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Decoding Bull Fertility Under Tropical Conditions: Multivariate Predictive Modelling From 25,512 Field Spermiograms

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ABSTRACT

This large-scale study evaluated 25,512 Nellore bulls raised under tropical field conditions to elucidate multivariate determinants of breeding soundness and to derive a data-driven risk stratification framework based on BSE outcomes. Macro- and microscopic semen traits (e.g., progressive motility, sperm morphology including major defects, scrotal circumference, vigour and mass activity), recorded clinical alterations and epidemiological factors (age and seasonality) were integrated using multivariable modelling and internal validation. Logistic regression identified progressive motility, proportion of major defects and scrotal circumference as independent predictors of BSE-based unfitness, with a cross-validated ROC AUC of 0.84 (95% CI 0.83–0.85), indicating high discriminative performance. Principal component and clustering analyses revealed three reproducible phenotypic profiles (optimal, intermediate and critical) combining functional, morphological and anatomical attributes. These components were synthesised into a Breeding Soundness Risk Index (BSRI) showing consistent calibration and stability under five-fold cross-validation, enabling objective stratification of bulls by probability of BSE-based unfitness. Advancing age and seasonal heat load were associated with increased odds of BSE-based unfitness, consistent with cumulative thermal and physiological stress under tropical management. Collectively, these results support progressive motility, major sperm defects and scrotal circumference as cornerstone indicators of breeding soundness in large-scale field settings, and the BSRI provides an objective, field-applicable score to support sire selection and management decisions under tropical conditions.

1 | Introduction

Reproductive efficiency is a key determinant of sustainability and profitability in beef cattle production, particularly in tropical systems based on natural mating, where a small number of bulls exert a direct impact on herd fertility (Bettencourt and Romão 2009; Wettemann et al. 2020). It is estimated that up to 90% of beef cows in Brazil are bred through natural mating following fixed-time artificial insemination (FTAI) protocols, making the reproductive soundness of bulls one of the main bottlenecks to productivity. Consequently, individual failures in bulls result in broad economic repercussions, including prolonged calving intervals, reduced pregnancy rates and substantial financial losses in tropical beef production systems (Barth 2013; Cooke et al. 2017).

The andrological examination is the reference tool for estimating the reproductive potential of bulls, encompassing clinical assessment, scrotal circumference measurement and both macroscopic and microscopic semen evaluation

(Chenoweth 2002a, 2002b; Vale Filho et al. 2013). In Nellore bull populations, andrological failure rates may reach 50%–53%, primarily due to seminal defects, testicular hypoplasia, degeneration and clinical lesions of the prepuce and penis (Vale Filho et al. 2013).

Scrotal circumference, traditionally included in breeding evaluations, is recognised as a robust marker of testicular function and spermatogenic capacity, showing correlations with motility, concentration and semen volume, as well as moderate to high heritability (Fonseca et al. 1992; Wahyudi et al. 2022; Silva et al. 2018). In Zebu cattle, reduced scrotal circumference is associated with an increased risk of subfertility, whereas higher measurements reflect greater testicular biomass and sperm production potential (Wahyudi et al. 2022; Silva et al. 2018).

Sperm motility is a key component of bull breeding soundness evaluation and has been associated with reproductive performance in field studies; therefore, minimum thresholds are typically used within BSE frameworks., both in conventional assessments and in computer-assisted approaches (CASA, flow cytometry), which enhance the accuracy and reproducibility of measurements (Verstegen et al. 2002). Likewise, sperm morphology—particularly the frequency of major defects—shows a direct relationship with fertility and early embryonic survival and is widely used as a clinical marker in breeding soundness evaluation and culling programs (CBRA—Colégio Brasileiro de Reprodução Animal 2013).

Beyond these classical parameters, epidemiological and environmental factors exert a strong influence on semen quality. Older bulls exhibit a higher prevalence of testicular degeneration, partial atrophy and reduced sperm vigour, compromising their breeding soundness (Barth 2013). Similarly, exposure to elevated ambient temperatures promotes testicular heat stress, increasing the incidence of morphological defects and reducing motility, with effects observed 4 to 8 weeks after the heat event (Wettemann et al. 1988). These factors indicate that fertility assessment should not rely on isolated variables but rather integrate age, seasonality and the interaction among multiple markers.

Despite the importance of these determinants, most published studies have been based on relatively small sample sizes (< 1000 bulls), univariate analyses or evaluations restricted to a subset of seminal variables. Large-scale population studies simultaneously integrating multiple seminal indicators, scrotal metrics and epidemiological factors under tropical conditions remain scarce—particularly those applying multivariate modelling and modern risk stratification techniques (Barth 2013; Weller et al. 2023).

To address this gap, the present study analysed a very large dataset comprising 25,512 andrological examinations of Nellore bulls raised under tropical Brazilian production systems. To our knowledge, this is among the largest BSE-derived datasets reported for *Bos indicus* cattle, integrating multiple herds, years and field conditions to describe population-level patterns of breeding soundness traits under tropical management. The objectives were to characterise the frequency of seminal and clinical conditions recorded in routine examinations, evaluate epidemiological associations (age and seasonality), identify multivariate phenotypic profiles through clustering and derive an internally validated, BSE-based risk stratification index from key andrological variables. This approach aims to strengthen conventional breeding soundness evaluation by providing objective, field-oriented tools for large-scale sire selection and management.

Accordingly, this study aimed to analyse, in a large population of Nellore bulls ($n = 25{,}512$) managed under tropical conditions, the prevalence and distribution of major sperm defects and recorded clinical andrological abnormalities; evaluate biological factors associated with BSE-based unfitness, including age, scrotal circumference, progressive motility and the proportion of major defects; identify multivariate patterns of ejaculate composition and clinically meaningful andrological profiles; and develop and internally validate an objective Breeding Soundness Risk Index (BSRI) based on seminal and morphofunctional markers to support decision-making in tropical beef cattle systems.

2 | Materials and Methods

The study was conducted in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and Council 2010) and the ethical guidelines of the Brazilian Federal Council of Veterinary Medicine for the humane management of beef cattle (CFMV 2013). All evaluations were performed by licensed veterinarians using standard andrological examination techniques (Chenoweth 2002a, 2002b; CBRA 2013), with procedural measures intended to minimise stress and transient discomfort.

The assessments were carried out in commercial herds, at the request of producers, to evaluate bull breeding soundness prior to the breeding season, since semen from these bulls would be used for natural mating or artificial insemination of cows during the upcoming reproductive period.

The andrological examination comprised two main stages: (1) clinical evaluation of the genitalia and (2) semen analysis. During the first stage, the scrotum and testes were inspected and palpated to record scrotal circumference (cm), testicular symmetry and position, parenchymal consistency and tone, and the presence of lesions, scars or adhesions. The epididymides, spermatic cords and accessory glands were examined for enlargement, asymmetry, thickening or abnormal sensitivity. The penis and prepuce were evaluated by exteriorization to identify adhesions, phimosis, paraphimosis, penile deviation, hematomas, fibrosis or other anatomical abnormalities potentially affecting copulation (Chenoweth 2002a, 2002b). All findings were systematically recorded as part of the clinical andrological dataset used in subsequent multivariate analyses.

The second stage involved semen collection and evaluation, performed initially on-farm to assess macroscopic appearance, colour, volume, whirl formation, mass and individual motility, sperm vigour and odour. Aliquots were then transported to the andrology laboratory for detailed examination of sperm morphology (major and minor defects), concentration, viability and specific morphological abnormalities, following the standards of the *Colégio Brasileiro de Reprodução Animal* (CBRA 2013) and the Society for Theriogenology (SFT) (2018). This methodological approach ensured diagnostic standardisation, data reliability and full compliance with animal welfare and ethical standards, without any experimental intervention.

2.1 | Study Population and Geographical Coverage

A total of 25,512 andrological examinations (spermiograms) from Nellore bulls (*Bos indicus*) were analysed. The animals originated from approximately 180 farms owned by nearly 100 producers located in the Araçatuba region, São Paulo State, Brazil (central coordinates: 21°12′ S, 50°25′ W). This region is recognised as one of the main beef cattle production hubs in Brazil, characterised by predominantly *Bos indicus* herds raised under extensive tropical grazing systems, thus conferring high epidemiological and commercial representativeness to the data collected.

The dataset spans a 20-year period (2004–2024) and includes evaluations of sexually active breeding bulls as well as young males selected for natural service following fixed-time artificial insemination (FTAI) programs (Baruselli et al. 2017). This long-term integration allows the identification of robust population-level trends, minimises short-term or seasonal variation and strengthens the applicability of the results to large-scale tropical production systems.

2.2 | Inclusion and Exclusion Criteria

Andrological records were included when they provided complete and consistent information on macroscopic and microscopic semen parameters, along with clinical findings from the external and accessory genitalia (scrotum, testes, epididymides, penis, prepuce and accessory glands). All records were manually reviewed to ensure diagnostic consistency and data quality.

Exclusion criteria comprised: (i) missing or inconsistent data (e.g., absence of volume, motility or morphology records); (ii) systemic non-reproductive illnesses (e.g., fever, weakness, infectious or metabolic conditions) at the time of sampling that could bias semen quality interpretation; and (iii) duplicate entries referring to the same individual within a short interval.

After quality control, 864 records were excluded (3.3% of the total), resulting in 25,512 validated andrological examinations included in the statistical analyses. This curated dataset provided a robust basis for multivariate analyses of semen quality, clinical andrological traits and the fertility index (IPF) described below.

2.3 | Animal Management and Housing Conditions

All bulls were maintained under extensive grazing systems on tropical forage pastures (mainly *Brachiaria* and *Panicum* species), with free access to clean water and mineral supplementation. Participating farms followed typical reproductive management practices for the Araçatuba region (São Paulo State), using bulls as the main agents of natural mating after fixed-time artificial insemination programs (Baruselli et al. 2017) in the cow herds. The evaluated categories included sexually active breeding bulls and young candidates undergoing pre-selection for natural service lots.

2.4 | Semen Collection and Evaluation

Semen was collected on-farm using portable electroejaculators (Valinhos—São Paulo—Brazil) under strict hygienic conditions to prevent contamination, following standard procedures described by Chenoweth (2002a, 2002b). Samples were collected in graduated tubes pre-warmed to 37°C and immediately evaluated for macroscopic and microscopic parameters. Macroscopic assessment included ejaculate volume, appearance and colour, odour and the presence of contaminants (urine, blood, pus), as well as whirl formation. Microscopic evaluation was performed using bright-field optical microscopy with pre-warmed slides (37°C) to preserve sperm viability, assessing mass and progressive motility, sperm vigour, concentration ($\times 10^6$/mL) and individual morphology, according to CBRA (2013) and SFT (2018) guidelines.

2.5 | Morpho-Anatomical Measurements

Preputial length (cm) was measured with the bull standing still, using a flexible tape from the ventral border of the umbilicus to the preputial tip in a relaxed position, without traction, as described by Lopes et al. (2015). Preputial length was classified into three categories (Table 1).

TABLE 1 | Classification of preputial length categories used in the study.

Preputial category	Typical length (cm)	Notes
Short (C)	≤30	Associated with lower risk of trauma or infection, but may reduce mating efficiency with taller females.
Medium (M)	31–39	Functionally ideal range; balance between protection and penile extension.
Long (L)	≥40	Characteristic of *Bos indicus*; higher risk of injuries, phimosis and prolapse, but facilitates mating in taller females.

Note: CBRA (2013), Lopes et al. (2015), and Baruselli et al. (2017).

2.6 | Statistical Analysis

The study was designed to explore, on a very large scale, a large dataset of 25,512 andrological examinations from Nellore (*Bos indicus*) bulls evaluated under tropical commercial conditions, integrating epidemiological, multivariate and predictive modelling approaches. The analytical framework aimed to characterise population patterns of seminal defects, identify key predictors of BSE-based unfitness, assess complex biological interactions and develop high-accuracy predictive models for application in precision reproductive selection programs. Descriptive and exploratory analyses were initially performed for all andrological variables, including means, standard deviations, percentiles and frequency distributions. The Shapiro–Wilk test and graphical methods (histograms and Q–Q plots) were used to assess the normality of continuous variables (motility, vigour, scrotal circumference and proportion of major defects). Categorical variables were compared using Pearson's chi-square test or Fisher's exact test (when expected frequencies were <5), with Bonferroni correction for multiple comparisons.

Associations between clinical and seminal traits were explored using bivariate and multivariate logistic regression models, estimating adjusted odds ratios (OR) and 95% confidence intervals (CI), while controlling for potential confounders such as age (>5 years), season (summer vs. winter) and bull category (active vs. pre-selection). To account for management differences among herds, generalised linear mixed models (GLMMs) were fitted (Bolker et al. 2009), including farm as a random effect to ensure population-level robustness. Second-order interactions (e.g., age × major defects, age × motility) were tested to detect physiological synergisms related to BSE-based unfitness risk. Given the 20-year longitudinal structure (2004–2024) of the dataset, generalised linear models (GLMs) were used to evaluate temporal trends in the frequency of seminal defects and to estimate the impact of seasonality and potential genetic or management improvements over time. These models assumed a binomial distribution with a logit link, including year and season as fixed effects, allowing inference on the temporal evolution of BSE-based unfitness risk.

For predictive modelling, Principal Component Analysis (PCA) was applied to continuous variables to reduce dimensionality and identify latent axes of seminal quality (motility, vigour, major defects, scrotal circumference), following Jolliffe and Cadima (2016). The extracted components were used as inputs for unsupervised clustering (k-means algorithm), which identified three homogeneous ejaculate profiles: optimal, intermediate and critical. Cluster stability was evaluated by the average silhouette coefficient and bootstrap resampling (1000 iterations). Subsequently, multivariate predictive models for BSE-based unfitness were fitted using classical logistic regression and penalised models (LASSO and ridge regression) according to Friedman et al. (2010). Discriminative performance was assessed by the area under the receiver operating characteristic curve (AUC), interpreted according to Fawcett (2006), with AUC ≥ 0.90 considered excellent predictive ability. Classification thresholds (fit vs. unfit) were defined by the Youden index, maximising both sensitivity and specificity.

From the final models, a Breeding Soundness Risk Index (BSRI) was constructed by weighting variables with the highest discriminative loadings derived from the multivariate and principal component analyses. The BSRI was internally calibrated and validated, enabling objective stratification of bulls into risk strata for practical application in genetic selection programs. Rare outcomes ($<0.1\%$), such as isolated testicular atrophy or severe penile lesions, were analysed using Firth's penalised logistic regression to avoid bias from small-sample effects and to ensure stable risk estimation. Subsequently, multivariate predictive models for BSE-based unfitness were fitted using classical logistic regression and penalised models (LASSO and ridge regression) according to Friedman et al. (2010). The final BSRI was computed as a weighted linear combination of normalised predictors: $\mathrm{BSRI} = w_1 \cdot \mathrm{Motility}_n + w_2 \cdot (100\text{—}\mathrm{MajorDefects})_n + w_3 \cdot \mathrm{SC}_n + w_4 \cdot \mathrm{MassMotility}_n$, where variables were scaled to [0–1], and weights (w_i) were derived from the principal component loadings and penalised model coefficients. Internal calibration was evaluated through 1000 bootstrap resamplings, and model performance was assessed by the area under the ROC curve (AUC), Brier score and calibration curve. An AUC ≥ 0.90 was interpreted as excellent discriminative ability (Fawcett 2006). All analyses were performed in R software (R Core Team 2025) using the packages tidyverse, lme4, epiR, FactoMineR, caret, glmnet and pROC. A 5% significance level ($p<0.05$) was adopted for all inferential tests.

3 | Results

The main clinical and andrological categories recorded in 25,512 Nellore bull examinations are summarised as absolute counts (n) and relative proportions (%). The category previously described as 'no abnormality' indicates that no major clinical/andrological diagnosis among the predefined categories was recorded at the time of examination; importantly, this does not imply the complete absence of sperm defects, minor abnormalities or subclinical findings. Therefore, these frequencies should be interpreted as distributions of the principal recorded diagnostic labels, whereas continuous semen quality traits (e.g., progressive motility) are summarised separately in the descriptive analyses and Figure 1. Terms such as aspermia/azoospermia and testicular hypoplasia are reported as field-recorded categories and are operationally defined in the Methods to avoid implying definitive diagnoses under electroejaculation-based collection (Table 2).

Table 3 presents the association analysis between macroscopic ejaculate variables and the final classification of breeding soundness. The evaluated parameters included mass motility score, semen appearance, preputial type (short, medium or long), penile alterations and testicular symmetry. For each variable, the chi-square test indicated a p-value <0.001, demonstrating a significant association with reproductive classification. The strength of association was expressed by Cramer's V coefficient, which ranges from 0 (no association) to 1 (perfect association). The mass motility score showed the highest value (0.42), indicating a moderate-to-strong association with breeding soundness. Semen appearance presented an intermediate value (0.18), representing a weak-to-moderate association. The remaining variables—prepuce (0.06), penile alterations (0.04)

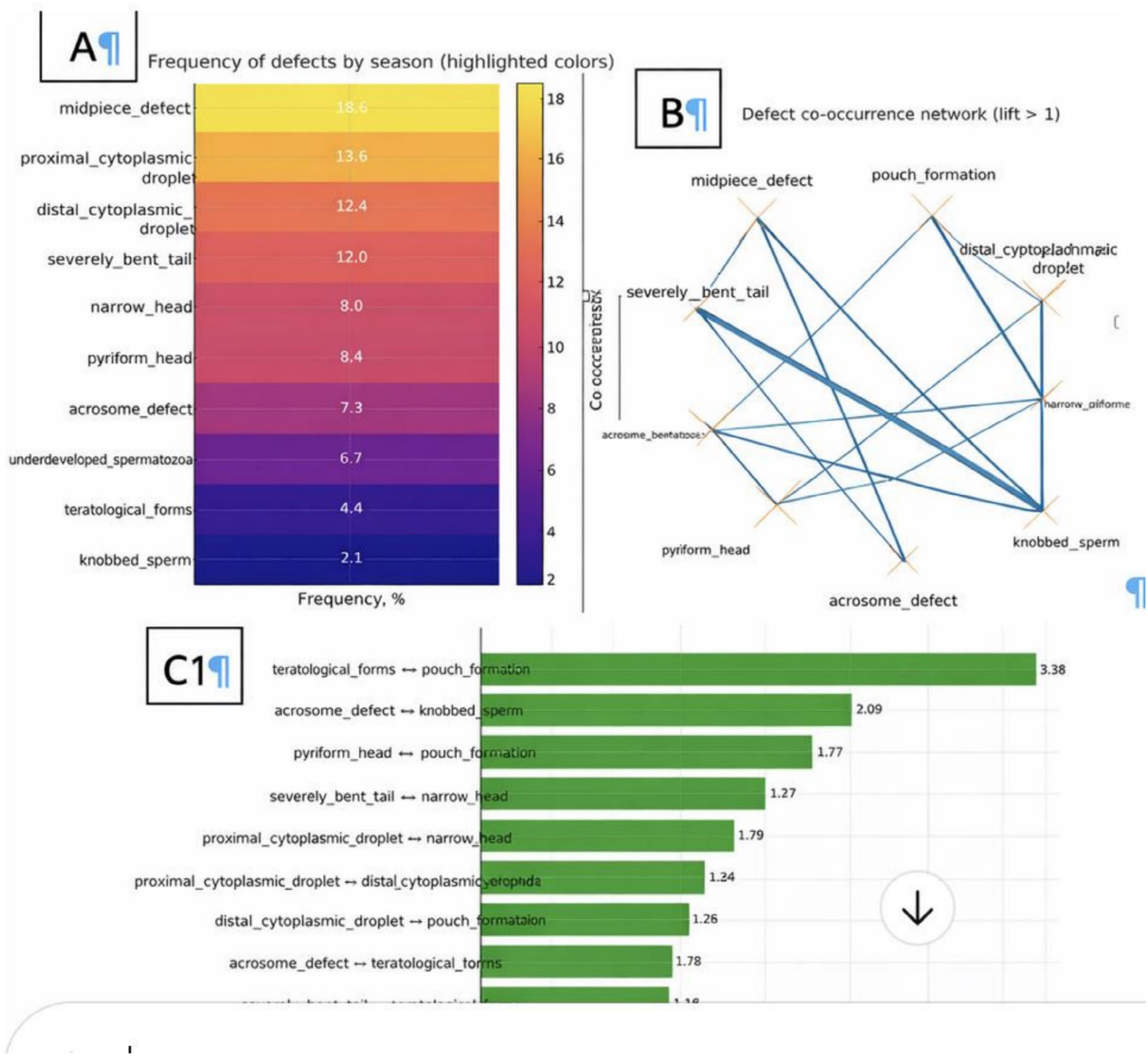

FIGURE 1 | Multilevel characterisation of sperm defect patterns in Nellore bulls under tropical conditions. (A) Seasonal frequency distribution (%) of individual sperm defects identified in 25,512 ejaculates. Colours highlight relative prevalence, with warmer tones indicating higher occurrence. (B) Co-occurrence network among morphological defects considering associations with lift > 1. Nodes represent individual defects and edges indicate positive associations; edge thickness is proportional to the magnitude of lift, reflecting the strength of non-random co-occurrence. (C) Ranking of the most relevant defect pairs according to lift values, illustrating combinations that occur more frequently than expected by chance. Higher lift values denote stronger epidemiological interdependence between abnormalities.

and testicular symmetry (0.05) showed weak, although statistically significant, associations.

These results demonstrate that macroscopic variables can serve as useful screening indicators of reproductive potential, with the mass motility score emerging as the most relevant macroscopic parameter, whereas the other factors exert minor but statistically detectable effects.

The prevalence and interdependence of sperm abnormalities were further explored through multivariate visualisation (Figure 1). The seasonal frequency distribution (Figure 1A) highlighted intermediate-piece defects (18.5%), proximal cytoplasmic droplets (15.6%), and distal cytoplasmic droplets (12.6%) as the most prevalent categories under tropical conditions. The defect co-occurrence network (Figure 1B) revealed strong connections among acrosomal, teratoid and cytoplasmic-droplet abnormalities, indicating shared etiological mechanisms likely associated with thermal or spermatogenic stress. The ranking of the top paired defects (Figure 1C) identified *teratoid forms* × *pouch formation* (lift = 3.38) and *acrosomal defect* × *knobbed sperm* (lift = 2.09) as the most frequent associations. Collectively, these findings demonstrate that sperm defects tend to cluster in non-random patterns, reflecting integrated biological processes that underlie semen quality in *Nellore* bulls.

TABLE 2 | Frequency of the main defects observed in 25,512 spermiograms of Nellore bulls.

Defect	Frequency (*n*/%)
No abnormality	22,077/25,512 (86.54)[a]
Major sperm defects ≥ 30%	2470/25,512 (9.68)[b]
Azoospermia	525/25,512 (2.06)[c]
Necrospermia	141/25,512 (0.55)[d]
Testicular hypoplasia	42/25,512 (0.16)[d]
Acrobustitis/balanoposthitis	36/25,512 (0.14)[d]
Aspermia	34/25,512 (0.13)[d]

TABLE 3 | Association between macroscopic ejaculate variables and final breeding soundness classification in Nellore (*Bos indicus*) bulls.

Variable	*p*-value (Chi^2)	Cramer's V	Interpretation
Mass motility score	< 0.001	0.42	Moderate to strong
Semen appearance	< 0.001	0.18	Weak to moderate
Prepuce type (short, medium, long)	< 0.001	0.06	Weak
Penile alterations	< 0.001	0.04	Weak
Testicular symmetry	< 0.001	0.05	Weak

TABLE 4 | Multivariate logistic regression model identifying independent predictors of BSE-based unfitness in Nellore (*Bos indicus*) bulls.

Variable	OR	95% CI (Lower)	95% CI (Upper)	*p*-value
Motility	0.89	0.82	0.97	0.0085
Major defects (%)	1.32	1.28	1.37	< 0.001
Scrotal circumference (cm)	0.53	0.50	0.55	< 0.001
Mass motility 1.0	0.07	0.02	0.28	0.0001
Mass motility 2.0	0.005	0.001	0.017	< 0.001

The multivariate logistic regression model summarised (Table 4) identified the main independent predictors associated with BSE-based unfitness in Nellore bulls. The analysis incorporated key functional and morphological parameters, sperm motility, proportion of major defects, scrotal circumference and mass motility score (1.0 and 2.0), with the final breeding soundness classification (fit vs. unfit) defined as the outcome variable. All estimates are presented as odds ratios (OR) with corresponding 95% confidence intervals (CI) and *p*-values for statistical significance. Values of OR > 1 indicate an increased likelihood of unfitness, whereas OR < 1 denote a protective or inverse association.

Sperm motility exhibited a protective effect (OR = 0.89; 95% CI: 0.82–0.97; $p = 0.0085$), indicating that higher motility substantially reduced the probability of unfitness. The percentage of major sperm defects emerged as a strong risk factor (OR = 1.32; 95% CI: 1.28–1.37; $p < 0.001$), significantly increasing the likelihood of BSE-based unfitness with each additional percentage point. Scrotal circumference showed a marked protective effect (OR = 0.53; 95% CI: 0.50–0.55; $p < 0.001$), confirming that larger testicular dimensions are closely linked to superior breeding soundness.

The mass motility scores of 1.0 (OR = 0.07; 95% CI: 0.02–0.28; $p = 0.0001$) and 2.0 (OR = 0.005; 95% CI: 0.001–0.017; $p < 0.001$) were strongly and inversely associated with BSE-based unfitness, underscoring that ejaculates with more intense mass movement are far less likely to be classified as inadequate. For interpretative clarity, each 10-percentage-point increase in major sperm defects raised the probability of BSE-based unfitness approximately 13-fold ($OR_{10}\% \approx 1.32^{10}$), whereas every additional 5 cm in scrotal circumference markedly reduced the risk ($OR_5 cm \approx 0.53^5$). These adjusted estimates emphasise the strong and opposing gradients of morphological and anatomical predictors on overall breeding soundness.

Overall, the model demonstrates that motility, sperm morphology, scrotal circumference and macroscopic ejaculate quality (as reflected by mass motility) constitute the principal determinants of breeding soundness in tropical beef bulls. Together, these parameters provide a quantitative, adjusted estimate of the risk of BSE-based unfitness, integrating both anatomical and functional dimensions of andrological evaluation.

Table 5 summarises the relationship between specific clinical abnormalities identified during the andrological evaluation and the final classification of BSE-based unfitness. The table presents the absolute occurrences (*n*), chi-square test *p*-values and risk estimates expressed as odds ratios (OR) for each clinical condition.

All abnormalities listed showed a significant association with BSE-based unfitness ($p < 0.001$), indicating a strong relationship between these defects and failure in the breeding soundness evaluation. Among the conditions with the highest impact, azoospermia exhibited an extremely high odds ratio (1.16×10^{13}), followed by aspermia (7.54×10^{11}) and major sperm defects ≥ 30% (1.26×10^4), representing an exponentially increased risk of being classified as unfit. Other abnormalities such as necrospermia, podal lesions, and acrobustitis/balanoposthitis, though less frequent, also showed elevated OR values (ranging from 10^2 to 10^3), confirming a strong association with BSE-based unfitness. These results demonstrate that the presence of any of these clinical abnormalities dramatically increases the likelihood of BSE-based unfitness, emphasising the importance of systematic detection of such conditions during the andrological examination to ensure accurate selection of breeding bulls.

Table 6 describes three distinct seminal quality profiles in Nellore (*Bos indicus*) bulls, identified through multivariate analysis using Principal Component Analysis (PCA) followed by unsupervised k-means clustering. PCA was applied to reduce the dimensionality of the andrological dataset, extracting the components that explained the greatest variability among individuals. Subsequently, the clustering algorithm grouped bulls into homogeneous profiles according to similarities in semen quality parameters.

Each cluster represents a group of bulls with comparable reproductive characteristics. The Cluster 0 (Optimal) profile exhibited high mean sperm motility (85.4% ± 5.2%), a low proportion of major defects (5.6% ± 2.1%), adequate scrotal circumference (38.2 ± 1.9 cm) and high sperm vigour (4.3 ± 0.5), characterising animals with excellent seminal quality and elevated reproductive potential. The Cluster 1 (Intermediate) profile showed moderate sperm motility (68.9% ± 6.8%), an intermediate proportion of major defects (15.2% ± 3.5%), moderate scrotal circumference (35.7 ± 2.0 cm) and vigour of (3.2 ± 0.6). This group represents bulls with intermediate seminal quality, in which the impact on fertility may range from mild to moderate depending on management and mating conditions. The Cluster 2 (Critical) profile was characterised by low motility (45.7% ± 7.4%), a high proportion of major sperm defects (32.5% ± 5.8%), reduced scrotal circumference (33.1 ± 2.4 cm) and low vigour (2.1 ± 0.7), indicating bulls with markedly compromised seminal quality and a high probability of BSE-based unfitness if used in natural mating or artificial insemination programs.

All values are presented as mean ± standard deviation for each variable. The combined application of PCA and clustering enabled the identification of hidden patterns of seminal variation within the evaluated population, allowing objective classification of bulls into andrological performance groups with potential applicability in reproductive management and genetic selection programs.

Table 7 presents the stratification of Nellore bulls into risk strata based on a breeding soundness risk index (BSRI) developed through the integration of four key andrological variables: sperm motility, percentage of major defects, scrotal circumference and mass motility score of the ejaculate. The index was derived from a weighted multivariate model, assigning greater weight to the variables with the highest discriminative power for breeding soundness. Two main risk strata were defined according to the predictive index. The Low risk stratum exhibited a mean index value of 0.32 ± 0.09, characterised by reduced motility (57.8%), a higher proportion of major sperm defects (8.8%), smaller scrotal circumference (32.5 cm) and a moderate mass motility score (3.0). These parameters indicate limited reproductive potential and an increased risk of suboptimal outcomes in natural mating or artificial insemination programs.

The Intermediate risk stratum showed a mean index value of 0.46 ± 0.05, with higher sperm motility (78.1%), fewer major defects (5.5%), moderate scrotal circumference (35.4 cm) and a mass motility score of (3.3). This group represents bulls that, on average, meet minimum BSE standards and present an intermediate estimated probability of BSE-based unfitness. Values are reported as mean ± standard deviation for the index and as percentages or absolute values for component parameters. The Breeding Soundness Risk Index (BSRI) provides an objective, quantitative estimate of BSE-based unfitness probability and supports standardised stratification for decision-making in large-scale sire selection and management. To ensure consistency with the three seminal quality profiles identified in

TABLE 5 | Statistical association between specific clinical abnormalities and final BSE-based unfitness classification in Nellore (*Bos indicus*) bulls.

Clinical abnormality	Occurrences (*n*)	*p*	Odds ratio (OR)
Azoospermia	525	< 0.001	1.16×10^{13}
Aspermia	34	< 0.001	7.54×10^{11}
Major sperm defects ≥ 30%	2465	< 0.001	1.26×10^{4}
Necrospermia	135	< 0.001	4.69×10^{2}
Podal lesions	19	< 0.001	1.27×10^{2}
Acrobustitis/Balanoposthitis	34	< 0.001	1.14×10^{2}

TABLE 6 | Three distinct seminal quality profiles identified by multivariate analysis (PCA) and unsupervised clustering (*k*-means). Values are expressed as mean ± standard deviation (M ± SD).

Cluster	Motility (%)	Major defects (%)	Scrotal circumference (cm)	Vigour (1–5)
Cluster 0 (Optimal)	85.4 ± 5.2	5.6 ± 2.1	38.2 ± 1.9	4.3 ± 0.5
Cluster 1 (Intermediate)	68.9 ± 6.8	15.2 ± 3.5	35.7 ± 2.0	3.2 ± 0.6
Cluster 2 (Critical)	45.7 ± 7.4	32.5 ± 5.8	33.1 ± 2.4	2.1 ± 0.7

TABLE 7 | Breeding Soundness Risk Index (BSRI) calculated from sperm motility, major sperm defects, scrotal circumference and mass motility score in Nellore (*Bos indicus*) bulls.

Fertility class	Index (M ± SD)	Motility (%)	Major defects (%)	Scrotal circumference (cm)	Mass motility (1–5)
Low	0.32 ± 0.09	57.8	8.8	32.5	3.0
Intermediate	0.46 ± 0.05	78.1	5.5	35.4	3.3

Table 6, the BSRI strata were aligned with the cluster-based profiles and expressed as Low, Intermediate and High risk strata, providing an intuitive and comparable interpretation across analytical levels.

This composite index represents an innovative quantitative framework for estimating BSE-based unfitness risk, integrating motility, morphological integrity and scrotal metrics into a unified predictive score. Its calibration and internal validation highlight its potential as an objective and scalable tool for breeding soundness classification in beef bulls.

Table 8 provides an epidemiological overview of the main seminal defects observed in Nellore (*Bos indicus*) bulls, highlighting their frequency, age-adjusted odds ratios (OR) for bulls older than five years, statistical significance, mean semen quality index (SQ) and seasonal distribution (proportion of cases in summer and winter). A population-level analysis revealed that advanced age, reduced SQ and exposure to high ambient temperatures were major contributors to the occurrence of severe seminal anomalies, particularly major sperm defects and azoospermia. Bulls over five years old showed elevated odds ratios for major defects (2.8) and azoospermia (3.2), confirming the progressive decline in sperm quality associated with aging. The synergistic interaction between age-related testicular degeneration and seasonal heat stress constitutes a newly characterised epidemiological signature of fertility decline in *Bos indicus* bulls, emphasising the compounded vulnerability of aging animals exposed to tropical summer conditions. The mean semen quality index (SQ) was markedly lower in groups affected by major defects or azoospermia, indicating a global functional collapse of spermatozoa in these animals. This SQ reduction likely reflects chronic heat exposure, testicular degeneration and reduced progressive motility. Population-level data further revealed a negative correlation between morphological defects and semen vigour, sperm swirling and concentration, reinforcing the multifactorial nature of fertility loss in tropical herds.

Table 9 presents the stratification of bulls into distinct clinical profiles, defined through multivariate analysis using clustering techniques. This approach enabled the classification of animals into homogeneous groups based on the combination of andrological defects identified during evaluation and allowed the estimation, for each profile, of the percentage risk of infertility, culling rate and predictive model accuracy, expressed as the area under the ROC curve (AUC).

Two major profiles were identified in the evaluated population:

- The Cluster 1 – High motility, no defects included 22,077 bulls (86.5%), characterised by the absence of significant abnormalities in their spermiograms. This profile showed a very low estimated BSE-based unfitness risk (2%), a culling rate of only 1% and a high predictive accuracy (AUC = 0.92), demonstrating excellent reliability in classifying these bulls as reproductively sound.
- The Cluster 2—Multiple severe abnormalities comprised 2470 bulls (9.7%), showing combined occurrences of necrospermia and testicular hypoplasia, both conditions with substantial negative impact on breeding soundness. This group exhibited an estimated BSE-based unfitness risk of 65%, a culling rate of 58% and a predictive model AUC of 0.87, indicating good discriminative performance for identifying animals with a high probability of BSE-based unfitness.

The combined application of clustering analysis and predictive modelling allowed for the objective classification of bulls into clinical risk profiles, providing valuable support for large-scale reproductive management and selection decisions. The AUC

TABLE 8 | Absolute and relative frequency of seminal defects in Nellore (*Bos indicus*) bulls, with odds ratios (OR) adjusted for age > 5 years, mean seminal quality index (SQ) and seasonal distribution.

Defect	*n*/*N* (%)	OR (age > 5 years)	*p*	Mean SQ index	Summer (%)	Winter (%)	Rank
No abnormality	22,077/25,512 (86.5)	1.0	—	95	85	15	1
Major sperm defects ≥ 30%	2470/25,512 (9.7)	2.8	< 0.001	55	65	35	2
Azoospermia	525/25,512 (2.1)	3.2	0.002	45	68	32	3

TABLE 9 | Clinical profiles of Nellore (*Bos indicus*) bulls grouped according to combinations of andrological defects, with estimated BSE-based unfitness risk, culling rate and predictive model accuracy (AUC).

Cluster	*n*/*N* (%)	Predominant abnormalities	Infertility risk (%)	Culling rate (%)	Model AUC
High motility, no defects	22,077/25,512 (86.5)	None	2	1	0.92
Multiple severe abnormalities	2470/25,512 (9.7)	Necrospermia + Testicular hypoplasia	65	58	0.87

TABLE 10 | Frequency of major seminal defects, co-occurrence patterns, mean seminal quality index (SQ), seasonality and relative culling risk (odds ratio) in Nellore (*Bos indicus*) bulls.

Defect	*n*/*N* (%)	Co-occurrence	Mean SQ index	Season (%)	Culling OR
Major sperm defects ≥ 30%	2470/25,512 (9.7)	Necrospermia (18%)	55	Summer (62%)	3.2
Azoospermia	525/25,512 (2.1)	Testicular hypoplasia (15%)	45	Summer (68%)	3.5

TABLE 11 | Predictive clinical profiles of Nellore (*Bos indicus*) bulls obtained through multivariate modelling, showing the predominant seminal and clinical characteristics, estimated BSE-based unfitness risk, culling rate and model discriminative performance (area under the ROC curve, AUC).

Profile	*n*/*N* (%)	Predominant features	Infertility risk (%)	Culling rate (%)	Model AUC
High motility, no defects	22,077/25,512 (86.5)	No abnormalities	2	1	0.92
Rare and atypical cases	10/25,512 (< 0.1)	Isolated testicular atrophy or severe penile lesions	90	100	0.95

values approaching 1.0 confirmed the model's high accuracy in distinguishing fit and unfit bulls based on clinical and laboratory parameters.

Table 10 provides an expanded epidemiological overview of the main seminal abnormalities, integrating their frequency, co-occurrence patterns, mean seminal quality index (SQ), seasonality and the relative risk of culling estimated through adjusted logistic regression. The table includes the two most relevant defects in both clinical and population contexts, based on their high frequency and pronounced negative impact on breeding soundness.

Major sperm defects (≥ 30%) were observed in 2470 bulls (9.7%), frequently co-occurring with necrospermia in 18% of cases. The mean SQ index was 55 (scale 0–100), indicating moderate to low seminal quality. The highest incidence occurred during the summer (62%), and the relative risk of culling (OR = 3.2) revealed a marked increase in the probability of BSE-based unfitness when this defect was present.

Azoospermia was detected in 525 bulls (2.1%), often associated with testicular hypoplasia in 15% of cases. The mean SQ index was 45, lower than that of major sperm defects, reflecting a more severe impairment of seminal quality. A predominance of cases occurred during the summer (68%), and the relative culling risk was even higher (OR = 3.5), indicating a strong likelihood of failure in breeding soundness evaluations among affected bulls.

The Seminal Quality Index (SQ) is a composite measure ranging from 0 (worst quality) to 100 (best quality), integrating motility, vigour, mass motility and the proportion of major defects. Lower SQ values correspond to ejaculates with greater functional impairment. The odds ratio (OR) for culling quantifies the relative increase in the likelihood of a bull being classified as unfit when a given defect was present, compared with animals showing no abnormalities.

Only these two variables are displayed in the table, as they represent the most prevalent and clinically relevant defects in the evaluated population. Other abnormalities, due to their low frequency and limited statistical weight, did not meet the threshold for inclusion in this advanced epidemiological synthesis.

Table 11 presents the results of the multivariate predictive modelling, which integrated multiple clinical and laboratory variables to stratify Nellore bulls into BSE-based risk profiles. The model incorporated parameters such as motility, proportion of major defects, scrotal circumference and clinical findings, enabling the estimation of infertility probability, culling rate and predictive accuracy (AUC – Area Under the ROC Curve). Two major profiles were identified: Profile 1—High motility, no defects included 22,077 bulls (86.5%) without relevant abnormalities in their spermiograms. This group exhibited a very low estimated BSE-based unfitness risk (2%), a culling rate of 1%, and excellent discriminative performance (AUC = 0.92). No significant clinical abnormalities were detected among these animals. Profile 2—Rare and atypical cases comprised only 10 bulls (< 0.1%), characterised by severe and uncommon abnormalities, such as isolated testicular atrophy or severe penile lesions. This group presented an extremely high BSE-based unfitness risk (90%), a culling rate of 100%, and a very high predictive accuracy (AUC = 0.95), emphasising the severity of these defects and their incompatibility with safe reproductive use. AUC values approaching 1.0 indicate the model's high capacity to differentiate fit and unfit bulls, including rare but clinically critical conditions. The inclusion of these extreme cases in the predictive model allows for statistically robust identification of animals with an almost absolute probability of BSE-based unfitness, thereby justifying their immediate culling from selection and breeding programs.

Profiles summarise the predominant seminal and clinical characteristics, BSE-based unfitness risk, culling rate and discriminative performance of the final model (area under the ROC curve, AUC). Rare and atypical cases include isolated testicular atrophy or severe penile lesions associated with near-total BSE-based unfitness.

In addition to these predictive profiles, further multivariate analyses were performed to visualise interrelations among sperm defects and to evaluate the predictive model's calibration and discriminative ability (Figures 2 and 3).

Figure 2 illustrates the network of co-occurring sperm defects identified in 25,512 ejaculates. The analysis revealed highly interconnected clusters involving proximal cytoplasmic droplets, coiled tails and acrosomal abnormalities, indicating potential common etiological pathways. This network revealed syndromic arrangements of sperm abnormalities, suggesting shared etiopathogenic links among oxidative damage, thermal stress and degenerative spermatogenic processes. A total of 21 distinct defects were mapped (blue = head, red = midpiece, green = tail, grey = other), with node size reflecting combined centrality (degree + betweenness). Head-shortened sperm occupied the highest centrality position, followed by tail-folded and proximal cytoplasmic droplet defects. The strongest positive

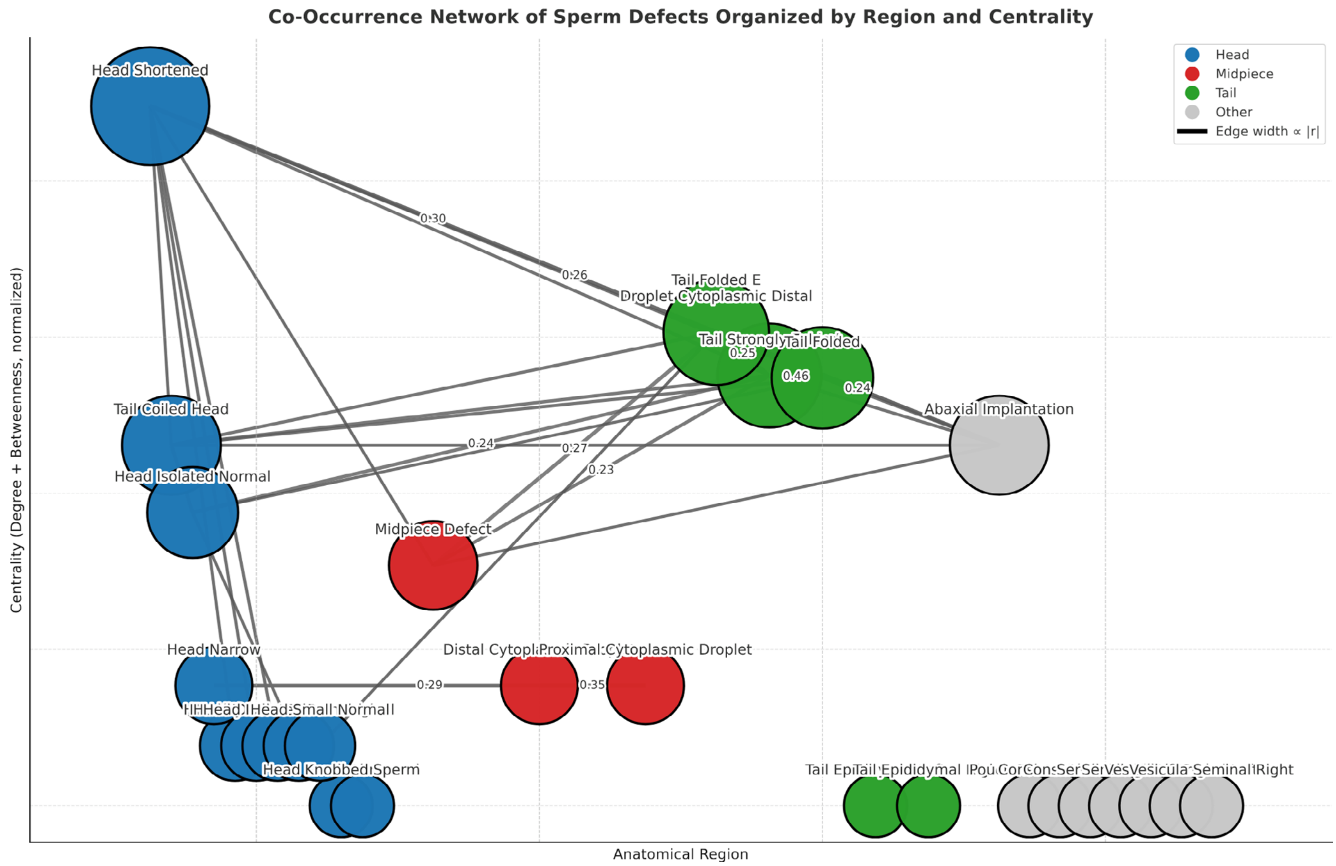

FIGURE 2 | Large-scale co-occurrence network of sperm defects organised by anatomical region and centrality. Nodes represent distinct sperm morphological defects recorded in 25,512 andrological examinations. Node size is proportional to combined network centrality (degree plus betweenness), and nodes are colour-coded by anatomical region (blue = head, red = midpiece, green = tail, grey = other). Edges link pairs of defects with Spearman correlations ≥ 0.30 or among the 30 strongest associations; edge width reflects correlation strength. The 10 strongest connections are labelled with their respective coefficients (*r*). Horizontal position indicates anatomical region, while vertical position indicates node centrality, enabling rapid identification of highly connected defects and potential morphological syndromes.

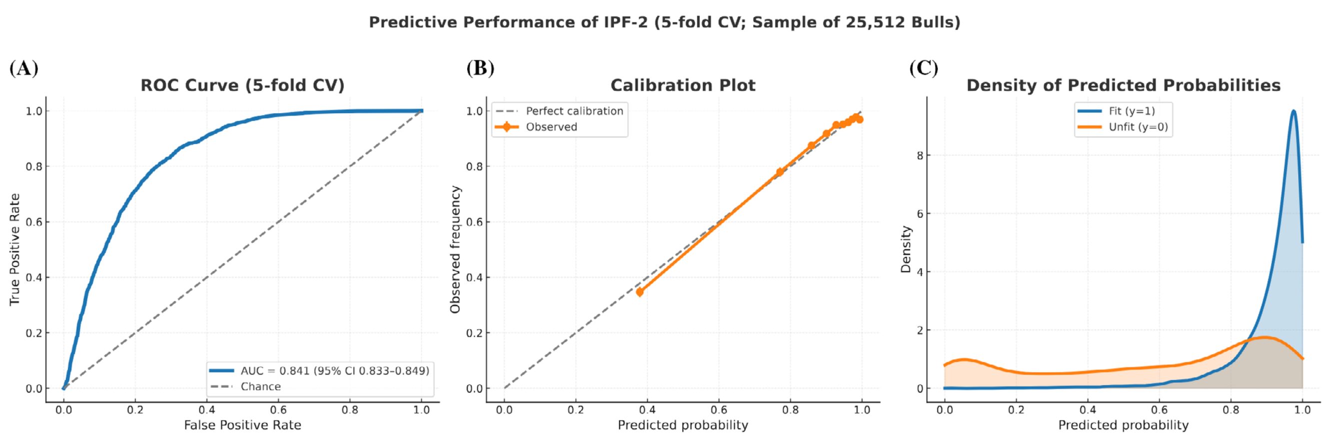

FIGURE 3 | Predictive performance of the BSRI (five-fold cross-validation; $n = 25{,}512$): (A) ROC curve (AUC 0.841, 95% CI 0.833–0.849); (B) calibration plot; (C) density of predicted probabilities.

associations were observed between proximal and distal cytoplasmic droplets ($r = 0.35$), between strongly folded tail and abaxial implantation ($r = 0.34$), and between tail-folded and distal droplet ($r = 0.29$). Correlations among head defects were generally lower ($r \approx 0.23$–0.27), forming a tightly clustered subnetwork. In contrast, midpiece defects exhibited fewer but stronger cross-regional connections, indicating their bridging role between head and tail abnormalities. This network layout allows rapid visualisation of co-occurring defects and highlights the anomalies most central within the overall sperm morphology landscape.

Figure 3 summarises the predictive performance of the IPF-2 model evaluated by 5-fold cross-validation on 25,512 bulls. The ROC curve (Panel A) achieved an area under the curve (AUC) of 0.841 (95% CI: 0.833–0.849), indicating strong discriminative ability. The calibration plot (Panel B) demonstrated excellent agreement between predicted probabilities and observed frequencies, with most points closely following the ideal 45° line across the full probability range. The density plot of predicted probabilities (Panel C) revealed a pronounced bimodal separation between bulls classified as Fit ($y = 1$) and Unfit ($y = 0$): Fit bulls exhibited a sharp density peak near predicted probability ≈1.0, whereas Unfit bulls showed density concentrated at lower predicted probabilities. This pattern underscores both the discriminatory power and calibration accuracy of the model across the entire dataset.

4 | Discussion

This study provides the first population-scale, data-driven assessment of reproductive soundness in *Bos indicus* bulls, integrating field andrological data with multivariate predictive modelling. Unlike traditional descriptive reports, this work identifies latent breeding soundness phenotypes and validates a breeding soundness risk index (BSRI) through internal calibration, thereby offering a quantitative framework for decision-making in tropical breeding systems (Table 2).

Recent studies have advanced the molecular understanding of this relationship: heat stress can induce Sertoli and Leydig cell dysfunction, reduce testosterone synthesis, trigger early activation of apoptotic pathways and promote intense oxidative stress in testicular tissue (Khan et al. 2023). Vanselow et al. (2024) demonstrated that semen collections performed during summer, under high temperature–humidity index (THI) conditions, result in ejaculates with reduced in vitro viability, providing direct evidence that thermal peaks compromise sperm integrity.

Additionally, deleterious effects at cellular and epigenetic levels—including mitochondrial dysfunction, sperm DNA damage and alterations in the expression of proteins involved in capacitation and fertilisation—have been recently described (Celeghini et al. 2024).

The observed association between sperm motility and scrotal circumference is corroborated by studies integrating morphometric and molecular indices, showing that a smaller effective scrotal surface area reduces heat dissipation and intensifies the accumulation of reactive oxygen species (ROS), thereby promoting oxidative damage to sperm membranes (Zhou et al. 2025). Furthermore, a recent study on proteomics and oxidative stress in Nellore bulls reported that increased seminal plasma concentrations of malondialdehyde (MDA) and carbonylated proteins were negatively correlated with sperm motility and membrane integrity.

Similarly, Alves, Gheller, et al. (2025); Alves, Cooke, et al. (2025) demonstrated that, across different bovine genotypes (Angus, Brangus, and Nellore), the THI preceding semen collection significantly affected both fresh and post-thaw sperm parameters, as well as pregnancy rates. Although the negative effect was more pronounced in taurine genotypes, Nellore bulls also exhibited detectable impairment under high THI conditions.

It is important to note that the impacts of heat stress do not necessarily manifest immediately but rather after a delay of several weeks, consistent with the duration and later stages of spermatogenesis (Vanselow et al. 2024). Moreover, prolonged exposure to high temperatures promotes sperm senescence and induces persistent oxidative responses even after thermal normalisation, a phenomenon described as 'residual damage' (Khan et al. 2023).

The analysis of macroscopic ejaculate variables provides a practical set of field-applicable indicators that serve as indirect markers of sperm quality and breeding soundness in bulls. Mass motility shows the strongest association with final breeding soundness classification, followed by the visual appearance of semen, whereas anatomical characteristics (preputial type, testicular symmetry) and penile alterations exhibited weaker but still significant correlations (Table 3). Mass motility represents a macroscopic manifestation of sperm density, progressive motility and flagellar vigour, integrating effects of structural integrity and flagellar beat coordination. Studies have indicated that mass motility formation is among the best visual indicators of semen quality and breeding soundness, showing positive correlations with pregnancy rates (Fonseca et al. 1992). A well-developed mass motility reflects high sperm concentration and synchronised motility, attributes dependent on intact spermatogenesis and proper epididymal maturation—processes that are highly sensitive to environmental and nutritional variations (Senger 2012; Barth and Oko 1989).

The visual appearance of the ejaculate (showing a more moderate correlation) remains a practical diagnostic marker, reflecting, to some extent, sperm concentration and the presence of contaminants such as pus, blood or cellular debris. Watery or excessively opaque ejaculates may indicate semen with low sperm density or a high proportion of degenerated cells (Arifiantini et al. 2024). Abnormal semen appearance has been associated with a higher prevalence of major defects and BSE-based unfitness in *Bos indicus* bulls (Vale Filho et al. 2013).

External anatomical traits (preputial type, testicular asymmetry and penile abnormalities) also display limited discriminative capacity for predicting short-term sperm quality, since these traits commonly reflect prior trauma, growth variations or mechanical limitations to copulation, rather than directly affecting sperm morphofunction in the immediate term (Palmer et al. 2005). The effects of heat stress indicate that damage to spermatogenesis or epididymal integrity often precedes visible external manifestations (Capela et al. 2022). These anatomical characteristics help anticipate mechanical failures during natural mating and anatomical compatibility during copulation (Azevedo et al. 2024) and constitute an integral component of breeding soundness evaluation protocols in bull selection programs (Chenoweth 2002a, 2002b).

Recent studies have demonstrated that the integration of functional and molecular methods has enhanced the interpretation of macroscopic variables. Microscopic and molecular parameters, such as motility, membrane integrity, DNA integrity and proteomic markers, show moderate correlations with fertility and should complement visual indicators (Bollwein and Bittner 2018). Convergently, proteomic analysis of semen has identified molecular signatures capable of distinguishing high- and low-fertility bulls, which are associated with macroscopic ejaculate patterns (Fernández-Montoro et al. 2025). Moreover, multivariate approaches to andrological evaluation have shown that combining multiple parameters—including sperm kinetics assessed by Computer-Assisted Sperm Analysis—outperforms the use of single, isolated variables (Azevedo et al. 2024).

The multivariate analysis (Table 4) identified sperm motility, proportion of major defects, scrotal circumference, and mass motility as independent predictors of BSE-based unfitness in *Bos indicus* bulls. Reproductive fitness results from the interaction between intrinsic sperm quality and testicular thermoregulatory capacity. Sperm motility exerted a significant protective effect against unfitness, as it depends on structural integrity of the flagellum, efficient mitochondrial function and preservation of the plasma membrane. Thermal stress compromises these systems, leading to reduced progressive motility (Rahman et al. 2022). Both classic and contemporary studies confirm a direct correlation between total or progressive motility and field pregnancy rates, especially in *Bos indicus* bulls, whose spermatogenesis is highly sensitive to thermal variations (Barth 2013; Capela et al. 2022).

The proportion of major defects emerged as the strongest predictor of BSE-based unfitness, reinforcing sperm morphology as a mirror of spermatogenic efficiency. Severe anomalies—such as pyriform heads, strongly folded/rigid tails and persistent proximal droplets—indicate dysfunctions in testicular differentiation and epididymal maturation phases (Barth and Oko 1989; Vale Filho et al. 2013). Under chronic heat stress, Sertoli cell damage, DNA fragmentation and persistent structural defects occur, compromising reproductive performance and increasing the risk of early embryonic losses (Rahman et al. 2022; Capela et al. 2022).

Scrotal circumference exhibited a robust protective association, corroborating broad evidence that larger scrotal size indicates greater testicular volume, higher density of active seminiferous tubules and improved thermoregulatory efficiency (Fonseca et al. 1992; Wahyudi et al. 2022). Genetic studies have shown a positive correlation between scrotal circumference and daily sperm production (Silva et al. 2018; Azevedo et al. 2024), whereas smaller scrotal sizes limit heat dissipation.

Mass motility (1.0–2.0) showed a strong inverse association with BSE-based unfitness. Higher mass motility scores correspond to greater sperm concentration and coordinated motility, reflecting sperm vigour suitable for efficient transport within the female reproductive tract (Fonseca et al. 1992; Fernández-Montoro et al. 2025). The sensitivity of the vortex to thermal and oxidative insults makes this parameter a valuable, rapid field-screening tool. Altogether, the data in Table 3 demonstrate that functional predictors of breeding soundness are interdependent: scrotal circumference supports thermoregulation, motility reflects structural and bioenergetic integrity, morphology expresses spermatogenic efficiency and the mass motility integrates a practical and accessible indicator.

The direct relationship between motility and scrotal circumference (Table 3) is visually confirmed in Figure 1, which shows a progressive increase in total motility across scrotal circumference terciles. This trend reinforces the role of testicular thermoregulation and tubular mass in spermatogenic efficiency, indicating that bulls with larger scrotal circumference maintain higher motility even under thermally challenging conditions (Rahman et al. 2022; Wahyudi et al. 2022).

Azoospermia represents the complete absence of spermatozoa in the ejaculate—caused by severe failures in spermatogenesis, obstruction of the genital tract or advanced testicular degeneration (Barth and Oko 1989; Pausch and Kadri 2023). Normal spermatogenesis depends on efficient thermoregulation, integrity of the seminiferous tubules and functional support from Sertoli and Leydig cells. The impairment of any of these mechanisms—due to thermal stress, somatic injury or hormonal dysfunction—leads to disruption in germ cell proliferation, differentiation and survival, resulting in persistent absence of spermatozoa in the ejaculate, thereby excluding the animal from reproductive use (Table 10). Recent studies have shown that heat exposure induces germ cell apoptosis, mitochondrial dysfunction, and sperm DNA damage, in addition to impairing Sertoli cell function (Maroto et al. 2025). Heat disrupts the structural integrity of the seminiferous epithelium, reducing germ cell viability and compromising the functionality of support cells (Capela et al. 2022).

Aspermia is associated with failure of semen emission during ejaculation. Its causes may include neurophysiological dysfunctions, obstructions of the spermatic ducts or traumatic sequelae (Chenoweth 2002a, 2002b).

The presence of ≥30% major defects appeared as a strong predictor of BSE-based unfitness, confirming the value of sperm morphology as a functional marker of germ cell quality. Severe abnormalities—such as distorted heads, strongly folded/rigid tails or retained proximal droplets—typically reflect intratesticular or epididymal dysfunction (Setchell 2006; Rahman et al. 2022). Under heat stress, oxidative damage, DNA strand breaks and persistent functional impairment are more likely to occur.

Necrospermia, characterised by a high proportion of dead spermatozoa, indicates a compromised testicular or epididymal environment. Frequently associated with genital infections, oxidative stress or extreme thermal insults, this condition suggests apoptosis and loss of membrane integrity (Vale Filho et al. 2013).

Foot lesions interfere with breeding soundness, as natural service requires adequate locomotor performance. Bulls experiencing pain or lameness tend to copulate less frequently, exhibit decreased libido and show reduced mating efficiency (Boakari et al. 2022). In beef cattle, limb lesions are associated with increased cortisol levels and decreased testosterone secretion, which can compromise spermatogenesis and sperm quality (Boakari et al. 2021) (Table 5). In extensive systems, this locomotor limitation acts as an additional restrictive factor, especially when bulls must travel long distances to reach females. Finally, acrobustitis/balanoposthitis mechanically compromises copulation by hindering intromission and increasing the risk of trauma during mating. Moreover, the chronic local inflammatory process may affect semen through the release of mediators, edema, or increased local temperature (Butkiewicz et al. 2025).

The multivariate analysis stratified bulls into three seminal quality profiles, Optimal, Intermediate and Critical (Table 6), demonstrating that integrating multiple biological variables enhances the understanding of breeding soundness determinants on a large scale. The Optimal Cluster is characterised by high motility (> 80%), a low incidence of major defects (< 10%), large scrotal circumference (> 38 cm), and pronounced vigour, a set indicative of testicular and epididymal integrity, efficient spermatogenesis and adequate thermoregulation, all recognised as indicators of satisfactory breeding soundness (Silva et al. 2018). The Intermediate Cluster comprises bulls with performance compatible with an intermediate breeding soundness profile, showing reduced motility and vigour and an increased rate of major defects (~15%), reflecting partially compromised spermatogenesis, possibly due to transient thermal or nutritional stress, with potential for recovery under proper management (Wettemann et al. 2020; Rahman et al. 2022). The Critical Cluster, in turn, includes animals with markedly reduced motility (< 50%), a high rate of defects (> 30%), small scrotal circumference and minimal vigour, typical characteristics of chronic testicular degeneration, hypoplasia or prolonged heat exposure (Barth and Oko 1989; Setchell 2006). Considering that the bovine spermatogenic cycle lasts about 61 days, persistent insults simultaneously impair sperm production, maturation and function.

The stratification obtained by PCA and k-means confirms the classical parameters of andrological evaluation and introduces a practical concept of andrological risk profiling, with potential applications in breeding soundness and sire selection programs (Azevedo et al. 2024; Losano et al. 2025).

Beyond the statistical integration of semen traits, the present findings redefine the interpretation of breeding soundness by introducing a predictive framework capable of quantifying BSE-based risk. The multivariate integration used here transcends conventional thresholds (e.g., motility < 60%), providing a reproducible basis for field-based precision selection, particularly under tropical constraints where environmental stressors dominate reproductive outcomes.

Breeding Soundness Risk Index (BSRI) integrates key fertility-related variables (motility, major defects, scrotal circumference and sperm swirling), proposing a composite metric that more accurately reflects the individual reproductive potential than isolated analyses. Studies advocate for the use of integrative tools to overcome the limitations of conventional exams (Azevedo et al. 2024), as well as multi-omic approaches applied to bovine fertility prediction (Costes et al. 2024) (Table 7).

The low-risk stratum includes bulls with significantly reduced motility (< 60%), smaller scrotal circumference (< 33 cm), and moderate vigour. Physiologically, this profile indicates partial impairment of spermatogenesis, often linked to environmental factors (chronic heat, poor nutrition) or subclinical genital tract conditions that affect mitochondrial metabolism and sperm motility (Setchell 2006; Rahman et al. 2022). Bulls in this group tend to exhibit low efficiency in depositing motile spermatozoa into the female reproductive tract, increasing the risk of fertilisation failure or early embryonic loss.

In the intermediate class, motility is higher (~78%), scrotal circumference approximates the reference values for *Bos indicus* (> 35 cm) and moderate sperm swirling is observed—a combination denoting functional spermatogenesis, though below the physiological optimum. This category likely includes bulls with preserved potential fertility but inconsistent reproductive performance under challenging environmental conditions, such as extreme heat, overcrowding or high mating load (Wettemann et al. 2020).

The use of the Breeding Soundness Risk Index (BSRI) proved particularly valuable by integrating multiple correlated parameters, recognising that fertility is not determined by a single factor but by the balance between sperm quantity, quality and functionality, combined with testicular thermoregulatory efficiency. This approach reduces the classification errors commonly observed when only one marker, such as motility or scrotal circumference, is used as a selection criterion (Fonseca et al. 1992; Silva et al. 2018).

Recent studies reinforce the validity of this integrative approach by demonstrating that multivariate predictive models increase the accuracy of fertile bull classification (Silva et al. 2018), and significant gains are achieved when combining kinetic and morphological parameters in composite models (Azevedo et al. 2024).

Population-level overview of the risk factors associated with severe seminal defects (major defects and azoospermia) in Nellore bulls, which can be evidenced by factors such as advanced age, mean semen quality (SQ) and the seasonal predisposition to these anomalies, is provided (Table 8). Bulls over five years old showed elevated odds ratios for major defects (2.8) and azoospermia (3.2), confirming the decline in sperm quality with aging (Barth 2013; Vale Filho et al. 2013). The synergistic interaction between age-related testicular degeneration and seasonal heat stress constitutes a newly characterised epidemiological signature of fertility decline in *Bos indicus* bulls, emphasising the compounded vulnerability of aging animals exposed to tropical summer conditions. The mean semen quality index (SQ) was lower in the groups with major defects and azoospermia, indicating a global functional collapse of spermatozoa in these animals. This SQ reduction is associated with chronic heat stress, tubular degeneration and decreased progressive motility (Capela et al. 2022; Khan et al. 2023). Population studies in *Bos indicus* have already demonstrated a negative correlation between high rates of morphological defects and parameters such as vigour, sperm swirling and concentration (Silva et al. 2018), reducing conception likelihood even when libido remains intact.

Seasonality was also a determining factor: during summer, a higher prevalence of severe defects and azoospermia was observed, reinforcing the impact of thermal stress on spermatogenesis. In tropical climates, ambient temperatures often exceed the scrotum's heat-dissipation capacity, raising intratesticular temperature by 4°C–6°C above the ideal, compromising the seminiferous epithelium, and inducing oxidative stress, DNA fragmentation and damage to sperm membranes (Khan et al. 2023; Capela et al. 2022). In such contexts, ***Bos indicus*** herds may exhibit varying degrees of thermotolerance and antioxidant defence (Marín-Urías et al. 2023; Gacem et al. 2023) (Table 9).

The second profile, characterised by multiple severe abnormalities such as necrospermia associated with testicular hypoplasia, showed an estimated BSE-based unfitness risk of 65% and a culling rate close to 60%. The combination of these disorders is particularly synergistic: testicular hypoplasia reduces the germ cell population and daily sperm output, while necrospermia indicates loss of cellular viability, commonly related to testicular degeneration, oxidative stress or subclinical infectious processes. The coexistence of these conditions drastically amplifies the likelihood of dysfunctional ejaculates and impaired fertility (Barth and Oko 1989; Vale Filho et al. 2013). In hot environments, persistent thermal exposure aggravates this scenario by compromising testicular thermoregulation and inducing sperm apoptosis (Setchell 2006; Wettemann et al. 2020).

An overview of the main seminal defects in Nellore bulls, highlighting the frequency of abnormalities, their co-occurrence patterns, correlations with mean semen quality (SQ), seasonality and relative risk of culling, is provided (Table 10). High proportions of major defects (≥ 30%) were observed in about 10% of bulls, often combined with necrospermia (18%). This may have originated from morphogenetic defects, degenerative processes, oxidative stress, failures in DNA packaging and secondary epididymal lesions acting simultaneously, thereby reducing membrane integrity and promoting cell death (Barth and Oko 1989; Setchell 2006). Such co-occurrences are described as markers of an adverse testicular environment, exacerbated under persistent heat or subclinical inflammation (Rahman et al. 2022; Malama et al. 2021). Although less frequent (2.1%), azoospermia was associated with testicular hypoplasia in 15% of cases, a condition of genetic origin or derived from perinatal insults affecting testicular development (Chenoweth 2002a, 2002b). Hypoplasia reduces tubular density and the number of Sertoli cells, resulting in insufficient sperm production, often yielding ejaculates devoid of spermatozoa. Identification of this association is crucial, as bulls with such characteristics have an extremely poor reproductive prognosis, showing no effective response to treatment or corrective management, thus justifying the high risk of culling.

Conversely, seasonality plays a decisive role. The prevalence of severe defects was higher in summer (62%–68%), reinforcing the role of heat stress as an exacerbating factor. Increased intratesticular temperature compromises spermatogenesis at multiple stages, impairs sperm DNA integrity, and may exacerbate preexisting pathological conditions such as tubular degeneration or marginal hypoplasia (Setchell 2006; Rahman et al. 2022). In tropical regions, prolonged exposure to ambient temperatures above 30°C–32°C, particularly when combined with high humidity, is associated with reduced motility, elevated morphological defects, and even temporary episodes of oligozoospermia or azoospermia in more sensitive bulls (Poclín-Rojas et al. 2025). The increased risk of culling (OR > 3) for bulls with severe defects confirms that these anomalies critically degrade reproductive capacity, rendering their use unsafe in natural mating programs. The presence of co-occurring abnormalities makes the scenario even more unfavourable, as it involves multiple failure pathways—defective production, reduced viability or total absence of spermatozoa—with low probability of functional recovery. These findings emphasise the importance of periodic andrological evaluations prior to the breeding season, especially in tropical climates, to detect bulls with severe or co-occurring defects at an early stage (Weller et al. 2023).

The co-occurrence network of morphological defects (Figure 2) reveals syndromic patterns among abnormalities of the head, midpiece and tail. The high centrality of defects such as proximal cytoplasmic droplet and bent tail indicates a common aetiology related to testicular degeneration and epididymal dysfunction. These findings complement the results in Table 10, demonstrating that sperm abnormalities do not occur in isolation but form interdependent networks that reflect the cumulative impact of thermal and oxidative stress (Zhou et al. 2025).

Finally, the stratification of bulls into predictive clinical profiles derived from multivariate modelling (Table 11) provides a refined clinical–population framework essential for precision breeding programs in tropical systems. The high-risk profiles are characterised by combinations of critical seminal alterations, for example, necrospermia associated with testicular hypoplasia or massive morphological defects combined with severely reduced motility. The simultaneous presence of these conditions reflects multiple pathophysiological mechanisms compromising fertility, ranging from spermatogenic failure (reduced germ cell population, Sertoli cell dysfunction) to loss of sperm viability due to oxidative stress or epididymal lesions. Previous and recent studies confirm that the concomitant occurrence of severe alterations has a multiplicative effect on infertility, drastically reducing conception probability even when libido remains high (Barth and Oko 1989; Vale Filho et al. 2013; Rahman et al. 2022). Rare cases, such as severe unilateral testicular atrophy, disabling penile lesions or persistent aspermatic ejaculates (< 0.1%), represent extreme-risk profiles, with an almost total probability of infertility and culling rates exceeding 90%. The irreversibility of these conditions results from advanced degeneration, fibrosis and destruction of the testicular parenchyma, making recovery of spermatogenesis unfeasible (Chenoweth 2002a, 2002b; Setchell 2006). BSRI confirms the discriminatory performance of the multivariate approach in identifying risk profiles, surpassing traditional methods that assess isolated variables. Neural-network and clustering-based models (Marín-Urías et al. 2023) exemplify this advancement in population-level andrological prediction. The ability to group animals by predictive clinical clusters contributes to more efficient reproductive-management programs, allowing early identification of bulls with compromised fertility, optimised use of reproductively sound individuals and reduction of waste and BSE-based unfitness.

Figure 3 summarises the internal validation performance of the BSRI-based model (five-fold cross-validation; $n = 25{,}512$), including discrimination (ROC AUC = 0.841; 95% CI 0.833–0.849), calibration and the distribution of predicted probabilities. The separation between BSE-satisfactory (Fit) and BSE-unsatisfactory (Unfit) bulls in the density plot supports the utility of the index for BSE-based risk stratification, rather than inference about realised reproductive performance.

In conclusion, this population-scale study demonstrates that breeding soundness can be accurately predicted through multivariate integration of conventional andrological traits. Motility, major sperm defects and scrotal circumference emerged as the most powerful predictors of reproductive soundness, while the mass motility score added a functional dimension related to ejaculate vigour and sperm concentration. The internally validated Breeding Soundness Risk Index (BSRI) developed herein provides an objective and reproducible metric for ranking sires according to reproductive aptitude. Beyond diagnostic classification, this predictive framework bridges classical andrology with precision reproductive analytics, offering a scalable and field-applicable tool to support evidence-based selection, culling and genetic improvement in *Bos indicus* herds under tropical conditions.

Author Contributions

Clovis Juk Fassano: conceptualization, data curation, investigation, resources, writing – review and editing. Pedro Henrique Lomba de Lima: conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing. Fernando Andrade Souza: methodology, formal analysis, writing – review and editing. Natalia Siqueira de Lara: validation, writing – review and editing. Tácia Gomes Bergstein-Galan: data curation, validation, writing – review and editing. Eriklis Nogueira: supervision, writing – review and editing. Luiz Ernandes Kozicki: supervision, writing – review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are not publicly available due to confidentiality agreements but are available from the corresponding author upon reasonable request and permission of the data owner.

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