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Optimizing semen collection, dilution, and thawing protocols to enhance frozen thawed sperm quality in Bach Thao bucks

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ABSTRACT

Artificial insemination and semen cryopreservation are key techniques for improving reproductive efficiency and conserving genetic resources in goats, but the success of these procedures depends on semen collection and processing protocols. This study aimed to optimize semen collection intervals, dilution ratios, and thawing conditions to enhance post-thaw sperm quality in Bach Thao bucks, an indigenous Vietnamese breed. Ejaculates were collected at different intervals, diluted with varying ratios of citrate-based extender, frozen, and thawed under several temperature-time combinations. Post-thaw quality of semen was evaluated through sperm motility, morphology, and membrane integrity assessments. Semen collected at moderate intervals resulted in better sperm motility and fewer abnormalities than daily collection. A balanced dilution ratio improved cryosurvival, while excessive dilution reduced sperm quality. Thawing at moderate temperatures for short durations resulted in higher motility and membrane stability compared with lower or prolonged conditions. Integrating an appropriate collection interval with optimal dilution and controlled thawing significantly improved frozen–thawed semen quality in Bach Thao bucks. These results provide practical recommendations for breeding centers and contribute to the conservation of indigenous goat genetic resources.

Keywords: Artificial insemination, Bach Thao goat, Cryopreservation, dilution ratio, Semen collection interval, Sperm motility

Artificial insemination (AI) is a cornerstone of goat breeding programs, enabling the dissemination of superior germplasm along with reduce sanitary risks (Leboeuf *et al.* 2008). When combined with semen cryopreservation, AI becomes a powerful biotechnological tool for genetic improvement and conservation of small ruminants. The use of frozen semen facilitates germplasm exchange, strengthens breeding strategies, and supports biodiversity preservation. However, the efficiency of AI in goats remains inconsistent because post-thaw semen quality is highly variable and sensitive to collection, dilution, freezing, and thawing procedures.

Semen production and quality are influenced by environmental and management factors, including photoperiod, temperature, and collection frequency (Leboeuf *et al.* 2003; Abecia *et al.* 2016). Yet, the number of reports on goat semen cryopreservation remains limited compared with cattle (Morrell *et al.* 2022), and existing studies often employ diverse, non-standardized protocols. Factors such as collection interval, dilution ratio, and thawing conditions are known to affect sperm viability and motility, but these parameters have not been systematically optimized for indigenous goat breeds.

The Bach Thao goat, a dual-purpose breed native to Vietnam, is valued for both meat and milk production, with most of the population in Ninh Thuan and Binh Thuan provinces (Hien, 2023). Despite its economic importance, studies on semen cryopreservation in this breed remain limited, and most available protocols have been adapted from exotic goat breeds without systematic evaluation of their suitability for the physiological characteristics of Bach Thao bucks (Kifaro *et al.* 2007). This lack of breed-specific optimization restricts the optimisation of effective artificial insemination programs for this indigenous breed.

Therefore, the objective of this study was to evaluate and optimize semen collection intervals, dilution ratios with freezing extender, and thawing conditions in order to improve post-thaw sperm quality in Bach Thao bucks. The findings are expected to provide practical insights for enhancing cryopreservation protocols and to support the wider application of artificial insemination in indigenous goat breeding programs.

MATERIALS AND METHODS

Study animals: This study was conducted from January 2025 to May 2025 using twelve healthy, sexually mature Bach Thao bucks maintained at a goat farm in Phong

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Dien commune, Can Tho city, Vietnam. The bucks were 18–20 months old with a body weight ranging from 41.5 to 43.0 kg, and were selected based on good body condition and reproductive soundness. The animals were housed individually in well-ventilated pens equipped with separate feeders and drinkers. Pens and equipment were cleaned daily, and the bucks were vaccinated regularly against common infectious diseases.

The animals were fed fresh forage supplemented with a pelleted concentrate mixture containing 19% crude protein (CP) and 3150 kcal/kg metabolizable energy. The concentrate was formulated from maize, broken rice, vegetable protein sources, rice bran, wheat bran, and supplemented with calcium (0.8–2.5%) and phosphorus (0.6–1.2%). Clean drinking water was available *ad libitum*. Prior to collection, all bucks were clinically examined to ensure good health and the absence of reproductive disorders.

Ethical approval: All animal care, handling, and semen collection were conducted in compliance with the Law on Animal Husbandry (No. 32/2018/QH14) issued by the National Assembly of the Socialist Republic of Vietnam. Animal welfare and health status were carefully monitored and maintained throughout the experimental period.

Experimental design: Twelve bucks were randomly divided into three groups (four bucks per group) according to collection frequency: every 1 day, every 2 days, and every 3 days. Semen characteristics including volume, concentration, motility, kinematic parameters, and abnormal morphology, were recorded.

Semen samples from all bucks were pooled immediately after within collection days and diluted at different semen-to-extender ratios (1:1, 1:3, 1:4, 1:5, 1:6, and 1:10) using a citrate extender (Chemineau and Cagnie, 1991). The extender was prepared with glucose, sodium citrate, hens' egg yolk, streptomycin, penicillin, and distilled water up to 100 mL. Diluted semen was cryopreserved, and post-thaw sperm motility was evaluated.

Frozen semen straws were thawed at three different temperatures (20°C, 37°C, and 42°C) and for four durations (30, 60, 90, and 120 seconds) in order to evaluate the combined effects of temperature and thawing duration on post-thaw sperm motility. These temperature–time combinations were selected to cover a wide range of thawing conditions, allowing the identification of optimal thawing parameters as well as the assessment of potential thermal stress associated with prolonged exposure at higher temperatures.

Semen volume: Ejaculate volume was measured immediately after collection using a graduated glass pipette. The semen sample was allowed to settle in a vertical position on a flat surface, and the volume was recorded by reading the lower meniscus. Only complete ejaculates with no contamination were considered for further analysis.

Motility (A: 0% < A ≤ 100%): Progressive motility was assessed under 200× magnification at 2, 3, and 5 hours post-collection. The percentage of motile spermatozoa was recorded manually through visual observation of forward movement.

Additionally, sperm motility characteristics were evaluated using a Computer-Assisted Sperm Analysis (CASA) system (Hamilton Thorne, USA). This automated system provided a comprehensive and objective analysis of sperm kinetics. The following CASA parameters were recorded:

Straight Line Velocity (VSL, μm/s): Linear distance travelled per unit time.

Curvilinear Velocity (VCL, μm/s): Total velocity along the actual path of the sperm.

Average Path Velocity (VAP, μm/s): Mean velocity along a smoothed trajectory.

Straightness (STR, %): Ratio of VSL to VAP, indicating directional accuracy.

Amplitude of Lateral Head Displacement (ALH, μm): Lateral movement of the sperm head during progression.

Beat Cross Frequency (BCF, Hz): Frequency with which the sperm head crosses the average path.

Sperm concentration: Concentration (C, $x10^9$/mL): Sperm concentration was measured using an SDM1 sperm densimeter (Minitube, Germany). Each semen sample was gently mixed and measured in triplicate to ensure accuracy. The average of the three readings was used for data analysis.

Abnormal sperm: The percentage of abnormal spermatozoa *(K, %)* was evaluated using the methylene blue staining method. A drop of fresh semen was placed on a clean glass slide and mixed with a few drops of 0.85% NaCl solution. The mixture was thoroughly mixed with a glass rod and gently smeared using a second slide to obtain a thin, uniform film and allowed to air-dry. The dried smear was then heat-fixed by briefly passing it over an alcohol flame. Slides were stained with methylene blue dye for approximately 10 minutes, rinsed with clean water, and examined under an Olympus microscope at 400× magnification. A total of 300–500 spermatozoa per slide were counted randomly including normal and abnormal sperm, and the proportion of morphologically abnormal cells was calculated.

$$K(\%) = \frac{n}{N} \times 100$$

Notes:

K%: Percentage of abnormal sperm morphology.

n: Number of abnormal sperm (of various types).

N: Total number of sperm counted, including both abnormal and normal sperm (N= 300-500).

Sperm Resistance: Sperm Resistance *(R)* was determined using the Milovanov chain dilution method (Milovanov, 1962). Briefly, semen samples were subjected to serial dilution with 1% NaCl solution. Progressive sperm motility was examined after each dilution step, and additional NaCl solution was added sequentially until forward sperm movement ceased. Sperm resistance was calculated based on the initial dilution level and the number of subsequent

dilution steps required to completely eliminate progressive motility.

$$R = r_o + r.n$$

Notes:

R: Sperm resistance.

r_o: Initial dilution level of sperm at the starting point.

r: Dilution level for each subsequent addition of 1% NaCl solution (r = 200).

n: Number of subsequent additions of 1% NaCl solution.

pH value

Semen pH: Determined using a pH/Ion meter (WINLAB, Japan). Each sample was measured three times, and the average value of the three measurements was recorded.

Semen processing, cryopreservation, and thawing: Fresh semen ejaculates were diluted using a citrate extender according to Chemineau and Cagnie (1991). The extender consisted of glucose, sodium citrate, hens' egg yolk, streptomycin, penicillin, and distilled water up to 100 mL. Immediately after collection, semen was initially diluted at a ratio of 1:1 (semen:extender) to facilitate cooling and equilibration, and then equilibrated at 4–5 °C for 2.5–4 h. After equilibration, the semen was further diluted to obtain the final semen-to-extender ratios of 1:1, 1:3, 1:4, 1:5, 1:6, and 1:10, which were used as experimental treatments to evaluate the effect of dilution ratio on post-thaw sperm motility. The diluted semen was subsequently cryopreserved.

Following equilibration, semen was packaged into 0.25 mL straws and frozen using a programmable freezer following the method of Evans and Maxwell (1987). Briefly, straws were exposed to liquid nitrogen vapor 16 cm above the surface for 2 min, then at 4 cm above the surface for 3 min, before being plunged directly into liquid nitrogen (-196°C) for storage.

Thawing was performed according to Corteel (1977). A total of 45 frozen semen samples were thawed at three different temperatures (20°C, 37°C, and 42°C; 15 samples per temperature) for four durations (30, 60, 90, and 120 seconds). Post-thaw sperm motility was evaluated under each thawing condition.

Statistical analysis: Data from individual ejaculates were analyzed using one-way analysis of variance (ANOVA) to evaluate the effects of experimental treatments. When significant differences were detected ($P < 0.05$), means were compared using Tukey's HSD test. Percentage data were arcsine square-root transformed prior to analysis, but results are presented as untransformed Mean ± SD for clarity. The assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene's tests. All statistical analyses were performed using Minitab software (version 16).

RESULTS AND DISCUSSION

Basic semen quality of Bach Thao bucks: The effect of collection frequency on semen quality of Bach Thao bucks is summarized in Table 1. Clear differences were observed among the three collection intervals, indicating that semen characteristics were strongly influenced by the time allowed between ejaculations

The semen volume recorded in Bach Thao bucks was slightly lower than that reported in other goat breeds. Yodmingkwan *et al.* (2016) observed an average volume of 1.15 mL in Boer goats, and Asanbekov (1983) reported 1.05 mL in goats raised in Kirgizia. More recently, Nhan *et al.* (2025) documented semen volumes of 1.24 mL in Boer goats and 1.16 mL in Alpine goats. In contrast, the present findings were higher than those reported by Vargas *et al.* (2022), who recorded an average of 0.51 mL in goats from Antioquia. These differences may be related to breed characteristics, management systems, or environmental conditions influencing reproductive performance. Furthermore, working with West African Dwarf (WAD) bucks, Iheukwumere (2008) reported similar findings, showed that frequent ejaculation resulted in a marked reduction in semen volume. This decrease was attributed to excessive physiological demand without sufficient time for recovery. Moyorga-Torres *et al.* (2015) further demonstrated a significant positive correlation between the length of sexual abstinence and semen volume, indicating that adequate rest between ejaculations is essential for volume accumulation. In line with these observations, the present study also showed that extending the collection interval led to increased semen volume and sperm concentration, plausibly reflecting replenishment of epididymal sperm reserves and recovery of accessory gland secretions. Additionally, the reduction in progressive motility observed under daily collection may be associated with the release of immature spermatozoa due to excessive collection pressure. Similar patterns of reduced semen quality under high ejaculation frequency, followed by recovery at moderate or longer intervals, have also been reported by Omasanya *et al.* (2021).

Assessment of sperm motility, morphology, and acrosomal status is a fundamental criterion for evaluating semen quality prior to its use in artificial insemination (Salamon and Maxwell, 2000). Spermatozoa typically exhibit two main phases of motility: active and hyperactivated. Active motility is characteristic of fresh ejaculate and is defined by a symmetrical, low-amplitude waveform that enables sperm cells to move progressively in a straight trajectory (Henkel *et al.* 2005). In the present study, sperm kinematic parameters improved as the collection interval increased (Table 1). Both straight-line velocity (VSL) and curvilinear velocity (VCL) were significantly higher in bucks collected every two to three days compared with those collected daily, indicating more vigorous and coordinated sperm movement. These trends suggested that longer recovery periods favor sperm maturation and energy balance, which enhance motility and fertilizing ability. The mean values recorded in Bach Thao bucks were comparable to those reported by Kozdrowski *et al.* (2007), who observed mean VSL and VCL of 68.46 and 129.20 µm/s, respectively, in frozen goat semen. Slight increases were also observed in the straightness ratio (STR) and

Table 1. Effect of collection time on semen quality of Bach Thao bucks (Mean ± SD)

Evaluation criteria	Every 1 days	Every 2 days	Every 3 days
Volume (V, mL)	0.71 ± 0.15^{c}	0.92 ± 0.09^{b}	1.08 ± 0.11^{a}
VSL (μm/s)	90.02 ± 2.71^{c}	92.14 ± 2.69^{b}	95.38 ± 2.73^{a}
VCL (μm/s)	132.35 ± 3.12^{c}	148.36 ± 3.14^{b}	154.17 ± 3.11^{a}
STR (%)	78.02 ± 2.21^{c}	79.63 ± 2.25^{b}	80.08 ± 2.21^{a}
ALH (μm)	2.85 ± 0.45^{c}	3.24 ± 0.46^{b}	3.82 ± 0.48^{a}
BCF (Hz)	12.03 ± 1.62	12.03 ± 1.64	12.04 ± 1.61
Concentration (C, ×10^{9}/mL)	2.66 ± 0.41^{c}	2.97 ± 0.45^{b}	3.06 ± 0.38^{a}
Abnormal sperm morphology (K, %)	13.48 ± 1.49^{a}	9.86 ± 1.46^{b}	8.25 ± 1.47^{c}
Sperm resistance (R)	6090.94 ± 275.32	6090.96 ± 270.11	6091.21 ± 271.89
pH	6.95 ± 0.16	6.96 ± 0.11	6.98 ± 0.15

Means with different superscripts in the same row differ significantly (P<0.05).

amplitude of lateral head displacement (ALH), implying improved directional motility and flagellar flexibility under moderate collection frequencies. In contrast, the beat cross frequency (BCF) remained relatively constant across all collection intervals, suggesting that this parameter is less sensitive to the frequency of semen collection. According to Mortimer (2000), higher BCF and linearity (LIN) values are generally associated with better sperm transport and penetration through cervical mucus, supporting the present observation that optimal collection spacing enhances the functional motility of Bach Thao buck spermatozoa.

Sperm concentration of Bach Thao bucks increased significantly with longer collection intervals, while the proportion of abnormal spermatozoa declined (Table 1). These results suggested that extended intervals between ejaculations allow for more complete spermatogenesis and epididymal maturation, resulting in a higher proportion of morphologically normal and viable sperm cells. In contrast, frequent semen collection may reduce the availability of mature sperm in each ejaculate due to insufficient replenishment of spermatozoa reserves. The sperm concentration recorded in the present study was within the range reported for other goat breeds. Bezerra *et al.* (2009) noted that sperm concentration in tropical goats can vary from 0.9 to 2.7 ×10^{9}/mL during the rainy season and from 0.96 to 2.15 ×10^{9}/mL during the dry season. Studies on Norwegian goats (Paulenz *et al.* 2005) and Florida goats (Hidalgo *et al.* 2008) indicated that highly fertile bucks generally produce sperm concentrations above 2000 ×10^{6}/mL. The proportion of abnormal spermatozoa in Bach Thao bucks was also comparable to the results of Bintara 2011, who found no significant differences between Kacang goats (8.2 ± 3.3%) and Peranakan Ettawa goats (8.6 ± 2.4%). These findings support the view that both sperm concentration and morphology are influenced by management practices and collection frequency rather than breed alone.

Regarding physicochemical traits, semen pH increased slightly with longer collection intervals (Table 1). This trend suggests a minor alteration in seminal plasma composition associated with reduced collection frequency. According to Fukuhara and Nishikawa (1973), an increase in pH may enhance sperm respiration and motility, although excessively high values could eventually compromise sperm viability. Sperm resistance, however, remained stable across all treatments, indicating that this parameter is relatively constant and less influenced by collection frequency. Previous studies examining the influence of seminal plasma biochemical components, including ions (Ca^{2+}, Mg^{2+}, PO_4^{3-}) and organic molecules such as total protein, citric acid, and fructose, on goat sperm characteristics have reported significant correlations between these parameters and sperm motility and vigor (Aguiar, 2008).

The results in Table 1 clearly demonstrate that semen quality of Bach Thao bucks improved when semen was collected at intervals of 2 to 3 days. Longer recovery times resulted in higher semen volume, concentration, and motility, together with reduced morphological abnormalities. Collecting semen every day reduced ejaculate quality, suggesting that a 2–3 day interval is optimal for semen collection in breeding and cryopreservation programs for this indigenous goat breed.

Semen processing, cryopreservation, and thawing: The effect of semen dilution ratio with freezing extender on post-thaw sperm motility of Bach Thao bucks is shown in Table 2. Significant differences were observed among the dilution ratios ($P < 0.05$), indicating that the degree of dilution plays an important role in cryosurvival of spermatozoa.

At the lowest dilution (1:1), post-thaw motility was 38.12 ± 0.75%, which was significantly lower compared with the higher dilution levels. Motility improved to 41.08 ± 0.96% at 1:03 and 41.37 ± 0.84% at 1:4, both of which were statistically higher than 1:1 but still lower than the optimal levels. At 1:5, sperm motility increased further to 43.57 ± 0.93%, while the highest motility was recorded at 1:6 with 46.28 ± 0.82%. This suggests that moderate dilution facilitates better penetration of extender components into sperm cells and reduces cell-to-cell interactions that

Table 2. Effect of semen dilution ratio with freezing extender on post-thaw sperm motility of Bach Thao bucks (Mean ± SD)

1:1	1:3	1:4	1:5	1:6	1:10
38.12 ± 0.75^{c}	41.08 ± 0.96^{b}	41.37 ± 0.84^{b}	43.57 ± 0.93^{ab}	46.28 ± 0.82^{a}	40.01 ± 0.77^{bc}

Means with different superscripts in the same row differ significantly (P<0.05).

may otherwise cause damage during freezing. When the dilution ratio was increased further to 1:10, post-thaw motility decreased again to 40.01 ± 0.77%, which was significantly lower than at 1:6. According to Purdy (2006), the dilution rate of goat semen can range from 1:1 to 1:23 (semen : extender). In practice, a ratio of 1:9 may be used when resources are not available to accurately determine the post-dilution concentration, which is expected to be approximately 200 $\times 10^{6}$ spermatozoa/mL (Nunes 2002). Excessive dilution likely reduces the protective capacity of seminal plasma and results in a lower number of sperm per volume, making them more vulnerable to cryo-injury. According to Gibbons (2002), an extender suitable for goat semen cryopreservation should include buffering agents such as Tris and sodium citrate; energy sources such as glucose and fructose; external cryoprotectants such as egg yolk or milk, or internal ones such as glycerol and ethylene glycol; as well as antibiotics, with penicillin and streptomycin being the most commonly used.

A semen-to-extender ratio of 1:6 provided the most favorable outcome for post-thaw sperm motility in Bach Thao bucks. This ratio appeared to be optimal for balancing the protective effects of the extender while maintaining sufficient sperm concentration for fertilization potential.

The influence of thawing temperature and duration on post-thaw sperm motility is presented in Table 3. Both temperature and thawing time had significant effects on motility ($P < 0.05$), with clear differences observed across treatments.

The effect of thawing temperature and duration on post-thaw sperm motility of Bach Thao bucks is summarized in Table 3. Thawing temperature had a pronounced influence on the recovery of motility after freezing. At the lowest temperature (20 °C), sperm motility remained consistently poor regardless of thawing time, indicating that slow warming failed to restore metabolic activity and might have prolonged ice-crystal damage during the warming process. Thawing at 37 °C produced a marked improvement in motility, particularly when samples were warmed for about one to one and a half minutes. This temperature approximated body conditions and provided an optimal balance between rapid reactivation of metabolism and minimal thermal stress. Similar trends have been observed in previous studies, where cryopreservation decreased sperm motility and kinematic parameters (Dorado *et al.* 2010; Batista *et al.* 2011). At higher temperatures, such as 42 °C, motility initially improved but declined with longer thawing durations, suggesting that prolonged exposure elevated heat caused structural and functional damage to spermatozoa. Comparable findings have been reported in goats, where thawing at 37 °C for 12–30 seconds yielded better results than slower thawing at 5 °C for 2 minutes (Purdy 2006). Tuli *et al.* (1991) found that a short, high-temperature treatment (70 °C for 7 seconds) provided superior progressive motility and plasma-membrane integrity compared with longer thawing at moderate temperatures, while Azerêdo *et al.* 2001 reported similar improvements when semen was thawed at 70 °C for 5 seconds.

The success of cryopreservation also depends on the composition of the dilution medium. Non-penetrating cryoprotectants protect cells from cold shock during cooling to 5 °C, whereas penetrating cryoprotectants such as glycerol safeguard spermatozoa against damage during freezing and thawing (Purdy 2006; Castelo *et al.* 2008).

Taken together, Table 3 indicated that both thawing temperature and duration are critical determinants of post-thaw sperm quality. Thawing at 37 °C for 60–90 seconds appeared to be the optimal condition, balancing rapid ice crystal dissolution with minimal thermal stress. These conditions are therefore recommended for routine use in artificial insemination programs employing frozen-thawed semen of Bach Thao bucks. Although higher temperature–shorter duration thawing protocols have been reported in previous studies, the optimal thawing condition identified in the present study (37 °C for 60–90 s) should be interpreted as being specific to the straw size, extender composition, and freezing procedure employed. Differences in cryopreservation protocols may account for the variation in optimal thawing conditions among studies.

Semen quality of Bach Thao bucks improved when collected every 2-3 days, with higher volume, concentration,

Table 3. Effect of thawing temperature and duration on post-thaw sperm motility of Bach Thao bucks (Mean ± SD)

Thawing temperature	Sperm motility (%)			
	30 seconds	60 seconds	90 seconds	120 seconds
20°C	18.02 ± 1.21^{c}	19.36 ± 1.74^{c}	20.48 ± 1.82^{c}	25.14 ± 1.77^{c}
37°C	32.18 ± 1.21^{b}	40.57 ± 2.89^{b}	45.02 ± 1.81^{a}	44.17 ± 1.68^{a}
42°C	40.46 ± 1.21^{a}	42.51 ± 1.69^{a}	40.02 ± 1.54^{b}	35.73 ± 1.67^{b}

Means with different superscripts in the same column differ significantly (P<0.05).

and motility, and fewer abnormalities compared with daily collection. A dilution ratio of 1:6 yielded the best post-thaw motility, while excessive dilution reduced sperm survival. Thawing at 37°C for 60–90 seconds provided optimal recovery of motility. These results offer practical guidelines to optimize semen collection and cryopreservation protocols for artificial insemination in Bach Thao goats.

REFFERENCES

Abecia JA, Arrebola F, Macias A, Laviña A, González-Casquet O, Benitez F and Palacios C. 2016. Temperature and rainfall are related to fertility rate after spring artificial insemination in small ruminants. *International Journal of Biometeorology* **60**(11): 1603–09. https://doi.org/10.1007/s00484-016-1150-y

Aguiar GV 2008. *Efeito individual e da época do ano sobre a composição do plasma seminal e a qualidade do sêmen caprino resfriado a 4 ºC por 48 horas do estado do Ceará 114p.* Tese (Doutorado em Zootecnia), Universidade Federal do Ceará, Fortaleza.

Asanbekov OA. 1983. A study of reproductive traits in down goat in Kirgizia. *Trudy Kirg NPO po Zhivotnovod* **35**: 92–98.

Azerêdo GA, Esper CR and Resende KT. 2001. Evaluation of plasma membrane integrity of frozen-thawed goat spermatozoa with or without seminal plasma. *Small Ruminant Research* **41**: 257–63.

Batista M, Niño T, Santana M, Alamo D, Castro N, Reyes R, González F, Cabrera F and Gracia A. 2011. Influence of the preservation temperature (37, 20, 4, −196 °C) and the mixing of semen over sperm quality of Majorera bucks. *Reproduction in Domestic Animals* **46**: 281–88. https://doi.org/10.1111/j.1439-0531.2010.01659.x

Bezerra FQG, Neto LMF, Filho CRA, Chaves RM, Santos MHB, Neves JP and Oliveira MAL. 2009. Avaliação espermática de caprinos jovens da raça boer nascidos nas estações chuvosa e seca. *Ciência Animal Brasileira* **10**(4): 1256–62.

Bintara S. 2011. Ratio of X:Y Spermatozoa and Sperm Quality of Kacang and Ettawa-Crossed Breed Goats. *Sains Peternakan* **9**(2): 65–71.

Castelo TS, Frota TR and Silva AR. 2008. Considerações sobre a criopreservação do sêmen de caprinos. *Acta Veterinaria Brasilica* **2**(3): 67–75. https://doi.org/10.21708/avb.2008.2.3.885

Chemineau P and Cagnie Y. 1991. *Training manual on artificial insemination in sheep and goats*. FAO, Aminal production and Health **83**.

Corteel JM 1977. *Production, storage and insemination of goats semen.* Proceedings of the symposium: management of reproduction in sheep and goat, Madison, 24–25.

Dorado J, Munoz-Serrano A and Hidalgo M. 2010. The effect of cryopreservation on goat semen characteristics related to sperm freezability. *Animal Reproduction Science* **121**: 115–123. https://doi.org/10.1016/j.anireprosci.2010.04.182

Evans G and Maxwell WMC 1987. *Salamons' artificial insemination of sheep and goats*. Sydney, Australia, Butterworths.

Fukuhara R and Nishikawa Y 1973. Effect of pH, sperm concentration, washing and substrate concentration on respiration and motility of goat spermatozoa. *Nihon ChikusanGakkaiho* **44**: 266–270. https://doi.org/10.2508/chikusan.44.266

Gibbons A. 2002. Inseminación artificial con semen congelado en cabras de raza Angora. *Revista Taurus* **4**(16): 24–32.

Henkel R, Maaß G, Bödeker RH, Scheibelhut C, Stalf T, Mehnert C, Schuppe HC, Jung A and Schill WB. 2005. Sperm function and assisted reproduction technology. *Reproductive Medicine and Biology* **4**: 7–30. https://doi.org/10.1111/j.1447-0578.2005.00087.x

Hien NTT. 2023. Evaluation of estradiol and progesterone contents of Bach Thao and Boer goats during the oestrous cycle. *Vietnam Journal of Science and Technology* **65**(12): 52–55. https://doi.org/10.31276/VJST.65(12).52-55

Hidalgo M, Rodriguez I and Dorado J. 2008. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Animal Reproduction Science* **100**: 61–72. https://doi.org/10.1016/j.anireprosci.2006.07.003

Iheukwumere FC. 2008. Effect of Different Intensities of Semen Collection and Biochemical Evaluation of the Seminal Plasma of West African Dwarf Bucks. *Journal of Agriculture and Social Research* **8**(1): 34–44.

Jiménez-Rabadán P, Ramón M, García-Álvarez O, Maroto-Morales A, Álvaro-García PJ, Del Olmo E, Pérez-Guzmán MD, Fernández-Santos MR, Garde JJ and Soler AJ. 2013. Improved cryopreservation protocol for Blanca-Celtibérica buck semen collected by electroejaculation. *Cryobiology* **67**: 251–57.

Jobling S, Coey S, Whitmore JG, Kime DE, Van Look KJ, McAllister BG, Beresford N, Henshaw AC, Brighty G, Tyler CR and Sumpter JP. 2002. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biology of Reproduction* **67**(2): 515-524. https://doi.org/10.1095/biolreprod67.2.515

Khadiga MG, Mohamed KG and Doaa FT. 2005. The hormonal profile during the estrous cycle and gestation in Damascus goats. *Small Ruminant Research* **57**(1): 85–93. https://doi.org/10.1016/j.smallrumres.2004.07.009

Kifaro GC, Eik LO, Mtenga LA, Mushi DE, Safari J, Kassuku AA, Kimbita EN, MaedaMachang'u AD, Kanuya NL, Muhikambele VRM, Ndemanisho E and Ulvund MJ. 2007. The potential use of artificial insemination in sustainable breeding of dairy goats in developing countries: A case study of Norwegian dairy goats in Tanzania. *Tanzania Journal of Agricultural Sciences* **8**: 19–24.

Kozdrowski R, Dubiel A, Bielas W and Dzięcioł M. 2007. Two protocols of cryopreservation of goat semen with the use of computer-assisted semen analysis system. *Acta Veterinaria Brno* **76**(4): 601–4.

Leboeuf B, Restall B and Salamon S. 2003. Production et conservation de la semence de bouc pour l'insémination artificielle. *INRA Productions Animales* **16**(1): 91–99.

Leboeuf B, Delgadillo JA, Manfredi E, Piacère A, Clement V, Martin P, Pellicer-Rubio MT, Boué P and de Cremoux R. 2008. Place de la maîtrise de la reproduction dans les schémas de sélection en chèvres laitières**.** *INRA Productions Animales* **21**(4): 391–402.

Mayorga-Torres BJM, Camargo M, Agarwal A, Du Plessis SS, Cadavid ÁP, and Maya WDC. 2015. Influence of ejaculation frequency on seminal parameters. *Reproductive Biology and Endocrinology* **13**(1): 1–7.

Memon AA, Wahid H, Rosnina Y, Goh YM, Ebrahimi M and Nadia FM. 2013. Effect of ascorbic acid concentrations, methods of cooling and freezing on Boer goat sperm cryopreservation. *Reproduction in Domestic Animals* **48**: 325–330. https://doi.org/10.1111/j.1439-0531.2012.02155.x

Milovanov VK 1962. *Biology of reproduction and articial insemination of animals*. Moscow, Russia: Selhozizdat.

Morrell JM, Malaluang P, Ntallaris T and Johannisson A. 2022.

Practical method for freezing buck semen. *Animals* **12**: 352. https://doi.org/10.3390/ani12030352

Mortimer ST 2000. CASA-practical aspects. *Journal of Andrology* **21**(4): 515–524.

Nhan P, Chuc NT, Mo TTH, Phuong NTM and Tri NM. 2025. Some quantitative, qualitative, and physicochemical parameters of semen from Boer and Alpine breeding bucks. *Journal of Animal Science and Technology* **149**: 52–61. https://doi.org/10.70408/nias.i149-y2025-593

Nunes JF 2002. *Inseminação artificial em caprinos*. In: Gonçalves PBD, Figueiredo JR, Freitas VJF (eds.), Biotécnicas aplicadas à reprodução animal. São Paulo: Varela, p. 340.

Omasanya OK, Hassan JO, Oloye AA, Oyewusi IK, Oni OO, Olurode SA, Oloruntuga OO, Adeusi AA, Bassahwa AP, Adetomiwa AS and Mustapha L. 2021. Effects of ejaculation frequency on semen characteristics and serum testosterone concentration in Red Sokoto bucks. *Nigerian Journal of Animal Production* **48**(6): 34–45. https://doi.org/10.51791/njap.v48i6.3275

Paulenz H, Soltun K, Ådnøy T, Berg KA and Söderquist L; 2005. Effect of different extenders on sperm viability of buck semen stored at room temperature. *Small Ruminant Research* **59**: 89–94.

Purdy PH. 2006. A review on goat sperm cryopreservation. *Small Ruminant Research* **63**(3): 215–225. https://doi.org/10.1016/j.smallrumres.2005.02.015

Salamon S and Maxwell WMC. 2000. Storage of ram semen. *Animal Reproduction Science* **62**(1–3): 77–111. https://doi.org/10.1016/S0378-4320(00)00155-X

Tuli RK, Schmidt-Baulain R and Holtz W. 1991. Influence of thawing temperature on viability and release of glutamic oxaloacetic transaminase in frozen semen from Boer goats. *Animal Reproduction Science* **25**: 125–31.

Vargas DH, Escobar MC, Cadavid HC, Maya WDC, Mayorga-Torres JM, López-Pérez J and Davila DA. 2022. Konvencionalna i funkcionalna procjena sjemena mužjaka mliječnih koza. *Veterinarska Stanica* **54**(2): 129–36. https://doi.org/10.46419/vs.54.2.1

Yodmingkwan P, Guntaprom S, Jaksamrit J and Lertchunhakiat K 2016. Effects of extenders on fresh and freezing semen of Boer goat. *Agriculture and Agricultural Science Procedia* **11**: 125–30.

Zhao BT, Han D, Xu CL, Luo MJ, Chang ZL and Tan JH. 2009. Protocol optimization for long-term liquid storage of goat semen in a chemically defined extender. *Reproduction in Domestic Animals* **44**: 865–72.