).

The absence of correlations between sperm concentration and motility parameters ($r=0.12$, $P=0.58$) indicates that concentration and motility represent independent aspects of semen quality in this context [61]. This statistical independence supports the biological interpretation that bromhexine affects motility through environmental modification rather than through changes in sperm production or intrinsic sperm characteristics.

Similarly, the lack of correlation between testosterone and motility ($r=0.08$, $P=0.71$) reinforces that the observed improvements occurred independently of hormonal status. This is particularly important given the seasonal context of the study, as it suggests that interventions targeting seminal plasma properties can improve functional outcomes even when hormonal drivers of reproduction remain suppressed [62].

4.8 Seasonal Context and Practical Implications

The demonstration of significant improvements in functionally important semen parameters (liquefaction

time, viscosity, progressive motility) during the challenging non-breeding season has particular practical relevance for Damascus goat production systems. Seasonal infertility represents a major constraint to year-round breeding programs and limits the efficiency of genetic improvement schemes through artificial insemination [6, 27].

Traditional approaches to managing seasonality include hormonal treatments (melatonin, GnRH, gonadotropins), photoperiod manipulation, and introduction of the "male effect" [13, 14]. While these methods can be effective, they often involve complex protocols, significant costs, specialized facilities, or welfare considerations. Photoperiod control requires expensive light-tight housing, hormonal treatments necessitate repeated administration and veterinary oversight, and the male effect has limited utility when males themselves are the limiting factor [50]. Bromhexine supplementation represents a comparatively simple, economically viable intervention that could be readily incorporated into routine management protocols for breeding males during periods of anticipated reproductive decline [51]. The oral administration route is practical for on-farm application, the drug is relatively inexpensive and widely available (originally marketed for respiratory indications), and no special handling or storage requirements are needed beyond those of standard pharmaceuticals [38].

The improvements observed in this study—particularly the 26% increase in progressive motility—could have tangible impacts on breeding outcomes. Progressive motility is among the most critical predictors of fertility in both natural breeding and artificial insemination programs [42, 63]. Ejaculates with >60% progressive motility are generally considered acceptable for AI, while those with <50% are often rejected [64]. The treatment effect observed in this study (moving from 52.8% to 66.3%) could potentially convert marginal-quality ejaculates into acceptable ones, reducing semen wastage and increasing the efficiency of AI programs.

For natural breeding, the enhanced motility could improve conception rates during the non-breeding season, enabling more flexible breeding schedules and year-round kidding patterns. This flexibility is increasingly important in intensive dairy goat operations where consistent milk production throughout the year is economically advantageous [1, 65]. Furthermore, bromhexine treatment might improve the success of semen processing and cryopreservation. Elevated viscosity complicates semen processing procedures, interfering with accurate volume measurement, dilution, and loading into straws [66]. It may also impair cryoprotectant penetration and contribute to suboptimal post-thaw quality [67]. By reducing viscosity, bromhexine could facilitate these technical procedures and potentially improve post-thaw outcomes, though this requires direct investigation.

4.9 Comparison with Previous Studies and Species Differences

The present findings are partially consistent with and partially divergent from previous research on bromhexine effects in other species, highlighting important species-specific differences in responses. In rams, bromhexine supplementation was reported to improve not only semen viscosity and motility but also sperm concentration and viability [14, 22]. In bulls, improvements in semen flowability, motility, and post-thaw quality were observed [21]. In roosters, enhanced semen volume, concentration, and motility were documented [12, 20].

The more selective effects observed in the present study with Damascus bucks—significant improvements in liquefaction time, viscosity, and motility, but not in concentration, viability, or morphology—may reflect several factors. First, species differences in seminal plasma composition and coagulation characteristics could influence the magnitude and scope of bromhexine's effects [68]. Goat semen differs from that of other ruminants in its higher viscosity and more prominent gel fraction [69], potentially making physical properties particularly limiting for motility. Second, methodological differences across studies may account for some apparent discrepancies. Many previous studies did not employ the same rigorous approach to measuring viscosity and liquefaction time, potentially missing these as primary mechanisms. Conversely, apparent

improvements in parameters like concentration may have reflected artifacts of improved sample handling rather than true biological effects [35, 36].

Third, the seasonal timing of studies may influence outcomes. The present study was specifically conducted during the non-breeding season when seminal plasma properties are particularly problematic [10, 11]. Studies conducted during peak breeding season might observe different effect profiles if viscosity is less limiting under optimal reproductive conditions. Fourth, dosage and treatment duration differences could explain variation in results. The present study employed 1 mg/kg body weight for 60 days based on previous work in small ruminants [22], but optimal dosing parameters may differ across species and breeding conditions [40]. Future dose-response studies in goats could refine recommendations.

4.10 Study Limitations and Future Research Directions

While this study provides robust evidence for bromhexine's effects on semen physical properties and motility, several limitations should be acknowledged that suggest directions for future research.

First, the study evaluated semen quality parameters but did not assess actual fertility outcomes through natural breeding or artificial insemination. While progressive motility is strongly correlated with fertility [42, 63], direct fertility trials are needed to confirm that the observed improvements translate to enhanced conception rates and kidding outcomes [70]. Controlled breeding trials comparing pregnancy rates between does breed to bromhexine-treated versus control bucks would provide definitive evidence of practical benefit. Second, the study duration was limited to 60 days covering the core non-breeding season. Longer-term studies extending through the transition into the breeding season would clarify whether bromhexine's benefits persist, diminish, or become unnecessary as photoperiod-driven reproductive function naturally recovers [71]. Additionally, year-round supplementation studies would determine whether continuous treatment maintains benefits without tolerance development or adverse effects [72].

Third, while the study employed 1 mg/kg body weight based on previous research, the optimal dosage for Damascus bucks remains incompletely defined. Dose-response studies examining a range of bromhexine concentrations (e.g., 0.5, 1.0, 2.0, 5.0 mg/kg) would identify the minimum effective dose and maximum safe dose, optimizing cost-effectiveness and safety margins [40, 73].

Fourth, the study focused on immediately post-collection semen parameters and did not evaluate effects on semen preservation or cryopreservation success. Given that elevated viscosity can interfere with cryopreservation procedures and post-thaw quality [66], [67], investigating whether bromhexine treatment improves freeze-thaw outcomes would have significant practical implications for AI programs using frozen-thawed semen. Fifth, while the study measured several biochemical parameters, more sophisticated molecular analyses would provide deeper mechanistic insights. Proteomic profiling of seminal plasma could identify specific proteins altered by bromhexine treatment [57]. Glycomic analyses could characterize changes in mucopolysaccharide composition [74]. Rheological studies could precisely quantify changes in seminal plasma flow properties beyond simple viscosity measurements [75].

Sixth, the study did not evaluate potential synergistic effects of combining bromhexine with other interventions. For example, combining bromhexine (to optimize seminal plasma properties) with antioxidant supplementation (to protect sperm membranes) or melatonin treatment (to partially restore hormonal drive) might produce additive or synergistic benefits exceeding those of single interventions [76, 77].

Seventh, individual animal variation in response to bromhexine treatment was not systematically explored. Some bucks may respond more favorably than others due to genetic differences, age, body condition, or baseline semen quality [78]. Identifying predictors of treatment response could enable targeted application to animals most likely to benefit. Eighth, potential effects on offspring quality and production

were not assessed. While bromhexine's localized physical mechanism makes significant offspring effects unlikely, confirming the health, growth, and productivity of offspring sired by treated bucks would provide complete reassurance regarding the safety and utility of this intervention [79].

4.11 Mechanistic Integration and Theoretical Model

Integrating the findings of this study, a coherent mechanistic model emerges: During the non-breeding season in Damascus bucks, photoperiod-mediated suppression of the hypothalamic-pituitary-gonadal axis reduces androgen-dependent secretory activity of accessory sex glands [2, 3]. This hormonal suppression alters the composition and properties of seminal plasma, potentially resulting in suboptimal ratios of coagulating proteins to lytic enzymes and elevated concentrations or altered structures of mucopolysaccharides [10, 80].

These compositional changes manifest as prolonged liquefaction times and elevated seminal plasma viscosity [11]. The viscous microenvironment physically restricts sperm motility by increasing hydrodynamic drag forces and potentially entrapping spermatozoa in mucoid networks [12, 41]. Even spermatozoa with functionally normal flagellar apparatus cannot achieve their full motility potential under these high-viscosity conditions [43]. Oral bromhexine supplementation exerts mucolytic effects on seminal plasma mucopolysaccharides through chemical depolymerization, breaking disulfide bonds and reducing polymer molecular weight [16, 17]. This mucolytic action accelerates the breakdown of the semen coagulum, reduces liquefaction time, and lowers residual seminal plasma viscosity [18]. The improved physical environment enables spermatozoa to swim more freely and efficiently, resulting in enhanced progressive motility and more linear swimming trajectories [30, 39].

Critically, this entire chain of effects occurs without altering spermatogenic processes, sperm cellular properties, accessory gland secretory activity, or reproductive hormone concentrations [81]. Bromhexine does not "improve" sperm quality in a biological sense; rather, it removes physical constraints that prevent existing spermatozoa from expressing their inherent functional capabilities [46]. This mechanistic model has broader theoretical implications beyond Damascus goats. It suggests that in seasonal breeders, the relative contribution of seminal plasma properties versus intrinsic sperm quality to overall fertility potential may vary across seasons [82]. During the breeding season, when hormonal support is optimal, intrinsic sperm characteristics may be most limiting. During the non-breeding season, seminal plasma properties may become the primary limiting factor, and interventions targeting these properties may yield disproportionate benefits [83]. This theoretical framework could explain why some interventions show season-dependent efficacy and suggests that optimal reproductive management strategies should be seasonally adjusted based on the primary limiting factors in each period [84].

4.12 Broader Implications for Reproductive Management

Beyond the specific context of Damascus goats and bromhexine, this study contributes to a broader understanding of reproductive management in seasonal breeders. The findings show that semen quality depends not only on spermatogenic efficiency and intrinsic sperm characteristics but also on the physical and chemical properties of the seminal plasma environment [85]. This perspective suggests that comprehensive semen quality assessment and optimization should address both cellular and environmental components [86]. Traditional semen evaluation heavily emphasizes sperm-centric parameters such as concentration, motility, morphology, and viability. However, it often gives insufficient attention to seminal plasma characteristics, which profoundly influence functional outcomes [87]. The significant improvements achieved through a relatively simple intervention targeting seminal plasma highlight the potential value of this often-overlooked component [88]. Future research could explore other approaches to optimizing seminal plasma. These may include nutritional interventions affecting glycoprotein synthesis, enzymatic supplements to enhance liquefaction, or physical treatments to modify viscosity [89, 90].

Additionally, this study demonstrates the value of mechanistically targeted interventions over empirical or untargeted approaches. By understanding bromhexine's specific mucolytic mechanism, we were able to predict its likely effects. This allowed us to design an appropriate experimental framework and interpret results within a coherent physiological context [91]. Such a mechanistic approach contrasts with purely empirical compound screening and offers greater potential for rational optimization and application [92].

5. Conclusions

Bromhexine can be used as a management strategy to improve reproductive performance in beef and dairy herds. Improving semen quality without altering hormonal levels makes it a safe and practical option to achieve consistent and sustainable production of livestock products. These selective effects provide strong evidence that bromhexine acts primarily through mucolytic modification of seminal plasma physical properties rather than through biological effects on spermatogenesis, sperm cellular function, or endocrine regulation. The 48% reduction in liquefaction time and 39% reduction in viscosity directly reflect bromhexine's pharmacological mechanism of mucopolysaccharide depolymerization. The resulting 26% improvement in progressive motility represents physical liberation of spermatozoa from viscous constraints, enabling them to achieve their inherent motility potential despite persistent photoperiod-mediated reproductive suppression. From a practical perspective, bromhexine supplementation offers a simple, economically viable intervention to partially mitigate seasonal reproductive decline in Damascus bucks. The treatment could improve the utility of non-breeding season ejaculates for artificial insemination programs, reduce semen wastage, and potentially extend the effective breeding season. The hormonal independence and peripheral mechanism of action suggest favorable safety profiles and compatibility with other reproductive management strategies. Future research should evaluate fertility outcomes through controlled breeding trials, optimize dosing protocols, assess effects on semen cryopreservation success, explore potential synergies with complementary interventions, and extend findings to other seasonal breeding species. The present study provides a foundation for evidence-based recommendations regarding bromhexine supplementation as a tool for optimizing male reproductive performance in seasonal breeders.

5.References

[1] Meadows, A., et al. (2022). The role of small ruminants in global food security. Global Food Security Journal, 33, 100632.

[2] Aljumaah, R. M., et al. (2021). Genetic and management factors affecting milk production and reproduction of Damascus goats in Saudi Arabia. Tropical Animal Health and Production, 53(2), 312.

[3] Kheradmand, M. H., et al. (2022). Seasonal variation in semen quality and reproductive hormone concentrations in Damascus bucks under semi-arid climate. Small Ruminant Research, 213, 106732.

[4] Delgadillo, J. A., et al. (2022). The photoperiodic control of reproduction in the buck: A neuroendocrine perspective. Domestic Animal Endocrinology, 78, 106685.

[5] Casao, A., et al. (2021). Seasonal variations in the quality of spermatozoa from bucks: A comprehensive review. Theriogenology, 168, 78-89.

[6] 28. Zarazaga, L. A., et al. (2022). Photoperiodic regulation of reproduction in domestic ruminants. Domestic Animal Endocrinology, 81, 106742.

[7] Jansen, M., et al. (2021). Seasonal effects on buck semen viscosity and its correlation with fertility outcomes. Veterinary Medicine International, 2021, 6684752.

[8] Karaca, F., et al. (2020). Effects of season on seminal plasma composition and oxidative stress parameters in rams. Animal Reproduction Science, 216, 106345.

[9] Chemineau, P., et al. (2016). Seasonality of reproduction and its control in sheep and goats. Domestic Animal Endocrinology, 56, S90-S98.

[10] Rizzoto, G., et al. (2021). Reproductive technologies for improved year-round breeding in small ruminants. Reproduction, Fertility and Development, 33(2), 73-85.

[11] M. Jansen et al., "Seasonal effects on buck semen viscosity and its correlation with fertility outcomes," *Veterinary Medicine International*,

vol. 2021, pp. 6684752, 2021. DOI: 10.1155/2021/6684752.

[12] U. Ipek et al., "Effects of bromhexine hydrochloride on semen quality parameters in roosters," *British Poultry Science*, vol. 62, no. 5, pp. 678-684, 2021. DOI: 10.1080/00071668.2021.1912288.

[13] M. A. Abecia et al., "Hormonal control of reproduction in small ruminants," *Animal Reproduction Science*, vol. 130, no. 3-4, pp. 173-179, 2012. DOI: 10.1016/j.anireprosci.2012.01.011.

[14] González-Bulnes et al., "Manipulation of reproductive seasonality in sheep and goats," *Reproduction in Domestic Animals*, vol. 50, no. Suppl 4, pp. 16-23, 2015. DOI: 10.1111/rda.12591.

[15] M. C. Peris-Frau et al., "Accessory sex glands and their function in the male reproductive tract," *Animals*, vol. 11, no. 6, pp. 1810, 2021. DOI: 10.3390/ani11061810.

[16] P. G. Noone et al., "Primary ciliary dyskinesia: Diagnostic and phenotypic features," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 4, pp. 459-467, 2004. DOI: 10.1164/rccm.200303-365OC..

[17] N. Rauthan et al., "Mucolytic agents: Clinical pharmacology and therapeutic role," *Indian Journal of Chest Diseases and Allied Sciences*, vol. 50, no. 2, pp. 227-235, 2008.

[18] S. Yasuda et al., "Structural and functional properties of bromhexine as a mucolytic agent," *Journal of Pharmacy and Pharmacology*, vol. 72, no. 11, pp. 1589-1600, 2020. DOI: 10.1111/jphp.13350.

[19] Y. D. Zhang et al., "Glycoproteins in seminal plasma and their roles in sperm function," *Journal of Proteomics*, vol. 231, pp. 103993, 2021. DOI: 10.1016/j.jprot.2020.103993.

[20] O. Adebiyi et al., "Bromhexine supplementation improved fertility parameters in heat-stressed roosters," *Poultry Science*, vol. 100, no. 12, pp. 101473, 2021. DOI: 10.1016/j.psj.2021.101473.

[21] R. Partyka et al., "Effect of bromhexine hydrochloride on seminal characteristics and enzymatic activity in Simmental bulls," *Animal Science Papers and Reports*, vol. 39, no. 3, pp. 253-262, 2021.

[22] F. Karaca et al., "Influence of bromhexine treatment on ram semen quality and antioxidant status during breeding and non-breeding seasons," *Reproduction in Domestic Animals*, vol. 56, no. 10, pp. 1298-1306, 2021. DOI: 10.1111/rda.13995.

[23] M. Boe-Hansen et al., "Computer-assisted sperm analysis: Capabilities and potential developments," *Theriogenology*, vol. 137, pp. 11-18, 2019. DOI: 10.1016/j.theriogenology.2019.05.035.

[24] S. A. Henkel et al., "Impact of reactive oxygen species on spermatozoa," *Andrologia*, vol. 53, no. 1, pp. e13656, 2021. DOI: 10.1111/and.13656.

[25] S. Bucak et al., "Oxidative stress and antioxidant defense in ram semen and their relation with semen quality," *Small Ruminant Research*, vol. 204, pp. 106531, 2021. DOI: 10.1016/j.smallrumres.2021.106531.

[26] M. N. Bucak et al., "The effect of antioxidants on post-thawed Angora goat (Capra hircus ancryrensis) sperm parameters, lipid peroxidation and antioxidant activities," *Small Ruminant Research*, vol. 89, no. 1, pp. 24-30, 2010. DOI: 10.1016/j.smallrumres.2009.11.015.

[27] C. G. Rizzoto et al., "Reproductive technologies for improved year-round breeding in small ruminants," *Reproduction, Fertility and Development*, vol. 33, no. 2, pp. 73-85, 2021. DOI: 10.1071/RD20265.

[28] H. Zarazaga et al., "Photoperiodic regulation of reproduction in domestic ruminants," *Domestic Animal Endocrinology*, vol. 81, pp. 106742, 2022. DOI: 10.1016/j.domaniend.2022.106742.

[29] C. Yániz et al., "Computer assisted sperm morphometry in mammals: A review," *Animal Reproduction Science*, vol. 156, pp. 1-12, 2015. DOI: 10.1016/j.anireprosci.2015.03.002.

[30] Agarwal et al., "Computer-aided sperm analysis (CASA): Technical parameters and clinical interpretation," *Panminerva Medica*, vol. 61, no. 4, pp. 382-394, 2019. DOI: 10.23736/S0031-0808.18.03532-5

[31] WHO, "WHO laboratory manual for the examination and processing of human semen," 6th ed., Geneva: World Health Organization, 2021.

[32] S. Elzanaty et al., "Semen viscosity: An important parameter for assessing male fertility," *Andrologia*, vol. 36, no. 1, pp. 22-26, 2004. DOI: 10.1046/j.0303-4569.2003.00596.x

[33] M. A. Swierstra, "Cytotoxicity of eosin-nigrosin stain to spermatozoa: A validation study," *Journal of Reproduction and Fertility*, vol. 89, no. 2, pp. 515-519, 1990. DOI: 10.1530/jrf.0.0890515

[34] V. N. Jeyendran et al., "Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics," *Journal of Reproduction and Fertility*, vol. 70, no. 1, pp. 219-228, 1984. DOI: 10.1530/jrf.0.0700219

[35] R. J. Keel, "Effect of semen viscosity on determination of sperm concentration," *Clinical Chemistry*, vol. 32, no. 2, pp. 383-384, 1986.

[36] P. F. Watson, "The causes of reduced fertility with cryopreserved semen," *Animal Reproduction Science*, vol. 60-61, pp. 481-492, 2000. DOI: 10.1016/S0378-4320(00)00099-3

[37] J. F. Mortimer et al., "Effect of seminal plasma on capacitation and hyperactivation in human spermatozoa," *Human Reproduction*, vol. 13, no. 8, pp. 2139-2146, 1998. DOI: 10.1093/humrep/13.8.2139.

[38] Leboeuf et al., "Management of reproduction in goats," *Animal Reproduction Science*, vol. 60-61, pp. 481-492, 2000. DOI: 10.1016/S0378-4320(00)00162-7

[39] D. J. Smith et al., "Human sperm accumulation near surfaces: A simulation study," *Journal of Fluid Mechanics*, vol. 621, pp. 289-320, 2009. DOI: 10.1017/S0022112008004953

[40] F. Alvarez-Rodriguez et al., "Dietary supplementation for male fertility in livestock," *Animal Reproduction Science*, vol. 220, pp. 106456, 2020. DOI: 10.1016/j.anireprosci.2020.106456

[41] H. C. Berg and L. Turner, "Movement of microorganisms in viscous environments," *Nature*, vol. 278, no. 5702, pp. 349-351, 1979. DOI: 10.1038/278349a0

[42] S. S. Suarez and A. A. Pacey, "Sperm transport in the female reproductive tract," *Human Reproduction Update*, vol. 12, no. 1, pp. 23-37, 2006. DOI: 10.1093/humupd/dmi047

[43] E. Lauga and T. R. Powers, "The hydrodynamics of swimming microorganisms," *Reports on Progress in Physics*, vol. 72, no. 9, pp. 096601, 2009. DOI: 10.1088/0034-4885/72/9/096601

[44] B. P. Setchell, "The Parkes Lecture: Heat and the testis," *Journal of Reproduction and Fertility*, vol. 114, no. 2, pp. 179-194, 1998. DOI: 10.1530/jrf.0.1140179

[45] H. Rodríguez-Martínez et al., "The physiological roles of the boar ejaculate," *Society of Reproduction and Fertility Supplement*, vol. 66, pp. 1-21, 2009.

[46] R. J. Aitken et al., "Reactive oxygen species as mediators of sperm capacitation," *Molecular Reproduction and Development*, vol. 84, no. 10, pp. 1039-1052, 2017. DOI: 10.1002/mrd.22871

[47] A. T. Bansal and A. J. Bilaspuri, "Impacts of oxidative stress and antioxidants on semen functions," *Veterinary Medicine International*, vol. 2010, pp. 686137, 2010. DOI: 10.4061/2010/686137

[48] B. T. Hinton et al., "The epididymis as protector of maturing spermatozoa," *Reproduction*, vol. 162, no. 6, pp. F63-F77, 2021. DOI: 10.1530/REP-21-0086

[49] E. R. S. Roldan and M. Gomendio, "The Y chromosome as a battle ground for sexual selection," *Trends in Ecology & Evolution*, vol. 14, no. 2, pp. 58-62, 1999. DOI: 10.1016/S0169-5347(98)01522-1

[50] R. E. Malpaux et al., "Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin," *Journal of Biological Rhythms*, vol. 16, no. 4, pp. 336-347, 2001. DOI: 10.1177/074873001129002051

[51] M. A. Kulaksiz et al., "Oral drug administration in livestock: Practical considerations," *Veterinary Medicine International*, vol. 2020, pp. 8458329, 2020. DOI: 10.1155/2020/8458329

[52] A. J. Conley and P. L. Corbin, "Steroid dynamics in male reproduction," *Domestic Animal Endocrinology*, vol. 56, pp. S19-S27, 2016. DOI: 10.1016/j.domaniend.2016.01.006

[53] M. K. Mann and J. R. Lutwak-Mann, "Male reproductive function and semen," *Springer-Verlag Berlin Heidelberg*, 1981.

[54] S. Durairajanayagam et al., "Medical management of male infertility," *Andrologia*,

vol. 53, no. 1, pp. e13791, 2021. DOI: 10.1111/and.13791

[55] B. Zini et al., "Correlations between prostatic secretions and semen characteristics," *Archives of Andrology*, vol. 39, no. 2, pp. 165-171, 1997. DOI: 10.3109/01485019708994886

[56] S. A. Krawetz, "Paternal contribution: New insights and future challenges," *Nature Reviews Genetics*, vol. 6, no. 8, pp. 633-642, 2005. DOI: 10.1038/nrg1654

[57] D. J. Druart et al., "Proteomic characterization of seminal plasma in bucks," *Journal of Proteomics*, vol. 77, pp. 293-305, 2012. DOI: 10.1016/j.jprot.2012.08.020

[58] M. J. Ford, "Energy costs of the immune response," *American Naturalist*, vol. 176, no. 2, pp. 167-173, 2010. DOI: 10.1086/653678

[59] P. Mortimer et al., "Effect of seminal plasma on stallion spermatozoa," *Animal Reproduction Science*, vol. 89, no. 1-4, pp. 245-248, 2005. DOI: 10.1016/j.anireprosci.2005.01.015

[60] R. A. P. Harrison, "Rapid PKA-catalysed phosphorylation of boar sperm proteins," *Molecular Reproduction and Development*, vol. 67, no. 3, pp. 337-352, 2004. DOI: 10.1002/mrd.20028

[61] G. N. De Iuliis et al., "DNA damage correlates with hyperactivation in human spermatozoa," *Reproduction*, vol. 133, no. 3, pp. 693-699, 2007. DOI: 10.1530/REP-06-0257

[62] M. P. Kouba et al., "Effect of season on reproductive hormones in Boer bucks," *Animal Reproduction Science*, vol. 195, pp. 249-256, 2018. DOI: 10.1016/j.anireprosci.2018.06.003

[63] J. P. Kastelic and J. C. Thundathil, "Breeding soundness evaluation and semen analysis," *Reproduction in Domestic Animals*, vol. 43, no. Suppl 2, pp. 368-373, 2008. DOI: 10.1111/j.1439-0531.2008.01186.x

[64] I. Casao et al., "Seasonal variations and freezability of sperm from Rasa Aragonesa rams," *Small Ruminant Research*, vol. 71, no. 1-3, pp. 34-41, 2007. DOI: 10.1016/j.smallrumres.2006.03.010

[65] J. F. Menzies, "Advances in the artificial insemination of sheep and goats," *Reproduction, Nutrition, Development*, vol. 36, no. 2, pp. 205-213, 1996. DOI: 10.1051/rnd:19960207

[66] F. Jiménez-Rabadán et al., "Effect of semen collection method on ram sperm cryosurvival," *Animal Reproduction Science*, vol. 136, no. 4, pp. 248-255, 2013. DOI: 10.1016/j.anireprosci.2012.11.005

[67] M. C. Peris-Frau et al., "Cryodamage in ruminant spermatozoa," *International Journal of Molecular Sciences*, vol. 21, no. 3, pp. 863, 2020. DOI: 10.3390/ijms21030863

[68] H. Rodríguez-Martínez, "State of the art in farm animal sperm evaluation," *Reproduction, Fertility and Development*, vol. 19, no. 1, pp. 91-101, 2007. DOI: 10.1071/RD06104

[69] M. Mahmoud et al., "Characteristics of fresh and frozen-thawed semen in Damascus bucks," *Small Ruminant Research*, vol. 109, no. 1, pp. 70-74, 2013. DOI: 10.1016/j.smallrumres.2012.07.022

[70] M. G. Diskin and J. M. Sreenan, "Fertilization and embryonic mortality," *Journal of Reproduction and Fertility*, vol. 43, pp. 417-424, 1991.

[71] J. Delgadillo et al., "Seasonality of reproduction in goats," *Animal Reproduction Science*, vol. 124, no. 3-4, pp. 195-204, 2011. DOI: 10.1016/j.anireprosci.2010.08.022

[72] P. M. Aponte et al., "Long-term drug effects on reproductive function," *Journal of Veterinary Pharmacology*, vol. 44, no. 3, pp. 412-421, 2021. DOI: 10.1111/jvp.12931

[73] G. C. Fahim et al., "Dose-response relationships in reproductive pharmacology," *Theriogenology*, vol. 85, no. 1, pp. 22-30, 2016. DOI: 10.1016/j.theriogenology.2015.09.017

[74] J. L. Tollner et al., "Glycans of the oviduct," *Molecular Human Reproduction*, vol. 17, no. 1, pp. 3-9, 2011. DOI: 10.1093/molehr/gaq071

[75] S. Elzanaty et al., "Viscoelastic properties of seminal fluid," *Biorheology*, vol. 41, no. 3-4, pp. 279-288, 2004.

[76] M. J. Martin et al., "Combined nutritional and photoperiod strategies in sheep," *Small Ruminant Research*, vol. 215, pp. 106777, 2022. DOI: 10.1016/j.smallrumres.2022.106777

[77] A. T. Lamond et al., "Antioxidant supplementation in rams," *Journal of Animal Science and Biotechnology*, vol. 12, pp. 47, 2021. DOI: 10.1186/s40104-021-00567-4

[78] M. Rickard et al., "Individual variation in buck reproductive parameters," *Journal of Animal Science*, vol. 99, no. 5, pp. skab098, 2021. DOI: 10.1093/jas/skab098

[79] F. Hernández-Cerón et al., "Paternal effects on offspring development," *Reproduction, Fertility and Development*, vol. 27, no. 1, pp. 1-12, 2015. DOI: 10.1071/RD14178

[80] I. Nur et al., "Seasonal changes in oxidative stress markers in rams," *Small Ruminant Research*, vol. 208, pp. 106615, 2022. DOI: 10.1016/j.smallrumres.2022.106615

[81] M. C. Peris-Frau et al., "Sperm-seminal plasma interaction," *Animals*, vol. 10, no. 8, pp. 1368, 2020. DOI: 10.3390/ani10081368

[82] S. Kaya et al., "Seasonal variations in Damascus buck reproduction," *Animal Reproduction Science*, vol. 229, pp. 106753, 2021. DOI: 10.1016/j.anireprosci.2021.106753

[83] M. A. Ramadan et al., "Effect of season on buck semen enzymatic activities," *Theriogenology*, vol. 168, pp. 28-35, 2021. DOI: 10.1016/j.theriogenology.2021.03.021

[84] A. González-Bulnes et al., "Seasonal reproductive management in sheep," *Animals*, vol. 11, no. 3, pp. 776, 2021. DOI: 10.3390/ani11030776

[85] D. J. Druart et al., "Seminal plasma proteins and fertility," *Journal of Reproductive Immunology*, vol. 83, no. 1-2, pp. 47-51, 2009. DOI: 10.1016/j.jri.2009.04.013

[86] H. Rodríguez-Martínez et al., "Seminal plasma: Relevance for fertility," *Theriogenology*, vol. 85, no. 1, pp. 150-161, 2016. DOI: 10.1016/j.theriogenology.2015.09.047

[87] R. J. Aitken, "Sperm function tests and fertility," *International Journal of Andrology*, vol. 29, no. 1, pp. 69-75, 2006. DOI: 10.1111/j.1365-2605.2005. 00630.x

[88] M. Khalifa et al., "Sperm metabolism and male infertility," *International Journal of Molecular Sciences*, vol. 22, no. 19, pp. 10855, 2021. DOI: 10.3390/ijms221910855

[89] R. C. Allrich et al., "Nutritional effects on reproduction," *Animal Reproduction Science*, vol. 42, no. 1-4, pp. 255-265, 1996. DOI: 10.1016/0378-4320(96)01490-0

[90] M. N. Bucak et al., "Enzymatic antioxidants and semen quality," *Systems Biology in Reproductive Medicine*, vol. 56, no. 1, pp. 80-85, 2010. DOI: 10.3109/19396360903456773

[91] S. A. Krawetz, "Paternal contribution insights," *Nature Reviews Genetics*, vol. 6, no. 8, pp. 633-642, 2005. DOI: 10.1038/nrg1654

[92] J. K. Vickram et al., "Emerging trends in male reproductive health," *Andrologia*, vol. 53, no. 1, pp. e13875, 2021. DOI: 10.1111/and.13875