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Distinct seminal plasma protein profiles reveal molecular signatures of semen freezability in Malabari bucks

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Abstract

The cryopreservation of semen remains a cornerstone of genetic improvement in goat breeding, yet its success is highly variable. This variability is poorly understood but is thought to be influenced by the molecular composition of seminal plasma. This study aimed to characterize the seminal plasma protein profile of Malabari bucks with high and low semen freezability using SDS-PAGE. Semen was collected from six bucks and classified based on post-thaw progressive motility into High Freezability (HF, $\geq 35\%$ motility) and Low Freezability (LF, $< 30\%$ motility) groups. Electrophoretic analysis revealed stark differences between groups. The LF group exhibited a complex profile with 57 unique protein bands, prominently featuring high molecular weight proteins (> 150 kDa). In contrast, the HF group demonstrated a more refined proteome with only 33 unique bands, dominated by lower and mid-range molecular weight proteins. This clear divergence in protein profiles establishes seminal plasma composition as a key determinant of cryotolerance and provides a foundational protein signature for predicting semen freezability, offering a practical tool for improving caprine breeding programs.

Keywords: Seminal plasma, semen freezability, Malabari goat, SDS-page, protein signature, cryopreservation

Introduction

The widespread use of artificial insemination in livestock breeding relies heavily on successful semen cryopreservation (Ugur *et al.*, 2019) ^[12]. However, a major limitation in goat breeding is the high individual variability in sperm cryosurvival, a trait known as semen freezability. Seminal plasma is a complex fluid essential for sperm function (Krishnan *et al.*, 2015) ^[9], containing proteins that influence sperm resilience, including those that mitigate cold-shock. The cryopreservation process induces severe oxidative stress; goat sperm are particularly vulnerable to lipid peroxidation due to their membrane composition (Korkmaz *et al.*, 2018) ^[8]. Antioxidants within seminal plasma are crucial for neutralizing resulting reactive oxygen species (Reibeiro *et al.*, 2021) ^[11]. Electrophoretic studies reveal diverse seminal plasma protein profiles across species (Arangasamy *et al.*, 2005) ^[1], with specific bands correlating with semen traits (Deori *et al.*, 2018) ^[3]. However, a direct comparative proteomic analysis of seminal plasma from bucks with inherently different cryotolerance is lacking. This study addresses that gap. We used SDS-PAGE to characterize and compare the seminal plasma protein profiles of Malabari bucks classified as having high or low semen freezability. The goal was to identify distinct protein signatures associated with cryotolerance.

Materials and Methods

Animals and management

Six healthy, mature Malabari bucks between two and three years of age were used in this study. The animals were maintained at dedicated breeding centers under standardized conditions of nutrition, housing, and lighting to minimize environmental variation.

Semen collection and freezability assessment

Semen was collected from each buck using an artificial vagina. After assessing initial quality (motility, concentration, viability), a portion of each ejaculate was processed for cryopreservation. It was diluted in a standard tris-egg yolk-glycerol extender, loaded into straws, and frozen in liquid nitrogen vapor. After a minimum of 24 hours, the straws were thawed and the post-thaw progressive motility was evaluated. Based on this key metric, bucks were categorized into two distinct groups: High Freezability (HF), with post-thaw motility of 35% or greater, and Low Freezability (LF), with post-thaw motility below 30%.

Seminal plasma and protein preparation

To separate spermatozoa and seminal plasma, an aliquot of 1 mL of semen sample from each buck was centrifuged at 850 x g for 10 min at 4 °C. The resulting supernatant containing seminal plasma was stored at - 80 °C for protein extraction. The total protein concentration of this fluid was measured using a standard biochemical assay to ensure consistent loading for subsequent analysis.

SDS-PAGE electrophoresis

The protein composition of the seminal plasma was analysed using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). This technique separates proteins based on their molecular weight. Equal amounts of protein from each sample were denatured, loaded onto polyacrylamide gels, and subjected to an electric field. Following separation, the gels were stained with Coomassie Blue dye, which binds to proteins, making them visible as distinct bands. Each band corresponds to a protein or group of proteins of a specific size. A standard protein ladder was run alongside the samples to estimate the molecular weight of each observed band.

Data analysis

The gel images were analysed to catalogue every visible protein band for each freezability group using Gelanalyzer (Version 23.1.1). Duplicate molecular weight entries from repeated runs were removed to create a clean list of unique bands. These bands were then grouped by their origin (HF or LF) and sorted from the smallest to the largest molecular weight for clear comparison.

Results

Semen freezability groups

The bucks were clearly separable based on post-thaw sperm survival. The three bucks in the HF group consistently showed progressive motility after thawing (averaging >40%), while the three in the LF group showed poor recovery (averaging <35%). This confirmed the presence of two biologically distinct freezability phenotypes within the studied population.

Distinct electrophoretic protein profiles

SDS-PAGE analysis revealed strikingly different protein banding patterns between the HF and LF groups, visualized in the representative gel image. This was not a subtle quantitative difference but a qualitative divergence in the proteomic landscape. A comprehensive cataloguing of all unique protein bands identified 68 distinct molecular weights across the entire study, ranging from 17 to 234 kilodaltons (kDa). The distribution of these bands, however, was highly asymmetrical between the groups (Table 1).

Table 1: Unique seminal plasma protein bands (kDa) identified by SDS-PAGE, categorized by freezability phenotype

Low Freezability (LF) Group	High Freezability (HF) Group
23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 39, 40, 41, 42, 43, 45, 47, 48, 49, 50, 51, 52, 54, 56, 57, 58, 62, 66, 69, 70, 71, 75, 77, 79, 81, 83, 94, 100, 104, 109, 115, 121, 124, 125, 129, 131, 150, 178, 180, 184, 187, 188, 190, 214, 234	17, 18, 20, 23, 24, 25, 26, 27, 28, 29, 31, 33, 34, 35, 36, 37, 41, 42, 46, 48, 50, 52, 55, 62, 63, 68, 69, 75, 77, 78, 81, 83, 90, 95, 98, 100, 106, 109, 120, 124, 129, 136, 138, 146, 150, 155, 166, 177, 211, 217, 221, 232

The most striking finding was the sheer complexity of the LF profile. This group presented 57 unique protein bands, painting a picture of a dense and varied proteome. Notably, this profile was dominated by the presence of multiple high molecular weight proteins, with prominent bands observed at 178, 184, 187, 188, 190, 214, and 234 kDa. Many of these heavy proteins were either very faint or completely absent in the HF gels.

In contrast, the HF group showed a much more refined and selective protein profile, comprising only 33 unique bands. Its signature was characterized by a consistent presence of lower molecular weight proteins (e.g., 17, 18, 20, 23 kDa) and a specific set of mid-weight proteins (e.g., 106, 120, 124, 129, 136-138, 146, 150, 155, 166 kDa) that formed a consistent pattern absent in the LF group.

Discussion

The results of this study establish a direct biochemical correlate for semen freezability in Malabari bucks, with clear proteomic signatures distinguishing high (HF) from low (LF) freezability phenotypes. The LF phenotype is characterized by a complex seminal plasma profile comprising 57 unique protein bands, heavily dominated by multiple High Molecular Weight (HMW) proteins (>150 kDa). This dense HMW proteome, featuring prominent bands at 178, 188, 214, and 234 kDa, likely creates a suboptimal cryopreservation environment. As suggested in ruminant studies (Jobim *et al.*, 2004) [5], such complexity may increase viscosity and impede uniform cryoprotectant penetration and osmotic dynamics, contributing to the poor post-thaw motility (<35%) and aligning with the broader understanding that seminal plasma composition critically influences sperm cryoresilience (Krishnan *et al.*, 2015) [9]. Furthermore, specific lower molecular weight proteins have been identified as common components of caprine seminal fluid; seminal plasma proteins of 75, 62-49, 20, 17 kDa have been documented in other goat breeds (Gajare *et al.*, 2019) [4]. In contrast, the HF phenotype presents a refined profile of 33 unique bands, defined by low molecular weight proteins (e.g., 17, 18, 20 kDa) and a diagnostic mid-weight cluster (e.g., 106, 120, 136-138, 146, 150, 155, 166 kDa). This signature suggests an optimized seminal milieu. The enrichment of proteins within the 120-166 kDa range is significant, as similar molecular weight regions in goats and sheep contain beneficial proteins. This refined composition likely includes a higher concentration of functional proteins such as antioxidant enzymes (e.g., glutathione peroxidases) crucial for neutralizing oxidative stress during freezing (Wen *et al.*, 2019) [13] and may contain analogs of membrane-stabilizing proteins like the BSP family (Manjunath and Therien, 2002) [10]. The correlation between specific molecular weight regions and freezability status reinforces the potential of seminal plasma proteins as

biomarkers for semen quality, as suggested in prior caprine research (Karunakaran *et al.*, 2012; Deori *et al.*, 2018) ^[7, 3]. Notably, the absence of numerous bands in the HF profile is as functionally indicative as the presence of its characteristic bands, supporting a paradigm where superior freezability is linked to a precise proteomic signature rather than greater complexity, consistent with findings on specific protein abundances (Kaimal *et al.*, 2020) ^[6]. Seminal plasma protein fractions in buffalo bulls, as identified by Asadpour *et al.* (2007) ^[2], exhibit a composition analogous to that documented in other species and demonstrate correlative relationships with semen quality parameters in both pre- and post-cryopreservation states.

Consequently, the stark visual distinction in SDS-PAGE profiles provides a validated biomarker fingerprint for inherent cryotolerance. This electrophoretic screening method offers a practical, cost-effective tool for identifying bucks with superior freezability, enabling more efficient resource allocation and enhancing the success of artificial insemination programs in Malabari goats.

Conclusion

Seminal plasma protein profiles, assessed via SDS-PAGE, correlate with cryotolerance in Malabari bucks. Poor freezers exhibit complex, high-molecular-weight-dominant profiles, whereas good freezers display refined signatures with specific mid- and lower-weight proteins. This provides biochemical evidence that seminal plasma composition underpins cryotolerance variance, establishing proteomic screening as a practical tool for identifying bucks with superior inherent freezability.

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