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Teratozoospermia in tropical stallions: integrating morphology, heat stress and molecular alterations

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Abstract. The fertility of stallions in tropical regions is strongly influenced by semen quality, with sperm morphology being one of the main indicators of reproductive performance. Structural alterations, grouped under the term teratozoospermia, compromise fertilizing capacity and are associated with subfertility. This study aimed to conduct an integrative literature review on teratozoospermia in stallions under tropical climate conditions, gathering morphological, methodological, environmental, and molecular evidence. Studies analyzed addressed racial variations in sperm morphology, staining and evaluation methodologies, the impact of thermal stress on seminal parameters and testicular hemodynamics, as well as comparisons with human models and proteomic analyses. Results showed that different breeds present distinct proportions of normal sperm and specific defects; that methodological variability can generate divergences of up to 15% in the identification of abnormalities; and that thermal stress in tropical climates reduces motility, increases defects and DNA fragmentation, compromising fertility. Comparative evidence in humans reinforces the universality of thermal damage mechanisms, while proteomic analyses reveal alterations in acrosomal enzymes and structural proteins, broadening the understanding of subfertility.It is concluded that teratozoospermia in tropical stallions is a multifactorial phenomenon, resulting from the interaction between racial predispositions, methodological variability, environmental stress, and molecular alterations. The integration of morphological analyses, thermal comfort indices, and proteomic approaches represents a promising strategy for reproductive diagnosis and management adapted to tropical conditions.
Keywords: teratozoospermia, sperm morphology, thermal stress, equine fertility, proteomics

Introduction

Equine fertility plays a strategic role in assisted reproduction and in the maintenance of genetic improvement programs, especially in tropical regions, where the environment exerts a direct influence on semen quality. Among the evaluated parameters, sperm morphology is considered one of the main indicators of reproductive performance, since structural alterations compromise fertilizing capacity and may result in teratozoospermia, a condition associated with subfertility in stallions.

Although the literature widely describes morphological patterns and evaluation methodologies, important gaps remain regarding the integration of these aspects with environmental and molecular factors. In tropical regions, thermal stress represents a constant challenge, capable of inducing cellular and molecular alterations that reduce fertility. Furthermore, variability among breeds and the lack of methodological standardization hinder direct comparisons between studies, limiting the practical applicability of results.

The relevance of this topic transcends the academic sphere: understanding the mechanisms of teratozoospermia in tropical stallions is essential to optimize reproduction programs, reduce economic losses, and improve management strategies

adapted to environmental conditions. In this sense, it is necessary to gather and critically discuss the available evidence, highlighting not only morphological and methodological aspects, but also climatic impacts and molecular alterations that underpin subfertility.

Given this scenario, the present study aims to conduct an integrative review on teratozoospermia in stallions under tropical conditions, addressing in an articulated manner racial, methodological, environmental, and molecular factors, and discussing their implications for reproductive diagnosis and management.

Contextualization and analysis

Scientific production on equine fertility in tropical regions has grown over the past decades but remains fragmented across different lines of investigation. In general, studies can be grouped into three main trends: classical morphological descriptions, methodological and diagnostic evaluations, and environmental and molecular approaches.

Morphological descriptions form the historical foundation of the field, providing parameters of normality and identifying recurrent defects in different breeds. However, these studies often lack integration with functional and molecular data, limiting their practical applicability.

Methodological evaluations, in turn, reveal the absence of consensus regarding staining techniques and diagnostic criteria, which generates significant variability among laboratories and hinders international comparisons. This methodological heterogeneity is pointed out as one of the main obstacles to consolidating universal indicators of semen quality.

Environmental and molecular approaches represent the most recent and promising trend. Studies on thermal stress in tropical climates demonstrate direct correlations between thermal comfort indices and seminal parameters, while proteomic and gene expression analyses broaden the understanding of subfertility by revealing the cellular and biochemical mechanisms involved. Nevertheless, there are still few studies that simultaneously integrate morphology, environment, and molecular aspects, highlighting an important gap in the literature.

Therefore, the analysis of the state of the art shows that, although relevant advances exist in each axis, an integrative vision that connects racial predispositions, methodological variability, climatic impact, and molecular alterations is still lacking. It is precisely at this point that the present review is situated, seeking to provide a critical synthesis that articulates these different levels of evidence.

Comparative studies show that sperm morphology varies significantly among breeds, reflecting both genetic predispositions and environmental adaptations. Kavak et al. (2004) observed that Estonian stallions presented a higher proportion of normal spermatozoa (74.4%) compared to Tori stallions (57.5%), with the latter exhibiting a higher prevalence of proximal droplets (17.3%). This finding suggests that certain breeds may be more susceptible to specific morphological defects. In Arabian horses, Waheed et al. (2015) identified that morphometric measurements of the sperm head (5.96 µm length and 3.06 µm width) correlate with fertility, indicating that reduced head dimensions favor fertilizing capacity. The comparison between studies shows that both proportions and structural measurements are relevant to predicting fertility, reinforcing the need for integrated analyses.

Table 1. Morphological Comparison Between Breeds

Breed	Normal specimens (%)	Main defects
Estonian	74.4 ± 3.8	Proximal droplets (2.9%)
Tori	57.5 ± 4.1	Proximal droplets (17.3%)
Arabian	Head size: 5.96 µm; Tail length: 48.88 µm	Smaller heads associated with greater fertility

Source: Adapted from Kavak et al. (2004) and Waheed et al. (2015).

Table 2. Staining Methods and Variation in Defect Identification

Method	Variation	Observations
Diff-Quik	+15% head defects	Overestimates abnormalities
Eosin-nigrosin	Intermediate	Influenced by evaluator experience
SpermBlue	Closest to unstained smears	Considered more reliable

Source: Adapted from Murcia-Robayo et al. (2018).

Figure 1. Normal spermatozoa. Source:Adapted from KAVAK, I. et al. *Sperm morphology in Estonian and Tori breed stallions.* Theriogenology, 2004.

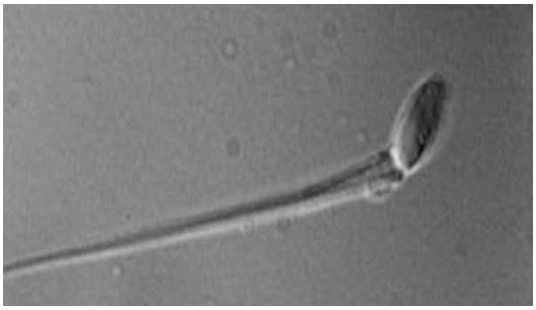

Figure 2. Double midpiece and proximal cytoplasmic droplet. Source:Adapted from KAVAK, I. et al. *Sperm morphology in Estonian and Tori breed stallions.* Theriogenology, 2004.

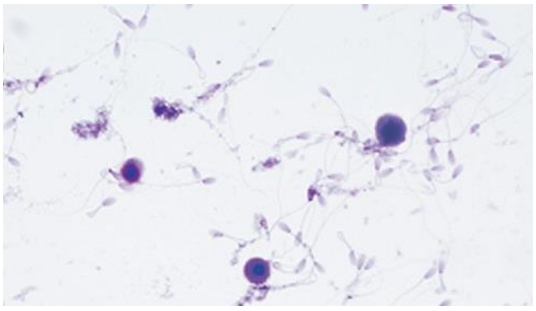

Figure 3. Stallion spermatozoa stained with the Diff-Quik method, showing head and tail defects. Technique widely used in morphological analyses, but with a tendency to overestimate abnormalities.Source:Adapted from MURCIA-ROBAYO, R.; OLIVIERI, B. T.; SAMUEL, M. *Evaluation of stallion sperm morphology.* Theriogenology, v. 113, p. 1-9, 2018.

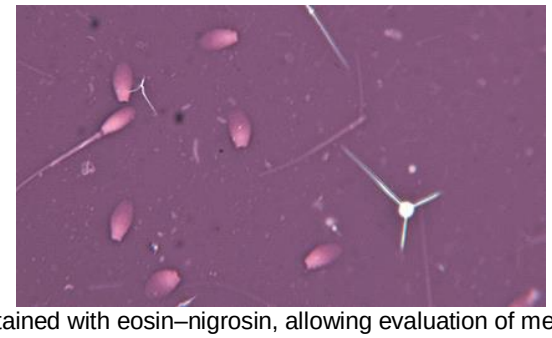

Figure 4. Stallion spermatozoa stained with eosin–nigrosin, allowing evaluation of membrane integrity and morphology. Intermediate method in terms of reliability, with significant influence from the evaluator's experience. Source:Adapted from MURCIA-ROBAYO, R.; OLIVIERI, B. T.; SAMUEL, M. *Evaluation of stallion sperm morphology.* Theriogenology, v. 113, p. 1-9, 2018.

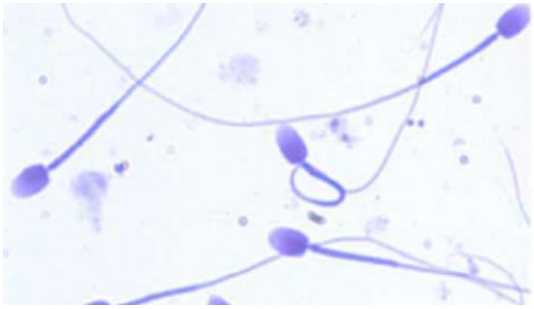

Figure 5. Stallion spermatozoa stained with SpermBlue®, a universal technique that provides more consistent results, closer to unstained smears. Considered more reliable for automated sperm morphology analyses. Source: Adapted from VAN DER HORST, G.; MAREE, L. (2009).

Thereliability of morphological analysis strongly depends on the staining technique. Murcia-Robayo et al. (2018) compared Diff-Quik, eosin-nigrosin, and SpermBlue®, showing up to 15% variation in defect identification. Diff-Quik overestimated head defects, while SpermBlue® produced results closer to unstained smears. Additionally, evaluator experience had a significant influence: less experienced clinicians classified a higher proportion of spermatozoa as abnormal. These results highlight that methodological variability can generate inconsistent diagnoses, reinforcing the need for international standardization.

Thermal stress is one of the main environmental factors compromising fertility in tropical regions. Shakeel & Yoon (2023) highlighted the role of heat shock proteins (HSP60, HSP70, HSP90, and HSP105) in cellular protection, assisting mitochondrial protein folding, motility maintenance, and capacitation signaling. In a later study, Shakeel & Yoon (2024) showed that in cryptorchid testes there was increased expression of UTF-1 and MCM2 (undifferentiated stem cells), but reduced DAZL (germ cell differentiation), indicating that heat mainly affects advanced stages of spermatogenesis. Ribeiro et al. (2025) correlated thermal comfort indices with seminal parameters, showing that in summer (28.6 °C) motility decreased (64.2%) and total defects increased (22.4%), compared to winter (81.2% and 12.5%). These indices (THI and ThStress) prove to be practical tools to predict fertility decline. Complementarily, Griffin et al. (2026) identified that 13% of stallions showed a positive correlation between heat and DNA fragmentation, with reduced pregnancy rates, confirming that thermal stress compromises fertility via oxidative damage.

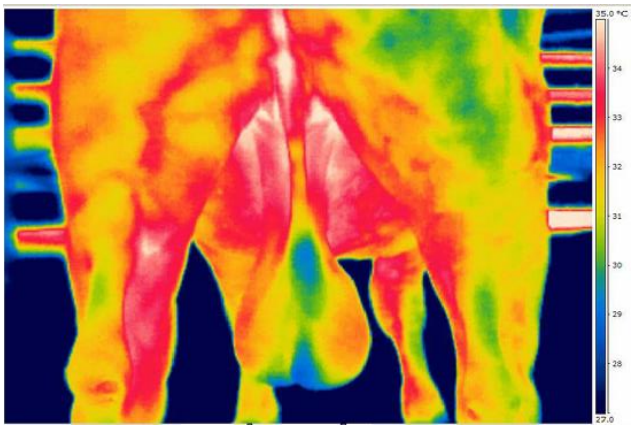

Figure 6. Thermographic image of the scrotal region of a bull, showing temperature gradients in the pampiniform plexus and scrotum. The technique allows the identification of thermal asymmetries and early changes in thermoregulation, correlating with seminal parameters. Although obtained in cattle, the methodology is also applicable to reproductive evaluation of stallions in tropical climates. Source: Adapted from PORTUGAL, E. S. (2014).

At the molecular level, Hernández-Avilés et al. (2024) identified 140 proteins differentially abundant between fertile and subfertile stallions, including reduced acrosomal enzymes such as ARSF, compromising the acrosome reaction and binding to the zona pellucida. These findings broaden the understanding of subfertility, showing that teratozoospermia is not only a consequence of visible morphological alterations but also of molecular mechanisms.

The results presented allow us to understand teratozoospermia in tropical stallions as a multifactorial phenomenon. Racial differences show that genetic predispositions modulate the occurrence of sperm defects, while methodological variability demonstrates that part of the divergences between studies stem from the lack of standardization in evaluation techniques. Thermal stress emerges as a determining factor, affecting both seminal parameters and cellular and molecular mechanisms. The correlation between thermal comfort indices and semen quality confirms that the tropical environment directly influences fertility. The comparison with humans reinforces that the mechanisms of thermal damage — DNA fragmentation, oxidative stress, and apoptosis — are universal, although reversibility is partial in equines. Finally, proteomic findings expand the discussion by revealing that subfertility is not limited to visible morphological alterations but also involves molecular modifications that compromise sperm function.

Table 3. Seasonal Differences in Stallions (Brazil, Tropical Climate)

Parameter	Summer (28.6 °C)	Winter (21.1 °C)
Motility (%)	64.2 ± 21.0	81.2 ± 8.5
Vigor (1–5)	2.7 ± 0.6	3.2 ± 0.4
Total defects (%)	22.4 ± 7.3	12.5 ± 9.4
Systolic velocity (cm/s)	24.4 ± 6.7	33.3 ± 9.3

Source: Adapted from Ribeiro et al. (2025).

Table 4. Scrotal Thermal Stress in Fertile Men

Parameter	Pre-SHS	During SHS (3 months)	After 3 months without SHS
Motility (%)	71.0 ± 14.7	25.7 ± 20.3	55.9 ± 22.0
Normal morphology (%)	21.1 ± 6.0	3.8 ± 3.1	21.7 ± 6.6
DNA fragmentation (%)	17.7 ± 3.6	77.9 ± 23.9	18.9 ± 6.6

Source: Adapted from Zhang et al. (2015).

Table 5. Comparison Between Species (Stallions vs. Humans)

Aspect	Stallions (tropical climate)	Humans (experimental SHS)
Morphological defects	↑ in summer (22.4%)	↑ during SHS (normal morphology <4%)
Motility	↓ in summer (64%)	↓ during SHS (25%)
Sperm DNA	Oxidative damage	Fragmentation + apoptosis (Caspase-3)
Reversibility	Partial (improves in winter)	Total after 3 months without heat

Source: Adapted from Ribeiro et al. (2025) and Zhang et al. (2015).

Table 6. Proteomic Differences Between Fertile and Subfertile Stallions

Category	Alterations in subfertile stallions	Implications
Acrosomal enzymes	Significant reduction	Lower acrosome reaction
Structural proteins	Altered abundance	Compromised motility
Signaling proteins	Expressive differences	Altered capacitation

Source: Adapted from Hernández-Avilés et al. (2024)

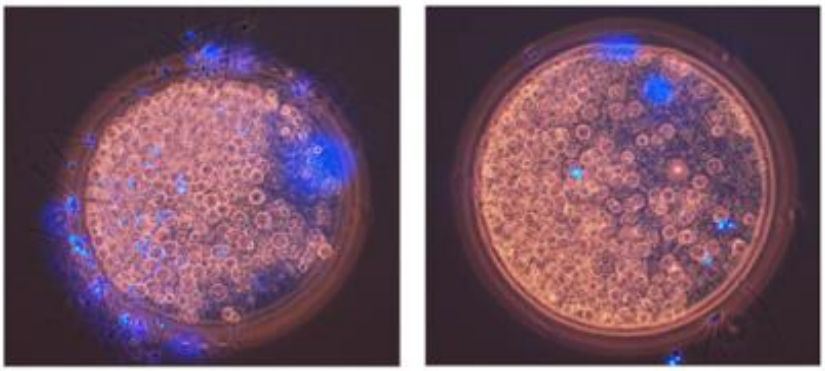

Figure 7. Differences in the binding of fertile and subfertile Thoroughbred stallion spermatozoa to the porcine zona pellucida, showing reduced interaction capacity in the subfertile group.
Source: Adapted from HERNÁNDEZ-AVILÉS, C. et al. (2024).

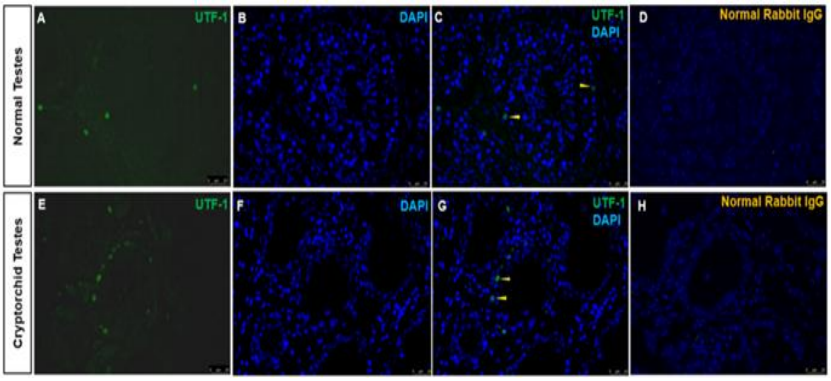

Figure 8. UTF-1 expression in germ cells of normal and cryptorchid stallion testes, demonstrating changes associated with heat stress.
Source: Adapted from ZHANG, Y. et al. (2025).

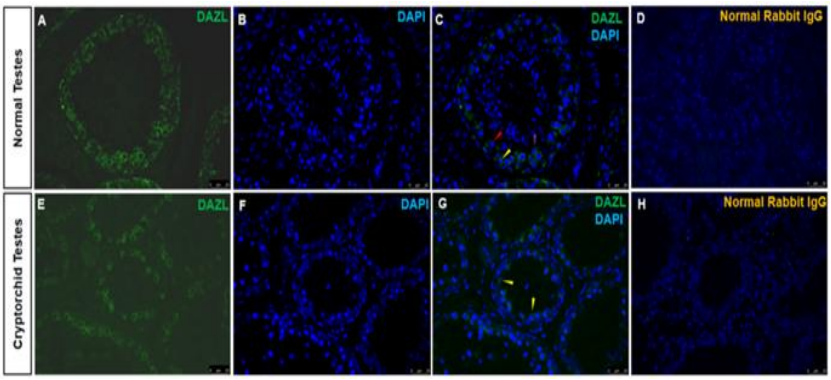

Figure 9. DAZL expression in germ cells of normal and cryptorchid stallion testes, showing reduced labeling under heat stress conditions.
Source: Adapted from ZHANG, Y. et al. *(*2025).

Conclusion

This integrative review demonstrates that teratozoospermia in stallions from tropical climates is a complex and multifactorial phenomenon, resulting from the interaction between racial predispositions, methodological variability, environmental stress, and molecular alterations. Morphological differences among breeds, as shown by Kavak et al. (2004) and Waheed et al. (2015), reinforce that genetic factors modulate semen quality. The methodological variability described by Murcia-Robayo et al. (2018) shows that standardization of staining techniques is essential for consistent and internationally comparable diagnoses.

Thermal stress, widely documented by Ribeiro et al. (2025) and Griffin et al. (2026), compromises seminal parameters and increases DNA fragmentation, confirming that the tropical environment exerts a direct influence on fertility. The comparison with humans (Zhang et al., 2015)

reinforces that the mechanisms of thermal damage — oxidative stress, chromatin fragmentation, and apoptosis — are universal, although reversibility is partial in equines. Finally, the proteomic findings of Hernández-Avilés et al. (2024) broaden the understanding of subfertility, showing that molecular alterations directly compromise sperm function.

In summary, teratozoospermia in tropical stallions should be understood as the result of the convergence of morphological, methodological, environmental, and molecular factors. The integration of these aspects is fundamental for the development of reproductive management strategies adapted to tropical conditions, including:

- ✓ Methodological standardization to reduce diagnostic variability.
- ✓ •Monitoring thermal comfort indices as a practical tool to predict fertility decline.
- ✓ Use of molecular and proteomic analyses to identify mechanisms underlying subfertility.

When applied jointly, these approaches can contribute to more accurate diagnoses and the implementation of management measures that minimize the impacts of the tropical environment on stallion fertility.

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