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

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ABSTRACT

The dynamics of reproductive performances of Damascus goats through seasonal changes are a major challenge to the production of milk and meat during year-round, which influences the sustainability of all the supply chains. Mucolytic agent Bromhexine has demonstrated a possible role in enhancing sperm quality by decreasing and viscosity therefore indirectly increasing the breeding efficiency and subsequently production of animal-derived foods. The present study examined the impact of bromhexine supplementation on the semen quality parameters in Damascus goat bucks during non-breeding season and implications of such research on reproductive management and subsequent impacts on milk and meat production. A total of twelve sexually fit Damascus bucks (2-3 years old, 45-55 kg) were randomly divided in a treatment group with oral bromhexine (1 mg/kg day) or a control group with placebo after 60 days during the non-breeding season. Sperm was pooled and assessed on volume, liquefaction period, viscosity, sperm density, motility, viability as well as morphology. The serum reproductive hormones and semen plasma biochemical constituents were also evaluated. Bromhexine massively lowered the 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, $P<0.01$). There was an improvement in progressive sperm motility (66.3+-3.4% vs. 52.8+-4.2%, $p<0.05$). There were no major differences in sperm concentration, viability, morphology, hormonal profiles or biochemistry of the seminal plasma. Bromhexine enhances some of the key semen physical characteristics, and this might increase the breeding efficiency in the non-breeding season. This can be used to improve year-round breeding that will result in stabilized production of milk and meat. The fact that there are no hormonal changes is an indication that bromhexine has no effect on the normal physiology of food producing animals, hence it is a possible alternative that can be incorporated into sustainable animal husbandry practices in an attempt to streamline the 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 Damascus bucks of sexually mature age, 2-3 years of age body weights of 45-55 kg were chosen from a commercial goat farm at position 35deg N latitude. Bucks were selected because of prior examination for breeding soundness based upon evidence of normal reproductive ability and semen quality during the breeding season. Animals were kept in individual pens (2.5 x 3 m) with natural photoperiod, having free access to fresh water. The diet was consisted of concentrate mixture designed to satisfy nutritional requirements of breeding males (16% crude protein, 2.8 Mcal/kg metabolizable energy), alfalfa hay and seasonal green fodder. All animals were vaccinated following regional protocols and also dewormed with ivermectin two weeks before the commencement of the experiment. Bucks had no clinical diseases during the experimental time, as could be verified through 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 The present study shows that oral bromhexine supplementation has a significant effect on improving some of the semen quality parameters of the Damascus bucks during the non-breeding season through a well-defined mucolytic mechanism. The selective effects of the improvements - dramatic improvement in liquefaction time and viscosity as well as higher progressive motility with no changes in sperm production, viability, morphology, or hormonal status - provide compelling evidence that bromhexine is acting primarily on the physical properties of seminal plasma rather than through biologic effects on spermatogenesis or endocrine function.

4.1 Mucolytic Mechanistic

The most striking result of this study is the 48% increase in the semen liquefaction time of bromhexine-treated bucks (8.2 vs. 15.7 minutes). This dramatic acceleration of the liquefaction process is a direct effect of bromhexine's principal pharmacological action, namely depolymerization of mucus polysaccharide complexes by disruption of disulfide bonds and reduction of glycoprotein molecular weight [16, 17].

Freshly ejaculated mammalian semen forms a coagulum or gel-like structure that is made up of

high-molecular-weight glycoproteins (in most cases semenogelins) secreted by the seminal vesicles, cross-linked via disulfide bonds and non-covalent interactions [7]. This temporary coagulation has evolutionary roles such as keeping the concentration of sperm at the site of deposition and perhaps protecting spermatozoa from the adverse environment of the female reproductive tract [8]. However, for spermatozoa to realize their full fertilizing potential they must be released from this matrix by a process of liquefaction. Physiological liquefaction occurs by the action of proteolytic enzymes, especially prostate-specific antigen (PSA), and other serine proteases secreted by the prostate gland, which cleave the semenogelin proteins [9]. In seasonal breeders during the non-breeding period, decreased androgen-dependent secretory activity of accessory sex glands may be the cause of suboptimal ratios of coagulating proteins to lytic enzymes, thus causing prolonged liquefaction periods and high viscosity [10, 11]. The mucolytic effect of bromhexine complements and enhances the physiological liquefaction process through a chemical breakdown of the structure of the mucopolysaccharide chains that occur in the formation of semen viscosity. By breaking the disulfide bonds and decreasing polymer chain length, bromhexine makes it easier for the coagulum structure to dissolve more rapidly [18,37]. The observed liquefaction time decrease from 15.7 to 8.2 minutes is a clinically

significant reduction bringing non- breeding season samples of closer to the liquefaction times generally found during peak breeding season (usually 5-10 min in healthy bucks) [38]. The parallel 39% decrease in seminal plasma viscosity (from 4.6 to 2.8 cP) is an independent confirmation of the mucolytic effect of bromhexine. Viscosity measurements were done after complete liquefaction and thus bromhexine not only helps in accelerating the liquefaction process, but also contributes to providing a final seminal plasma with fundamentally lower viscosity [32]. This residual effect leads to the possibility that the action of bromhexine is not only to hasten the process of enzymatic liquefaction, but rather to modify the final physical state of seminal plasma by decreasing the molecular weight and complexity of the mucopolysaccharide networks that remain even after enzymatic cleavage of the semenogelin proteins [39].

4.2 Physical Liberation and Increase of Sperm Motility

The significant improvement in progressive motility (52.8 to 66.3% - a 26% relative increase) is the most functionally important result of this study. This enhancement can be mechanistically referred to as physical release of spermatozoa from viscous constraints rather than an improvement in intrinsic sperm functions.

There are a number of lines of evidence to support this interpretation. First, the CASA analysis showed that although straight-line velocity (VSL) was significantly increased (from 41.7 to 52.4 mm/s) curvilinear velocity (VCL) did not change significantly (105.8 vs. 112.5 mm/s). VCL is the sum of distances covered by a spermatozoon in any direction and is indicative of the intrinsic ability of the flagellar apparatus to produce movements [23]. The lack of change in VCL suggests the basic machinery of motility generation of spermatozoa was not improved by bromhexine. 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 the metabolism, energy production, or function of sperm, alterations in these parameters would be expected [40]. Their lack is a strong indicator that the improvements in motility are due to environmental factors as opposed to modifications within the cells. Third, the enhancement in the linearity (LIN) and particularly in the straightness (STR) indices reflect that the spermatozoa in less viscous medium have more efficacious and directed swimming patterns [30]. For spermatozoa swimming in highly viscous medium, the forward progression of the spermatozoa is hindered by increased drag forces and swimming trajectories become more circular and less productive accordingly [41]. By lowering the viscosity, bromhexine lowered medium resistance so that spermatozoa could change their flagellar beating into more efficient forward motion. The strong negative correlations between viscosity and progressive motility ($r=-0.71$) and viscosity and VSL ($r=-0.64$) are statistical evidence supporting this mechanistic model. These correlations have proven the physical properties of seminal plasma have a significant effect on observed sperm motility independent of the inherent sperm quality. From a biophysical point of view, the swimming of sperm is governed by the Reynolds number (Re), which is the ratio of inertial and viscous forces [42]. At the microscopic scale of spermatozoa, re is very low (<10-4) and viscous forces dominate on which the propulsion of the spermatozoons is very sensitive to the viscosity of the medium [43]. Even relatively small decreases in viscosity can yield big increases in swimming efficiency and velocity as has been shown in this work.

The practical implications are very significant. During the non-breeding season, the semen of Damascus bucks naturally has increased level of viscosity which physically limits sperm motility even if the spermatozoa themselves are functionally competent [4, 5]. By pharmacologically lowering the viscosity, bromhexine makes the real motility potential of these cells available, which may improve the fertility of these cells, despite the photoperiod-induced suppression of reproductive function.

4.3 No effects with Sperm Production / concentration

The finding that the concentration of sperm was statistically similar between groups (3.2x109/ml vs. 2.9x109/ml, $P=0.58$) is of equal importance to the

positive findings since it helps define the boundaries of bromhexine's action. Sperm concentration is an indication of testicular spermatogenic efficiency and epididymal sperm reserves [34]. The lack of effects of treatment suggests that bromhexine does not affect spermatogenesis, which is in line with the lack of hormonal changes and the known mode of action of bromhexine [16].

This finding is in contrast to some previous reports about an increase in sperm concentration after bromhexine treatment [21, 22]. These apparent discrepancies are probably correlated to differences in counting methodologies in sperm counting. In the case of highly viscous semen, inadequate sample homogenization during dilution can lead to a non-uniform distribution of sperm and false low estimates of concentration [35, 36]. In addition, viscous semen may have clumps of sperm that are aggregated within the semen and counted as single sperm, further underestimating true concentration [37].

In the present study, concentration measurements were obtained post-complete liquefaction and using careful homogenization procedures, which likely gave more accurate estimates, which were less prone to viscosity-related artifacts. The similarity in concentrations between groups therefore probably reflects true biological equivalence and not technical limitations.

The unchanged ejaculate volume (1.08 ml vs. 0.95 ml, P=0.31) is worthy of comment. While some previous studies found increased ejaculate volume with bromhexine treatment [12, 20], these apparent increases may in part reflect better recovery of viscous samples from collection vessels rather than actual increases in secretory volume [33]. Highly viscous semen is likely to stick to the walls of vessels and may not flow entirely into graduated collections in collection tubes, thus underestimating true volume [44].

However, despite the possible collection artifacts, the findings of the present study indicate that bromhexine is not significantly more effective than placebo at improving accessory sex gland fluid secretion. This interpretation is supported by the finding that seminal plasma protein, fructose and citric acid concentrations, which are biochemical indicators of the activity of accessory glands [15], were statistically similar in both groups. If bromhexine had stimulated secretory epithelial cells, then increases in these parameters would be expected [45].

4.4 viability, Membrane Integrity & Morphology: Biological Equivalence

The lack 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) reveals important information about the mechanism of action and the practical usefulness of bromhexine. These results showed that bromhexine treatment during the non-breeding season does not significantly affect sperm biology, cellular integrity and structural quality. The spermatozoa are biologically equivalent between groups; it is the physical environment in which they swim [46]. This biological shared identity is encouraging from a safety standpoint and adds to the interpretation that the benefits of bromhexine are based on the physical rather than the cellular.

Of particular information are the unchanged viability percentages. If bromhexine had antioxidant effects or protected spermatozoa from oxidative stress (i.e. the mechanisms proposed in some previous studies [26]), improvements in viability would be expected, as oxidative damage is a primary cause of the permeabilization of sperm cell membrane and cell death [25], [47]. The lack of viability differences indicates that bromhexine may not have any significant antioxidant activity in this model, or that oxidative stress during this period of the experiment was not limiting sperm viability.

Similarly, no improvement of morphology, especially of cytoplasmic droplet retention (2.3% vs. 2.8%, P=0.47), suggests bromhexine did not benefit the epididymal maturation processes [36]. Cytoplasmic droplets are ordinarily shed during epididymal

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

An alternative explanation for some of the previous reports of lessened morphological abnormalities following bromhexine treatment is assessment artifacts in viscous samples. Mucoid material stuck to spermatozoa may give the appearance of morphological defects [49]. By decreasing the viscosity and the mucoid content, bromhexine may allow a better morphological evaluation rather than an actual decrease in the true abnormality rate. The rigorous morphological evaluation in the present study on properly liquefied samples probably represents a more accurate picture of the true morphological status.

4.5 Hormonal Independence and Peripheral Action

The The lack 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 present definitive evidence that effects of bromhexine are independent of hypothalamus-pituitary-gonadal axis. These hormonal results are consistent with bromhexine's known pharmacology, and with other reported results from other species [13, 14, 22].

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

Second, the site of action is peripheral so it is unlikely that bromhexine will cause systemic endocrine side effects or interfere with normal reproductive physiology. This safety profile is favourable for practical use in the commercial breeding operations [51]. The drug can potentially be used long term without worrying about hypothalamic-pituitary desensitization and testicular dysfunction that can happen with long-term use of hormonal treatments [52]. Third, low testosterone levels that were maintained over the non-breeding season for both groups (about 2.7-2.9 ng/ml, well below breeding season levels of 5-8 ng/ml from Damascus bucks [3]) confirm that the experimental time period was associated with true seasonal reproductive suppression. This validation is important because it shows that the benefits of bromhexine occurred in the face of persistent photoperiod-mediated suppression of the hormonal system and therefore may be practically useful at the time when it is most needed.

The absence of hormonal effects also assists in explaining why some parameters, especially those that are dependent on secretory processes regulated by androgens such as fructose production by the seminal vesicles [53], were not affected. Bromhexine has no effect on substituting or augmenting androgenic stimulation of accessory glands, but operates within the limits of existing hormonal status to optimize the physical characteristics of whatever secretions are produced [54].

4.6 Biochemical Composition: Physical + Secretory Effects

The demonstration 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 were statistically similar between groups is additional evidence that the effects of bromhexine are most likely of a physical nature rather than secretory or metabolic.

Fructose, released by seminal vesicles under androgenic influence, is the main energy source of ejaculated spermatozoa and is a popular marker of seminal vesicle function [36, 53]. Citric acid, secreted mostly by the prostate gland, besides being a buffer, is

a substrate for energy metabolism, reflecting prostatic secretory activity [55]. Total protein concentration gives an general index of secretory output of accessory glands [45].

If bromhexine acted as a secretagogue, i.e. by stimulating the secretion of epithelial cells as suggested in some studies [10], then increases in these biochemical markers would be anticipated. Their absence suggests that bacteria had not significantly increased the secretory activity of accessory sex glands in this study by bromhexine. This interpretation is consistent with no change in ejaculate volume and is consistent with a mechanism localized to modification of existing secretions rather than stimulation of additional secretion.

It is possible to note that bromhexine could theoretically be a way to modify the qualitative composition of secretions without modifying total quantities of major components. For instance, it may alter the glycosylation patterns of mucoproteins or may alter the relative amounts of different protein species [56]. Such subtle changes would not be registered by the gross biochemical measurements used in this study but could cause some of the altered physical properties. More advanced proteomic or glycomic analyses would be needed to detect such changes [57].

The biochemical makeup that is maintained is reassuring from a physiological point of view, as it implies that the treated semen still has normal metabolic support systems for spermatozoa. The improved motility of bromhexine treated samples cannot be explained by increased energy substrate availability (unchanged fructose) or changes in ionic environment (unchanged citric acid) and this further supports the interpretation that improvements arise from reduction in physical limitations rather than metabolic enhancement [58].

4.7 Correlation Patterns and Mechanistic Understanding

The good correlations described between physical parameters (liquefaction time, viscosity) and motility results are the convincing statistical support for the suggested mechanistic model. The negative correlation observed between liquefaction time and progressive motility ($r=-0.76$, $P<0.001$) shows that delayed liquefaction significantly decreases observed motilities in sperm cells, probably by extending the period of time during which spermatozoa are physically trapped in the coagulum [59].

Similarly, a strong negative correlation was found between viscosity and progressive motility ($r=-0.71$, $P<0.001$) which directly implicates medium viscosity as a determinant of sperm swimming efficiency. Such a relationship is predicted by basic laws of low Reynold's number hydrodynamics [42, 43], which controls the movement of sperm at microscopic sizes. The agreement between observed correlations and theoretical predictions gives 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 - presumably the quantity and molecular weight distribution of mucopolysaccharides and glycoproteins that cannot be degraded by enzymes and contribute to viscosity [60]. Samples having a high mucopolysaccharide content would be expected to have both a long liquefaction time (from the increased amount of substrate requiring enzymatic cleavage) and a higher residual viscosity (from the degradation of the polymers being incomplete).

The lack of correlation between sperm concentration and motility parameters ($r=0.12$, $P=0.58$) suggests that concentration and motility are independent parameters of semen quality in such a setting [61]. This statistical independence lends support to the biological interpretation of the effect of bromhexine on motility being the result of environmental modification, rather than environmental modification as a result of changes in sperm production or intrinsic sperm characteristics. Similarly, the non-correlation between testosterone and motility ($r=0.08$, $P=0.71$), makes it even more evident that the effects were independent of hormonal status. This is especially important in light of the seasonal context of the study as it does suggest that interventions targeting seminal plasma properties can help improve functional outcomes even when the hormonal drivers of reproduction remain suppressed [62].

4.8 Context of Season and Implications of Practice

The demonstration of significant improvements in functionally important semen parameters (liquefaction time, viscosity, progressive motility) during the difficult non-breeding season has specific practical relevance for Damascus goat production systems. Seasonal infertility poses a significant challenge to breeding programs that need to be conducted throughout the year and reduces the efficiency of genetic improvement schemes using artificial insemination [6, 27]. Traditional methods of seasonality management are hormonal therapy (melatonin, GnRH, gonadotropins), photoperiod and introduction of the "male effect" [13, 14]. While these methods can be successful, they are often complicated, involve large costs and special facilities or take into account the welfare issues. Photoperiod control involves costly light-tight housing, hormonal treatments must be repeated and vet supervised and the male effect is also of limited use when the males themselves are the limiting factor [50]. Bromhexine supplementation is a relatively simple, low cost intervention that can be easily added to routine management protocols for breeding males during times of predicted reproductive decline [51]. The route of administration through the mouth is sensible for on-farm application, the cost of the drug is relatively cheap and freely available (it was originally marketed for respiratory purposes), and special handling or storage requirements are not necessary beyond those of normal pharmaceuticals [38].

The improvements resulting from this study - especially the 26% increase in progressive motility - could have concrete consequences at the breeding level. Progressive motility is one of the most important predictors of fertility for both natural breeding as well as artificial insemination programs [42, 63]. Ejaculates with >60% progressive motility are usually deemed acceptable for AI and those with <50% often rejected [64]. The effect of treatment noted in this study (from 52.8% to 66.3%) could possibly improve the quality of ejaculates from marginal to acceptable quality, leading to a reduction in wastage of semen and greater efficiency of AI programs.

For natural breeding, the enhanced motility may lead to higher conception rates during the non-breeding season which would allow more flexible breeding schedules and year-round kidding patterns. This ability to be flexible is becoming increasingly so in intensive dairy goat operations where it may be economically advantageous to have consistent milk production throughout the year [1, 65]. Furthermore, treatment with bromhexine may enhance success from semen processing and cryopreservation. Elevated viscosity makes processing procedures in semen difficult, interfering with the correct measurement of processing volume, dilution, and loading into straws [66]. It may also affect the penetration of cryoprotectants and contribute to the poor post-thaw quality [67]. By decreasing viscosity, bromhexine may lead to easier completion of these technical procedures and may lead to better post-thaw outcomes, but this needs to be investigated directly.

4.9 Comparison with the Previous Studies and Species Differences

The current results are partly similar to and partly contrasting previous research on the effects of bromhexine in other species, which is important, as it indicates the presence of significant species-specific differences in response. In rams, the supplementation with bromhexine was reported to improve not only the semen viscosity and motility, but also the sperm concentration and viability [14, 22]. In bulls improvements in semen flowability, motility and post-thaw quality were observed [21]. In roosters, improved semen volume and concentration and motility were recorded [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, differences between species of the composition of seminal plasma and the coagulation characteristics could affect the extent and magnitude of the effects of bromhexine [68]. Goat semen is distinct from other ruminants in having higher viscosity and a more

prominent gel fraction [69], and hence, physical properties may be especially restrictive to motility. Second, methodological differences among studies may be the cause of some apparent discrepancies. Many previous studies were not conducted with the same rigor in measuring viscosity and liquefaction time, so these may have been missed as a primary mechanism in these studies. Conversely, apparent improvements in parameters such as concentration could have reflected artifacts of better handling of samples and not necessarily biological effects [35, 36].

Third, the time of year when studies are conducted may have an effect. The current investigation was specifically performed during the non-breeding season when the properties of seminal plasma are especially problematic [10, 11]. Studies performed during peak breeding season may see different effect profiles since viscosity is less of a limitation under optimal reproductive conditions. Fourth, differences in dosage and duration of treatment could explain differences in results. The current study used 1 mg/kg body weight for 60 days based on the results of other studies in small ruminants [22], but optimal dosing parameters may vary depending on the species and the breeding conditions [40]. Future dose-response studies in goats could refine recommendations.

4.10 Study Limitations and Future Research Directions

While this study presented strong evidence for the effects of bromhexine on semen physical properties and motility, there are a number of limitations that should be recognized that point to directions for future research. First, this study measured semen quality parameters and not actual fertility results by natural breeding or artificial insemination. While a strong correlation has been found between progressive motility and fertility [42, 63], direct fertility trials are required to determine whether the observed improvements result in increased conception rates and kidding results [70]. Controlled breeding trials of rates of pregnancy for does versus bucks when bred to bronoxine treated versus control bucks, would provide definite evidence of practical benefit. Second, the duration of the study was limited to the core non-breeding season of 60 days. Longer term studies continuing through the transition into the breeding season would elucidate whether the benefits of bromhexine are sustained, reduced or are no longer needed as photoperiod driven reproductive function spontaneously returns [71]. Additionally, year-round studies of supplementation would determine whether continuous treatment of Echinacea with or without additional herbs was superior to treatment using only Echinacea. maintains benefits with no tolerance development or adverse effects [72].

Third, although the study used 1 mg/kg body weight based on previous research, the optimal dosage for Damascus bucks is yet to be defined. Dose-response studies of a range of bromhexine concentrations (e.g. 0.5, 1.0, 2.0, 5.0 mg/kg) would define the minimum effective dose and maximum safe dose with optimal cost-effectiveness and margin of safety [40, 73].

Fourth, the study focused on the immediate post-collection semen parameters and did not assess impacts on semen preservation and cryopreservation success. Given that high viscosity can interfere with cryopreservation procedures and post-thaw quality [66], [67] any research into whether bromhexine treatment improves outcomes of freeze-thaw procedures would have significant practical implications for AI programs using frozen-thawed semen. Fifth, although the study measured a number of biochemical parameters, more sophisticated molecular analyses would give mechanistic insights. Proteomic profiling of seminal plasma could be used to detect certain proteins that are modified by bromhexine treatment [57]. The composition of mucopolysaccharides may be characterized by glycomic analyses [74]. Rheological studies could precisely quantify alterations in seminal plasma flow properties other than simple viscosity measurements [75].

Sixth, the study did not assess the possible synergistic effects of using bromhexine with other interventions. For example, a combination of bromhexine (to optimize seminal plasma properties) and antioxidant supplementation (to protect sperm membranes) or melatonin treatment (to partially restore hormonal

drive) may have additive or synergistic effects, which are greater than the single effects [76, 77].

Seventh, individual animal variation of the response to bromhexine treatment was not systematically explored. Some bucks might respond better than others depending on genetic differences, age, body condition or baseline semen quality [78]. Identification of predictors of response to therapy may allow for application to animals with the highest likelihood of benefit. Eighth, possible influences on quality and production of offspring

were not assessed. While the physical mechanism of bromhexine action is localised, and significant effects on offspring are unlikely, confirmation of the health, growth and productivity of the offspring sired by treated bucks would be reassuring of the safety and utility of such an intervention [79].

4.11 Integration Mechanistic and Theoretical Model

Integrating results of this study thus leads to a coherent mechanistic model: Photoperiod-mediated suppression of the hypothalamic-pituitary-gonadal axis in non-breeding season bucks in Damascus (2, 3) suppresses the accessory SGs from large secretions that are muscle testosterone dependent. This hormonal suppression changes seminal plasma composition and characteristics, which may lead to suboptimal ratios of coagulating proteins to lytic enzymes and high concentrations or altered structures of mucopolysaccharides [10, 80].

These changes in composition are reflected by an increase in liquefaction time and an increase in seminal plasma viscosity [11]. The viscous microenvironment also physically limits the motility of the sperm by increasing the hydrodynamic drag forces and also can trap the spermatozoa in mucoid networks [12, 41]. Even spermatozoa with functionally normal flagellar apparatus are unable to maximize their motility potential under these high viscosity conditions [43]. The oral bromhexine supplementation has mucolytic effect on seminal plasma mucopolysaccharides by chemical depolymerization, disruption of disulfide bonds and reduction of polymer molecular weight [16, 17]. This mucolytic action helps to accelerate the breakdown of the semen coagulum, thereby decreasing liquefaction time, and to decrease the residual seminal plasma viscosity [18]. The positive changes in the physical environment permit free and efficient swimming of the spermatozoa leading to increased progressive motility and more linear swimming trajectories [30, 39].

Critically, this whole chain of effects takes place without changing the spermatogenic processes, the sperm cellular properties, the secretory activity of the accessory glands or the concentrations of reproductive hormones [81]. Bromhexine does not "improve" the quality of sperm in a

biological sense; that is, it eliminates physical constraints that prevent existing spermatozoa from manifesting their inherent functional capabilities [46]. This is a mechanistic model with wider theoretical implications than that of Damascus goats. It suggests that in seasonal breeders, the relative importance of the properties of seminal plasma versus the intrinsic quality of sperm to overall fertility potential may change from one season to the next [82]. During the breeding season, when hormonal support is at a peak, the hormonal support of the sperm may be most limiting, and intrinsic characteristics in the sperm may be most limiting. During the non-breeding season seminal plasma characteristics may become most limiting and impacts aimed at these characteristics may have disproportionate benefits [83]. This theoretical framework could explain why some of the interventions have season-dependent efficacy and suggests that the best reproductive management strategies should be adjusted seasonally depending on the primary limiting factors in each period [84].

4.12 Wider Implications to the Reproductive Management

Beyond the point of the specific context of the Damascus goats and bromhexine, this study also contributes to the broader study of reproductive management in seasonal breeders. The findings reveal that the quality of semen is not only dependent on the efficiency of the spermatogenic process and intrinsic sperm characteristics but it is also dependent on the

physical and chemical nature of the seminal plasma environment [85]. This perspective proposes that a comprehensive assessment and optimization of semen quality needs to target the cellular and the environmental aspects [86]. Traditional semen evaluation gives a lot of attention to sperm-centric parameters (such as concentration, motility, morphology and viability). But it usually lays insufficient attention on the characteristics of seminal plasma, which have a profound inflammation on the functional results [87]. The profound improvements through a relatively simple intervention focused upon seminal plasma point to the potential value of this relatively neglected component [88]. Future studies could be done to look into other ways to optimise seminal plasma. These may include nutritional interventions related to synthesis of glycoprotein, enzymatic supplements to increase liquefaction, or physical treatments to alter viscosity [89, 90].

In addition, this study highlights the value of mechanistically targeted interventions in contrast to empirical or untargeted interventions. Through understanding the specific mucolytic mechanism of action of bromhexine, it was possible to predict the probable effects of bromhexine. This enabled us to create an appropriate experimental framework and describe results in a coherent physiological context [91]. Such a mechanistic approach is in contrast to the purely empirical compound screening and has a higher potential for rational optimization and application [92].

5. Conclusions

Bromhexine is an option as a management strategy for boosting reproductive performance in beef and dairy herds. Improving semen quality without manipulating hormonal parameters makes it a safe and viable choice to establish a consistent and sustainable production of livestock products. These selective effects are strong evidence that the action of bromhexine is insofar mediated by mucolytic modification of seminal plasma physical properties than it is by biological effects on spermatogenesis, sperm cell cellular function or endocrine regulation. The 48% reduction in liquefaction time and 39% reduction in viscosity is a direct reflection of the pharmacological mechanism of action of bromhexine, mucopolysaccharide depolymerization. The resulting 26% improvement in progressive motility is physical liberation of spermatozoa from the viscous constraints and allowing them to attain their inherent motility potential despite the continued photoperiod-mediated reproductive suppression. From a practical view point, the bromhexine supplementation provides an easy and economically feasible intervention strategy to partially counteract seasonal decline in reproductive health in Damascus bucks. The treatment may increase the utility of non-breeding season ejaculates for artificial insemination programs, decrease semen wastage and perhaps lengthen the effective breeding season. The hormonal independence and peripheral mechanism of action suggests favorable safety profiles to the way of compatibility with the other reproductive management strategies. Future studies should test the impact on fertility using controlled breeding trials, develop optimized dosing strategies, determine if the effect on the success of semen cryopreservation, investigate possible interactions with other complementary actions and expand the duration of the effects.

5.References

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