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Reproductive and biochemical dynamics of local Iraqi bucks across seasonal variations

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Abstract
The primary objective of this research was to assess how environmental shifts and thermal stress induced by summer and winter conditions affect seminal traits, seminal plasma biochemical profiles, testosterone levels, and reproductive success in local Iraqi bucks. The experiment was conducted using 14 healthy, sexually mature males, with semen samples collected weekly throughout the duration of the research work. Statistical analysis demonstrated a pronounced elevation ($p \leq 0.05$) in both maximum and minimum ambient temperatures during the summer months, , compared to a significant increase in relative humidity during the winter. Furthermore, samples collected during the winter demonstrated reaching statistical significance at $P \leq 0.05$ in terms of sperm concentration, mass , individual motility, and acrosome integrity. Total antioxidant capacity, testosterone concentration, and fertilization rate were higher, while the percentages of dead spermatozoa, abnormalities, and plasma membrane damage as well as the concentration of malondialdehyde (MDA) were significantly lower compared to the summer season. Furthermore, "High-performance liquid chromatography (HPLC) measurements demonstrated a pronounced elevation ($P \leq 0.05$) in total concentrations of both indispensable and dispensable amino acid profiles of the seminal fluid during the winter compared to the summer. This study highlights the severe adverse effects of summer the consequences of high temperatures on the semen characteristics and reproductive efficiency of local goats in a subtropical environment, underscoring the importance of these biomarkers and amino acids as precise metrics for assessing reproductive status under environmental stress.

Keywords: Season, Semen, Amino acids, Goat fertility

Introduction

In recent decades, the world has experienced significant changes in climatic conditions most notably rising temperatures and heat stress (Auboghaba *et al*., 2021) [1] which have impacted various productive and reproductive aspects of living organisms. Given that the reproductive performance of animals is the result of an interaction between environmental and genetic factors, it is expected that changes in the environment will have a clear effect on their reproductive efficiency (Kirsten *et al*., 2019) [2]. The domestic goat (*Capra hircus*) is considered one of the most important farm animals in Iraq and the Middle East, owing to its high adaptability to arid and semi-arid environments, as well as, its significant contribution to milk and meat production (Alkass *et al*., 2011) [3]. The reproductive efficiency of bucks is a fundamental pillar for the success of breeding and production improvement programs, as it is governed by multiple determinants, particularly nutritional status, physiology, genetics, and season (Blache *et al*., 2006 [4] ; Delgadillo *et al*., 2009 [5]; Cárdenas-Gallegos *et al*., 2012 [6]; Malejane *et al*., 2014) [7]. Evaluating semen characteristics in bucks is an essential procedure for selecting males with high fertility potential and culling those that are less capable. Although goats raised in tropical climates are generally considered year-round breeders, they often experience prolonged periods of reproductive inactivity when feed resources are limited (El-Sayed *et al*., 2018). Furthermore, Climatic heat stress represents one of the major most significant environmental challenges threatening animal productivity (Auboghaba *et al.,* 2021) [1], given that increased environmental temperatures impair both the reproductive capacity and the overall reproductive performance of the animals (Al-Kanaan *et al*., 2015; Isnaini *et al*., 2020) [8, 9]. Numerous Previous research has indicated the deleterious effects of thermal stress regarding male reproductive performance, encompassing andrological traits, androgen synthesis, sexual behavior, and overall fertility rates (Dias *et al*., 2017) [10]. Environmental factors including Environmental variables encompassing ambient thermal conditions, atmospheric moisture levels, and photoperiod dynamics influence animal reproduction. Owing to seasonal fluctuations variations, functional integrity of spermatozoa in male goats may change over time (Jamal *et al*., 2021) [11]. Impact and Effects a direct adverse effects on reproductive success through disrupting the cellular integrity the functions of reproductive cells (Shahat *et al*., 2020) [12]. It can also exert an indirect effect, as animals experiencing heat stress

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often exhibit reduced Nutritional consumption (Hedia *et al*., 2020) [13]. Furthermore, heat stress causes direct damage to sperm morphology Fertilization capacity levels (Sejian *et al*., 2018) [14]. It also leads to a reduction in Blood supply to the testes (El-Sherbiny *et al*., 2022) [15].

Goats are display remarkable adaptation capabilities toward thermal instability compared with conventional agricultural species; they adapt by lowering suppressing cellular metabolic activity, while simultaneously concurrently elevating their respiratory frequency heart rates (Al-Saki *et al*., 2018) [16]. Furthermore, the response of goats to stress induced by handling varies based on individual differences, breed, and geographical region (Salama *et al*., 2014 ; Samir *et al*., 2018) [17, 18]. Studies on the characteristics of goat semen have yielded varied results, modulated by determinants such as breed, Ambient factors and geographical location. Globally, most such studies have categorized data based on the four seasons or on a monthly basis, however, in the climate of Iraq, the spring and autumn seasons are relatively short and poorly defined. Furthermore, monthly evaluations alone may not accurately reflect direct climatic impacts, given that the process of spermatogenesis in goats spans approximately 48–60 days. Consequently, this This investigation was undertaken to evaluate the influence of thermal variations on the physical characteristics and certain biochemical components resulting from the seminal plasma in local Iraqi buck goats during the winter and summer seasons.

Materials and Methods

Experimental animals

This field experiment was conducted on a local goat herd owned by a private breeder in the Al-Musaib project, Babil Governorate. Laboratory analysis was performed at the Animal Production Laboratories within the Al-Musaib Technical College, in collaboration with the former Ministry of Science and Technology, to quantify amino acid concentrations in the seminal plasma of local bucks. This study utilized 14 healthy, sexually mature male goats (bucks), ranging in age from 3 to 5 years, with a mean body weight of 30 ± 40 kg. The animals were maintained under a grazing system in a subtropical climate, supplemented with a balanced ratio of grains, and received periodic vaccinations. Each male mated with 7 females per season all of which were sexually mature, free from any systemic or reproductive diseases, and aged between 3 and 4 years. Based on environmental temperature data (Table 1), these animal groups were studied over the period from winter 2024 to summer 2025. Climatic data including ambient temperatures and ambient relative humidity were systematically registered daily, both in morning and in evening.

Before the study began, The male goats were accustomed to ejaculating into an artificial vagina (AV) for semen collection. The samples selected for the study were based on fundamental sperm quality criteria: ejaculates displaying a volume of at least 0.2 mL and a sperm concentration exceeding 10^9 spermatozoa/mL, alongside, progressive motility of at least 60%. samples were collected once a week throughout the two study periods using an electro-ejaculator, and were then transported to the laboratory for the following analyses:

The volumetric yield of the semen sample was recorded using a conical, graduated glass collection tube, with a volumetric precision of 0.1 ml, whereas spermatozoal concentration was determined utilizing a 591B-type densimeter to quantify the number of sperm cells per milliliter.. Litmus paper (with 0.3-unit increments) was used to measure the pH. Mass motility was evaluated immediately after collection by depositing a micro-droplet of semen onto a pre-warmed glass slide (37 °C) and evaluating it under an optical microscope at 40x magnification; Spermatozoal motility intensity was scored using a 1 to 5 rating system, according to the protocol outlined by Avdi *et al.* (2004) [19]. Individual Spermatozoal motility percentage was determined through examining the sample under 400x magnification, following the method of Soltanpour and Moghaddam (2012). The proportion of non-viable spermatozoa was evaluated employing an eosin-nigrosin staining technique, where unstained (colorless) sperm were considered alive, and pink-stained sperm were considered dead. Sperm morphological defects (%) were quantified, with specific abnormalities involving the head, midpiece, and tail regions separately evaluated.

Additionally, a fast gree stain was used to assess acrosomal abnormalities; with this technique, the acrosome appears pale green, while the sperm head appears red (as described by Salamon *et al*., 1995) [20]. Spermatozoal viability and acrosomal membrane integrity were assessed in accordance with the protocol reported by Noor *et al*. (2005). Blood specimens were obtained via jugular venipuncture utilizing plain vacutainer tubes devoid of anticoagulants (Mohamed *et al*., 2021) [21]. Subsequently, the tubes underwent centrifugation at 3000 rpm for 15 min, after which the harvested plasma was aspirated and stored at -20 °C pending further assays. Furthermore, Seminal plasma total antioxidant capacity (TAC) was evaluated utilizing a commercial assay kit (Sigma-Aldrich Co., St. Louis, USA) in accordance with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging protocol. This test serves as a comprehensive assessment of the activity of various non-enzymatic antioxidant compounds, including α-tocopherol (vitamin E), ascorbic acid (vitamin C), and β-carotene as well as enzymatic antioxidants (including GPx, SOD, CAT, and glutathione reductase). Seminal plasma MDA levels were evaluated through the thiobarbituric acid (TBA) reaction and via the thiobarbituric acid (TBA) assay protocol, following the method described by Kumaresan *et al.* (2006) [22]. Plasma testosterone concentration was determined via enzyme-linked immunosorbent assay (ELISA) utilizing commercial reagent sets (Monobind Inc., Lake Forest, CA, USA; Cat. No. 3725-300) in accordance with the manufacturer's protocol. The fertilization rate was calculated for each group following the artificial insemination of females during both summer and winter; pregnancy was confirmed two months post-insemination using an ultrasound scanner. The mean values for all groups were calculated for each season, and a comparison was made between the two seasons.

Measurement of amino acid concentration

Quantification of essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, and lysine) alongside non-essential amino acids (aspartic acid, serine, glutamic acid, proline, glycine, alanine, cystine, tyrosine, and arginine) in cryopreserved seminal plasma was performed via high-performance liquid chromatography (HPLC), following the method described by Abadi *et al.* (2016) [23].

Statistical analysis

Data were statistically processed using the General Linear Model (GLM) procedure in SAS software (SAS, 2012) [24]. A Completely Randomized Design (CRD) under a one-way classification layout was implemented to evaluate the seasonal impact on seminal attributes, serum testosterone levels, as well as essential and non-essential amino acid profiles in local goat seminal plasma. The data were fitted using the following mathematical model:

$Y_{ij} = \mu + S_i + e_{ij}$

Where:

Y_{ij}: The outcome measurement recorded for the j-th replicate under the i-th seasonal period.

μ: The overall population mean for the evaluated parameter.

S_i: The main fixed effect of the i-th season (summer vs. winter).

e_{ij}: The uncontrolled random error component, distributed normally as N(0, σ^2_e)

To evaluate significant variations among group means, Duncan's (1955) [25] multiple range test was applied employing the least squares means procedure.

Results and Discussion

Temperature and relative humidity

Both maximum and minimum thermal records exhibited marked elevations during the summer season relative to winter (35.13 ± 2.2 and 44.5 ± 1.5, respectively), while the mean relative humidity values

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were markedly elevated during the winter months (41.33) compared to the autumn (60.14 ± 2.1) (*Table 1*).

Table 1. Statistical overview of temperature and humidity values across summer and winter months (Mean ±SE).

Traits	Temperature		Relative Humidity
Season	Maximum	Minamum	
summer	44.5 ± 1.5a	35.13 ± 2.2a	15.32 ± 1.3b
winter	20.12 ± 2.3b	9.33 ± 2.7b	60.14 ± 2.1a

Values labeled with varying superscript letters (a,b) exhibit statistically reliable differences.

Semen characteristics

The winter sampling phase yielded data indicating a significant an elevation in semen parameters: concentration, mass and individual sperm motility, the percentage of intact acrosomes, total antioxidant capacity, testosterone concentration, and fertility rate compared to the summer season. Conversely, A marked reduction was recorded in the proportion of dead and abnormal spermatozoa, plasma membrane abnormalities, and MDA concentration during the winter diverging from summer values (Table 2).

Table 2. Season effects on Semen traits of males domastic goats. (Means±SE).

Season summer winter significance
Traits
Volume (ml) 0.6 ± 1.2 a 1.0 ± 1.3a $p \geq 0.05$
Concentration(10^9 sperm /ml) 1012.3 ± 1b 1675.5a ✱
PH 6.8 ± 0.5a 6.4 ± 0.4a $p \geq 0.05$
Mass activity(score) 2.43 ± 0.93b 3.99 ± 0.83a ✱
Sperm individual activity (%) 62 ± 0.5b 79.00 ± 1.83a ✱
dead sperms (%) 27.33 ± 1.42a 16.66 ± 2.03b ✱
total Sperm abnormalites (%) 30.00 ± 0.1a 14.00 ± 0.2b ✱
Acrosome intact (%) 65.00 ± 0.8a 81.00 ± 0.5b ✱
Plasma membrane integrity (%) 11.3 ± 0.4 a 4.2 ± 0.3 b ✱
Total antioxdants capacity (µg/dl) 11.32 ± 1.2b 20.21 ± 2.2a ✱
Malndialdehyde (µm/10^9 sperm) 25.22 ± 3.3a 12.45 ± 2.7 b ✱
Test.erone (ng/ml) 4. 9 ± 0.1b 5.6 ± 0.2a ✱
Conception rate (%) 66.00 ± 0.02B 92.00 ± 0.01A ✱

✱: means significantly different ($P < 0.05$) with letter differencess.. $p \geq 0.05$: Non-significant.

El Kadili *et al.* (2019) [26] found that higher ambient temperatures and longer daylight hours during the summer are associated with a reduction in testicular dimensions in male goats including scrotal circumference, Testicular axial length, width, along with transverse breadth and overall testicular dimensions compared to the winter season. Furthermore, elevated environmental temperatures negatively affect the reproductive activity of males (Boni, 2019), which in turn impacts both collective sperm motility and individual sperm movement (Sejian *et al.*, 2018) [14]. The collective and individual activity of spermatozoa does not return to its normal state following exposure to heat stress until 8–10 weeks after the stress event. This is because heat stress affects all stages of spermatogenesis (Myerhoeffer *et al.*, 1985; Al-Dulaimi, 2018; Al-Kanaan *et al.*, 2015) [28, 27, 8], disrupting the process, reducing sperm production, and increasing the percentage of spermatozoa and morphological abnormalities (Kanter *et al.*, 2013; Al-Wahab *et al.*, 2015) [30, 29]. Prior evidence substantiates that spermatogenic dynamics exhibit a 14% decline per unit degree Celsius elevation in testicular heat (Durairajanayagam *et al.*, 2015) [31]. Reduced feed intake whether due to high temperatures or a shortage of feed leads to diminished blood flow within the testes (*Baptista et al., 2023*) [32]. This, in turn, causes a decline in calcium levels within the blood and seminal fluid, thereby adversely impacting sperm motility parameters (Samir *et al.*, 2018 ; *Shahat et al., 2020; Harrison et al., 2022; Hedia et al., 2020*) [18, 12, 33, 13]. An elevated frequency of sperm plasma membrane disruption may be attributed because of an increase in free radicals within the seminal fluid during the summer; these free radicals can damage the lipid membranes of the sperm and lead to an increased production of malondialdehyde, an end product of oxidative stress (*Eidan et al., 2015b ; Van Tran et al., 2017; Eidan et al., 2020; Yangngam et al., 2021*) [34, 35, 36, 37].

High temperatures lead to pathological histological changes in the testicular tissue of male goats (Gao *et al.*, 2012; Setchell, 2018) [38, 39]. Furthermore, heat stress reduces testicular blood perfusion (Hedia *et al.*, 2020; *Vicente-Carrillo et al., 2023*) [13, 40]. This is associated with a decline in the quantity, quality, concentration, motility, and viability of sperm produced during the summer a finding consistent with data generated in the current trial. This aligns is consistent with previous reports by *Ridler et al. (2019*), *Samir et al. (2020) [41], and Sinha et al. (2021) [42]*, who also reported seasonal variations in sperm quality and morphology in other goat breeds. The poor quality of goat semen during the summer can be attributed to a severe depletion of energy substrates such as triglycerides, glucose, and cholesterol caused by high temperatures. This disrupts the body's internal homeostasis, particularly when energy requirements are not met due to dehydration (common in subtropical regions) and nutritional deficiencies exacerbated by heat, low rainfall, or drought. Such conditions place stress on sertoli cells, which play a pivotal role in the formation of steroid male hormones essential for successful reproduction while simultaneously increasing harmful free radicals and malondialdehyde (MDA) levels, alongside a reduction in antioxidant capacity. Moreover, oxidative stress damages the genetic material (DNA) of the sperm, leading to lower fertilization rates (*Mundim et al., 2007 [43]; Sezen and Güney, 2010 [44]; Ribeiro et al., 2016 [45]; Ribeiro et al., 2018b, Ribeiro et al., 2018a; Al-Samarrae, 2020 [46]*). The decline in semen quality during the summer in this study may be attributed to a correlation between seminal parameters and circulating testosterone levels (*Ragab et al., 2023*) [47]. Reproductive hormone levels in the blood are considered the most common and reliable biomarker for determining the functional competence of the testes (Shi *et al.*, 2018) [48]. The decrease in testicular androgen during the summer substantiates the reported evidence by Delgadillo *et al.* (2004) [49] regarding the impact of environmental conditions on reproduction in goats, as well as the findings of Mandour *et al.* (2022) [50] who clearly demonstrated the impact of ambient stressors on circulating testosterone levels in sheep. Furthermore, previous literature has reported seasonal variations in the testicular architectural measurements; specifically, as temperatures drop during the winter, the dimensions of the testes in male goats increase a change accompanied by higher testicular androgen profiles during the sexually active season, ultimately upregulating the spermatogenic process (Ntemka *et al.*, 2019) [51].

Seminal plasma amino acid profilse

The concentrations of essential amino acids valine, threonine, isoleucine, methionine, phenylalanine, leucine, histidine and lysine, as well as , the total amino acids contents were significantly higher in winter. Similarly, the non-essential amino acids profile serine, aspartic acid, proline, glutamine, cystine and glycine, exhibited greater abundance in winter than in the summer months (Table 3).

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Table 3. Effect of season on semen amino acids contents of males domastic goats .(Means±SE).

Season summer winter significance
Amino acids
Valine 5.25 ± 3.11b 8.03 ± 2.11a ✱
Therionine 66.50 ± 0.02b 89.4 ± 0.11a ✱
Isoleucine 5.39 ± 0.10b 11.12 ± 2.01a ✱
Methionine 4.99 ± 2.14a 5.19 ± 0.51a $p \geq 0.05$
Phenylalinine 13.36 ± 0.61b 33.37 ± 0.15a ✱
Leucine 10.45 ± 1.31b 36.97 ± 1.16a ✱
Histidine 15.25 ± 1.11b 34.92 ± 2.11a ✱
Tryptophan 5.09 ± 1.33a 4.99 ± 1.05a $p \geq 0.05$
Lysine 11.51 ± 1.54b 30.37 ± 1.83a ✱
Serine 59.71 ± 1.91b 92.17 ± 1.75a ✱
Aspartic acid 9.70 ± 1.71b 25.34 ± 2.12a ✱
Proline 2.80 ± 1.75b 9.77 ± 2.00a ✱
Glutamine 90.20 ± 1.25b 140.33 ± 1.90a ✱
Alanine 12.27 ± 1.12a 12.57 ± 0.99a $p \geq 0.05$
Glycine 66.80 ± 1.42a 51.25 ± 0.60b ✱
Arginine 22.45 ± 1.33a 21.99 ± 1.21a $p \geq 0.05$
Cystine 1.99 ± 2.11b 3.72 ± 1.90a ✱
Essential amino acid 173.79 ± 1.07b 254.36 ± 1.14a ✱
Non- essential amino acid 214.12 ± 2.09a 208.35 ± 1.39a $p \geq 0.05$
Total amino acid concentration 387.909 ± 3.76b 462.71 ± 2.28a ✱

✱: means significantly different ($P < 0.05$) with letter differencess.. $p \geq 0.05$: Non-significant.

In recent years, small ruminants including goats in Iraq have been subjected to a harsh, semi-tropical climate characterized by rising temperatures, reduced rainfall, and water scarcity in the *Tigris* and Euphrates rivers. This has negatively impacted rangeland conditions and animal nutrition, further exacerbated by heat stress across Iraq, particularly in the central and southern regions. Consequently, the season exerts a direct influence on the animals through high temperatures, low relative humidity, and a lack of precipitation. Furthermore, the season affects plant growth and the complex interactions between the soil, vegetation, and livestock. These effects vary depending on the animal breed, geographical location, climatic and nutritional conditions, management practices, and the availability of high-energy feed which is essential for sustaining biological processes and providing the metabolites necessary for metabolic pathways (AL-Gebouri and Eidan, 2024; Riekeberg and Powers, 2017) [52].

Consequently, these metabolites can serve as indicators of the season, nutritional status, and semen quality. Amino acids serve as fundamental precursors for protein biosynthesis in ovine and caprine species (Khalid *et al*., 2021) [53]. Amino Acid Content of the Seminal Acellular Fraction participate in vital functions, such as attenuating oxidative stress and safeguarding spermatozoal structural integrity (Mann *et al*., 1981) [54]. Indispensable amino acids, including Tryptophan, leucine, and methionine—contribute to increasing the total sperm count, improving sperm quality, and enhancing reproductive health in rams and bucks (Abbasi *et al*., 2018; Ayyat *et al*., 2019; Pascal *et al*., 2023) [55, 56, 57], Dispensable amino acids / Endogenously synthesized amino acids as proline, glycine, arginine, alanine, and serine play a pivotal role in optimizing ejaculate attributes by augmenting spermatozoal motility, viability, plasma membrane architecture, and genomic structural functionality, acrosomal integrity, and DNA preservation (Smith *et al*., 2018; Shakouri *et al*., 2021; Oladejo *et al*., 2025) [51, 58, 59]. Meanwhile, branched-chain amino acids like leucine, isoleucine, and valine sustain reproductive efficiency under adverse environmental conditions by attenuating HPA-axis hyperactivation (Kujoana *et al*., 2023) [60]. It has been demonstrated that the inclusion of amino acids in semen extenders significantly preserves spermatozoal cryotolerance and membrane stability semen extenders, or their administration via dietary supplementation to animals, mitigates ROS-induced oxidative damage in spermatozoal structures. This includes maintaining sperm viability, mitigating peroxidative membrane damage, and preventing genomic DNA degradation. Furthermore, in ruminants, such amino acid treatments have been shown to enhance the developmental potential of embryos derived from these sperm (Gualtieri *et al*., 2014; Barbato *et al*., 2017) [62, 61], The optimal amino acid equilibrium / balanced amino acid profile can optimize ejaculate characteristics, endocrine homeostasis, and reproductive stress resilience, and reproductive stress management in males. Indispensable amino acids notably leucine and tryptophan, play vital roles for instance, by upregulating protein translation and biosynthesis. Leucine also promotes protein turnover and metabolic flux, while both amino acids contribute to neuro-immunological pathways, mediated via serotonergic and melatonergic receptor signaling (Wu, 2010) [63]. Furthermore, tryptophan augments spermatozoal viability and gonadal function in ovine and caprine species (Pascal *et al*., 2023) [57].

Indogenously synthesized amino acids namely proline, glycine, arginine, alanine, and serine substantially contribute to scavenging reactive oxygen species (ROS) and preserving DNA, Addition to play vital roles in enhancing sperm quality by influencing sperm motility, the integrity of the spermatozoal plasmalemma, the stability of genetic material, mitochondrial bioenergetics and antioxidant capacity; these factors collectively contribute to increased fertility and improved reproductive performance (Smith *et al*., 2018; Shakouri *et al*., 2021; Oladejo *et al*., 2025) [51, 58, 59].

Conclusion

The present empirical evidence indicates that ambient temperature and relative humidity operate as decisive bioclimatic constraints governing the reproductive performance of indigenous bucks under the hyperthermic conditions of central Iraq. Semen characteristics and various evaluation parameters including sperm concentration, individual and mass motility, morphological abnormalities, plasma membrane and acrosomal membrane integrity, the content of essential and non-essential amino acids, malondialdehyde (MDA) levels, total antioxidant capacity, testosterone concentration, and fertilization rates were clearly influenced by seasonal variations between winter and summer.

This variation is attributed to the direct impact of climatic conditions during the process of spermatogenesis (which precedes ejaculation), as well as the indirect impact resulting from seasonal changes in forage availability, which are linked to winter rainfall and summer drought.

Consequently, this study highlights the importance of utilizing these biomarkers and amino acids as precise metrics for assessing reproductive status under environmental stress. Furthermore, it underscores the need for additional research into the influence of other climatic factors, such as photoperiod and geographical location. Such research would contribute to the development and support of natural and artificial breeding programs for local goats, integrated with assisted reproductive technologies (ART).

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