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Immune–endocrine interactions in bovine testicular homeostasis: A conceptual framework for reproductive resilience

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Abstract

Bull fertility depends on coordinated regulation of spermatogenesis, endocrine function, immune tolerance, and tissue stress responses. This narrative review synthesizes direct bovine evidence with comparative mammalian mechanisms to evaluate a conceptual framework of coordinated immune–endocrine regulation in testicular homeostasis. At baseline, luteinizing hormone-dependent Leydig-cell steroidogenesis, follicle-stimulating hormone- and androgen-responsive Sertoli-cell functions, resident immune cells, cytokines, redox pathways, and the blood–testis barrier collectively support germ-cell development while limiting damaging immunity. Direct studies in cattle show that this balance is stress sensitive. In Nelore bulls, 48 h of scrotal insulation increased intratesticular temperature from 35.28 to 38.62°C, reduced tissue testosterone from 3,119 to 295.5 ng/g, and markedly suppressed STAR while inducing GPX1 and HSP70. Bovine Sertoli-cell experiments identify TLR4–NF-κB signaling as a causal link between danger sensing, inflammation, mitochondrial injury, and barrier-protein loss. Acute bovine besnoitiosis further demonstrates how vascular injury, inflammatory infiltration, impaired thermoregulation, and germ-cell degeneration can converge in vivo. Recent single-cell and spatial transcriptomic studies now resolve macrophage, T-cell, Leydig-cell, Sertoli-cell, and germ-cell populations in bovine testes, although they do not yet establish causal immune–endocrine communication. Three operational states – homeostatic tolerance, compensated stress, and decompensated inflammation – are used to describe regions of a continuous response spectrum rather than validated, discrete physiological categories. Within this framework, bull subfertility may partly reflect a reduced capacity to maintain coordinated testicular homeostasis. Longitudinal assessment of immune, endocrine, metabolic, and spatial parameters is therefore a priority for further mechanistic validation.

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1. Introduction

Male reproductive efficiency in cattle is biologically and economically important because individual bull can influence the genetics of many offspring. Conventional bull evaluation focuses on physical soundness, scrotal size, sperm concentration, motility, and morphology; and at the same time, molecular fertility phenotypes are getting more attention nowadays. Although these traits are still considered important, they primarily serve as measures of reproductive output rather than describing the regulatory mechanisms that protect testicular function before the deterioration of semen quality (Chenoweth and McPherson 2016; Butler et al. 2020; Klein et al. 2022). In mammals, the testis function as an endocrine organ and an immune-privileged tissue. At the same time, it acts as an immune-competent barrier organ, providing a metabolically specialized niche for germ cells. After meiosis and during post-meiotic development, the germ cells express antigens that may not have been developed during the establishment of central immune tolerance. These antigenic cells, however, are normally able to live alongside a fully operational local immune system. Such a coexistence is possible by the blood-testis barrier (BTB), Sertoli cells, Leydig cells, resident macrophages, lymphocytes, locally produced cytokines, and systemic immune-tolerance mechanisms (Babcock et al.

2026; Wang et al. 2026; Zhao et al. 2014). These cells also contribute to endocrine and metabolic control in mammals. Testosterone influences immune phenotypes at the local to the organ level, signals of macrophage origin may regulate the development of Leydig cells and steroidogenesis, and Sertoli cells respond to follicle stimulating hormone (FSH) and testosterone stimuli while producing a wide range of immunoregulatory factors and metabolites required by the germ cells (Alves et al. 2013; Wang et al. 2017; Martinez and Motrich 2025). Therefore, considering reproductive endocrinology and immunology of the testicle as independent systems is scientifically fallible.

The bovine context is particularly informative. Bull testes operate under high sperm-production demands and depend on efficient scrotal thermoregulation. Reproductive performance is also affected by infectious, nutritional, and environmental perturbations. Spermatogenesis in the bull has a highly ordered cellular architecture, and heat-related alterations can manifest in semen weeks after the original insult (Staub and Johnson 2018; Kastelic et al. 2018). Breed and environment further modify thermoregulatory and semen phenotypes (Nichi et al. 2006; Alves et al. 2025). This review proposes a conceptual and testable framework for bidirectional immune–endocrine regulation of bovine testicular homeostasis; the term "rheostat" is used heuristically to denote graded coordination rather than an established bovine physiological mechanism. In this model, physiological perturbations are initially buffered by endocrine, antioxidant, barrier and immunoregulatory responses; sufficiently intense or persistent perturbations shift the tissue toward an inflammatory state in which cytokine signaling, oxidative stress, mitochondrial dysfunction, steroidogenic suppression, and barrier failure reinforce one another. The objective is to distinguish what has been demonstrated directly in cattle from what remains comparative inference, and to identify measurable transitions that can make the conceptual framework experimentally falsifiable.

2. Materials and Methods

2.1. Narrative evidence identification

This article was prepared as a critical narrative review rather than a systematic review. Evidence was drawn from the peer-reviewed literature behind the recent reviews of testicular immune privilege, reproductive immunology, and immunometabolism. It was supplemented by targeted searches for primary bovine studies and recent omics studies in Scopus, PubMed, Elsevier, and Web of Science databases, up to 8 September 2026. Priority was given to studies that provided mechanistic, quantitative, cellular or histopathological evidence relevant to testicular immune–endocrine regulation. Search words were organized around combinations of bovine/cattle/bull, testis/testicular, immune/inflammation/macrophage/cytokine, endocrine/steroidogenesis/testosterone, blood–testis barrier/Sertoli cell, hyperthermia/heat stress, and single-cell/spatial transcriptomics, depending on the mechanistic topic being evaluated.

2.2. Evidence hierarchy and quantitative extraction

Evidence was interpreted at three levels. Level A comprised direct bovine *in vivo* evidence, including experimental hyperthermia, infectious pathology, and endocrine phenotyping. Level B comprised *in vitro* and single-cell or spatial-omics studies that directly characterized bovine testicular cells but did not necessarily establish *in vivo* causality. Level C comprised comparative mammalian mechanisms used only where the homologous causal relationship has not been tested in cattle. Statements derived from Level C are described as comparative evidence or hypotheses rather than bovine proof. This hierarchy was used to reduce species over-extrapolation and is summarized for the principal mechanistic nodes in Table 1.

Quantitative values were extracted only when they were directly reported by the cited primary study. No pooled effect size was calculated because the included evidence was heterogeneous in species, experimental design, and endpoint. The review therefore emphasizes convergence of mechanism rather than meta-analytic magnitude. No new dataset was generated. Because this was a critical narrative review rather than a systematic review, no PRISMA-style study-selection flow or formal risk-of-bias score was applied. Instead, evidence was appraised according to species relevance, experimental setting, causal versus descriptive design, and relevance to the mechanistic question.

3. Results: Mechanistic synthesis

3.1. Immune privilege and endocrine operating range

The standard interpretation of testicular immune privilege revolved around the concept of BTB. Sertoli cell junctional complexes partition the seminiferous epithelium into the basal and the adluminal compartments and limit the penetration of antibodies and leukocytes circulating in blood towards the advanced phase of germ cells inside the adluminal compartment. On the other hand, immune privilege is not the same as immunological silence. In the testicular interstitium, an infection or injury of tissues may lead to very strong local inflammation due the presence of immune cells. Physical separation must complement systemic tolerance, regulatory cells in the immune system, and locally produced immunoregulatory mediators such as IL-10 and TGF-β (Hedger and Meinhardt 2003; Wong and Cheng 2005; Cheng and Mruk 2012). This broader interpretation is supported by comparative studies of testicular autoimmunity and antigen tolerance. Disruption of tolerance can expose germ-cell antigens and recruit macrophage and T-cell responses, whereas regulatory pathways normally restrain these reactions (Jacobo et al. 2011; Harakal et al. 2022; Qu et al. 2020). The evidence base for immune privilege is extensive in rodents and humans, but fewer studies directly quantify these mechanisms in bulls.

The endocrine regulation is regulated by the hypothalamic-pituitary-testicular axis, which sets the operational levels of the testicular endocrine function. GnRH triggers the release of pituitary LH and FSH; LH stimulates the production of steroids by Leydig cells. In addition, FSH and androgens produced in the testes influence Sertoli cells to promote germ-cell development, provide energy to developing germ cells, and form tight junctions between Sertoli cells (Ramaswamy and Weinbauer 2014; Smith and Walker 2014; Zirkin and Papadopoulos 2018). Endocrine and nutritional conditions in a bull prior to puberty can determine the timing of puberty and the later sperm production (Kastelic 2014; Dance et al. 2015). The key point for the present synthesis is reciprocity. Androgen signaling can restrain inflammatory activity and contribute to immune privilege, whereas inflammatory mediators can directly inhibit Leydig-cell steroidogenic machinery (Fijak et al. 2011; Fijak et al. 2015; Walecki et al. 2015). Table 1 therefore treats endocrine, immune, metabolic, and barrier nodes as components of one regulatory system rather than independent pathways. The evidence hierarchy given in Table 1 compares the results in bovine studies with mechanistic studies from other mammals.

3.2. Leydig cells and testicular macrophages

Table 1. Evidence hierarchy for principal immune–endocrine nodes in bovine testicular homeostasis

Mechanistic node	Direct bovine evidence	Comparative mechanistic evidence	Homeostatic interpretation	Evidence status / key gap
Leydig-cell steroidogenesis	With acute hyperthermia in bulls, intratesticular testosterone and STAR are reduced; different approaches to insulation result in alteration of testosterone pattern (Rizzoto et al. 2020; Ferrer et al. 2022)	Signaling molecules released by cells of the immune system like cytokines, as well as the oxidative stress produced by macrophages, can lead to a decrease in StAR, cholesterol transport, and steroidogenic enzymes in the male gonad (Diemer et al. 2003; Allen et al. 2005)	Maintains high local androgen activity required for spermatogenesis and may contribute to a tolerogenic interstitial environment	Strong bovine endocrine evidence; causal bovine immune→Leydig signaling remains untested
Testicular macrophages	Macrophage populations have been identified in bovine testes using scRNA-seq analysis and spatial transcriptomics methods (Wang et al. 2024; Jin et al. 2026)	Resident macrophages are involved in Leydig cell development and steroidogenesis in the testis while at the same time displaying phenotypes associated with reduced inflammatory response in rodents (Yee and Hutson 1985; Wang et al. 2017)	Potential trophic and immunoregulatory component of the steroidogenic niche	The presence of these cells has been established in cattle; however, functional macrophage–Leydig crosstalk is comparative inference
Sertoli cells / BTB	Bovine Sertoli cells link TLR4–NF-κB activation to cytokine induction, mitochondrial injury and BTB-protein loss; selenium modifies regulatory/barrier genes (Adegoke et al. 2018a, 2018b; 2019)	In addition to immune privilege and barrier function, androgen signaling and several factors released by Sertoli cells have been implicated (Meng et al. 2011; Kaur et al. 2014)	Couples endocrine input, germ-cell metabolic support, innate sensing and immune compartmentalization	Strong bovine in vitro causal evidence; in vivo threshold and recovery kinetics need definition
Cytokine network	It is documented how Sertoli cells of the bovine testis respond by producing cytokines upon activation of toll-like receptor 4	IL-1β, TNF-α and IL-6 are capable of influencing both steroid hormone production and the provision of support to the process of sperm development. While IL-10 and TGF-β regulate and limit the effects of inflammation (Harakal et al. 2022; Gong et al. 2020)	Permits physiological paracrine signaling while limiting excessive inflammatory exposure	Bovine cell evidence exists; bovine macrophage/Leydig cytokine maps are incomplete
Lymphocyte tolerance	T cells appear as a population in bovine single-cell and spatial transcriptomic data	Tregs and antigen-specific tolerance play major roles in the control of autoimmune responses to germ-cell antigens (Harakal et al. 2022; Gong et al. 2020)	Limits adaptive immune attack on germ-cell antigens	Functional Treg studies in bulls are scarce
Immuno-metabolism	We have not found any direct study on the metabolic-flux of the testis of bovine animals	Immune activation states are coupled to glycolysis, mitochondrial metabolism and succinate/ROS signaling (Mills et al. 2016; Buck et al. 2017)	May set the energetic threshold for regulatory versus inflammatory immune behavior	Hypothesis in cattle; requires isotope tracing and extracellular-flux validation
Thermoregulation / vasculature	Exposure of bulls to hyperthermia results in immediate downregulation of steroidogenesis; besnoitiosis combines vascular damage with impaired cooling and degeneration (Rizzoto et al. 2020; González-Barrio et al. 2020)	Heat can induce oxidative stress, germ-cell death and stress-protein responses in mammalian testes	Maintains the temperature range required for endocrine and germ-cell function	Strong bovine evidence for thermal/endocrine coupling; local immune mediation remains unresolved
Cellular niche organization	Bovine scRNA-seq resolves germ and somatic populations; spatial transcriptomics adds anatomical context (Wang et al. 2024; Jin et al. 2026)	Human/mouse atlases demonstrate cell-state and niche reconstruction (Guo et al. 2018; Green et al. 2018)	Provides the anatomical substrate for local endocrine–immune communication	Descriptive evidence is strong; ligand–receptor predictions require functional validation

The Leydig-cell–macrophage interface represents one of the clearest examples of reciprocal immune–endocrine regulation within the testis. Testicular macrophages are located in the interstitial compartment and are often found in close proximity to Leydig cells. Evidence from rodents and other comparative models indicates that these macrophages can contribute to Leydig-cell development and steroidogenic function. Whereas, local steroids and lipid-derived mediators may in turn favor a relatively restrained macrophage phenotype (Yee and Hutson 1985; Nes et al. 2000; Wang et al. 2017). Functionally distinct interstitial and peritubular macrophage populations have also been identified, with different associations with steroidogenic and spermatogonial niches (Mossadegh-Keller et al. 2017; Wang et al. 2021; Matsuyama et al. 2026). However, whether these reciprocal interactions occur in the same manner in the adult bovine testis remains to be established. A relatively controlled inflammatory phenotype has been described for resident testicular macrophages under homeostatic conditions, particularly in rodent models. Constitutive production of IL-10 has been reported in resident populations, while excessive NF-κB activation appears to be constrained by the local testicular environment (Winnall et al. 2011; Bhushan et al. 2015; Meinhardt et al. 2018). Macrophage activation is now considered to occur along a context-dependent spectrum rather than within fixed M1 and M2 categories, with functional states being shaped by tissue-derived signals and experimental conditions (Murray et al. 2014).

Macrophages can supply steroidogenic support under conditions that are not related to inflammation. Testosterone synthesis has increased in cells exposed to macrophage-conditioned medium, and macrophage-derived 25-hydroxycholesterol has been identified as a potential substrate for testicular steroidogenesis (Yee and Hutson 1985; Nes et al. 2000). The involvement of tissue-resident macrophages in testicular developmental and homeostasis has also been reported in experimental models (DeFalco et al. 2015; Gu et al. 2023). Nevertheless, there is a lack of direct evidence for such mechanisms in adult bovine testis. A different pattern may emerge when inflammatory signaling becomes dominant. Exposure to IL-1β, TNF-α, reactive oxygen species (ROS), and related mediators has been associated with disruptions of cAMP signaling, StAR expression, mitochondrial cholesterol transport, and steroidogenic enzyme activity (Diemer et al. 2003; Allen et al. 2005). Macrophage activation may also contribute to orchitic injury through enhanced antigen presentation and cell-death signaling in experimental systems (Ma et al. 2024). A shift from supportive to inhibitory macrophage activity may therefore occur as the local inflammatory environment intensifies, although the extent of this transition in adult bulls still requires direct experimental validation. Thus, the proposed supportive-to-inhibitory shift remains a mechanistic hypothesis for adult bovine testes rather than an established causal process. Direct bovine evidence currently stops short of proving this causal switch. Recent cattle cell atlases identify macrophage and Leydig populations in the same tissue, but coexistence and transcriptomic proximity do not demonstrate functional communication. The testable prediction is that stress-induced changes in the state of bovine testicular macrophages shall be temporarily associated with reduced intratesticular steroidogenesis before extensive tubular degeneration becomes apparent. Accordingly, current bovine data support cellular coexistence and biological plausibility, but not yet direct causal macrophage–Leydig communication in the adult testis.

3.3. Sertoli cells and the blood–testis barrier

Sertoli cells form a second major immune–endocrine interface because they simultaneously maintain the BTB, support germ-cell metabolism, respond to FSH and androgen, and participate in local immune regulation. They are therefore better viewed as active integrators than as structural nurse cells (França et al. 2016; Kaur et al. 2014). During the passage of germ-cells, the blood-testis barrier (BTB) must undergo dynamic remodeling to maintain immunological compartmentalization. This process involves tight junctions, adherens junctions, gap junctions, along with related cytoskeletal networks being dynamically reorganized within the seminiferous epithelium (Stanton 2016; Cheng and Mruk 2012). Sertoli cells in mammals contribute to immunoregulatory mechanisms via mediators and pathways, including TGF- β, galectin-1, indoleamine 2,3-dioxygenase, and PD-L1 (Gualdoni et al. 2019; Kaur et al. 2014). Furthermore, the maintenance of this regulatory environment is also linked to androgen-receptor signaling. Sertoli-cell-specific androgen receptor deletion in laboratory mice revealed a decline in testicular immune privilege (Meng et al. 2011). Direct bovine cell studies are especially informative. Selenium supplementation of primary calf Sertoli cells at 0.25–0.50 mg/L increased expression of anti-inflammatory mediators and BTB-associated proteins, whereas higher concentrations were less favorable, demonstrating a dose-dependent connection among micronutrient status, redox biology and barrier phenotype (Adegoke et al. 2018b).

Conversely, microcystin-LR exposure at 40–80 μg/L reduced bovine Sertoli-cell viability, activated TLR4–NF-κB, increased pro-inflammatory cytokine expression and reduced BTB proteins during the first 48 h. A later increase in anti-inflammatory mediators and barrier proteins indicated partial compensatory regulation. Pharmacological TLR4 inhibition attenuated inflammatory signaling, mitochondrial injury, mitophagy and barrier-protein loss (Adegoke et al. 2018a; Adegoke et al. 2019). These experiments provide Level B causal evidence that danger sensing and barrier integrity are mechanistically linked in bovine Sertoli cells. Comparative toxicology supports the general plausibility of an inflammation–testosterone–BTB axis, but findings from mouse and other non-bovine models are used here to support mechanistic plausibility rather than to establish equivalent causal relationships in adult cattle. Deoxynivalenol disrupted BTB proteins, reduced testosterone synthesis and induced testicular inflammation in mice, while restoration of barrier signaling has improved spermatogenic phenotypes in diabetic models (Cao et al. 2020; Song et al. 2022).

3.4. Cytokines and immunometabolism

Cytokines are not exclusively markers of pathology. Within the testis they can participate in physiological paracrine regulation, whereas sustained or excessive signaling can suppress steroidogenesis, disturb Sertoli-cell function and promote germ-cell loss (Hedger and Meinhardt 2003; Wanjari and Gopalakrishnan 2023; Fomichova et al. 2025). This concentration- and context-dependence is central to a rheostat model. Under experimental inflammatory conditions, the production of pro-inflammatory cytokines, including IL-1β and TNF-α, has been linked to suppression of Leydig-cell steroidogenesis. Even in the presence of gonadotropin stimulation, decrease in steroidogenic enzyme activity, StAR-dependent cholesterol transport impairment, and reduced testosterone production were observed (Diemer et al. 2003; Allen et al. 2005). IL-6 and chemokine signaling may still affect immune-cell attraction and somatic-cell response. On the other hand, regulatory

immune suppression within the testicular environment is usually a result of IL-10 and TGF-β (Peng et al. 2022; Gong et al. 2020; Chen et al. 2016). Studies of bovine Sertoli cells have demonstrated that the expression of IL-1β, IL-6, and TNF-α respond to TLR4-NF-κB activation. Similar cytokine patterns in bovine testicular macrophages and Leydig cells could be investigated, as they remain inadequately characterized at the moment.

Immunometabolism provides a plausible additional layer, but the bovine evidence is currently indirect. In general immunology, inflammatory macrophage activation can involve increased glycolysis, succinate accumulation and mitochondrial ROS, whereas regulatory states may depend more heavily on oxidative metabolism (Jha et al. 2015; Mills et al. 2016; Buck et al. 2017). Contemporary frameworks emphasize continuous, stimulus-dependent macrophage states rather than a binary metabolic classification (Murray et al. 2014; O'Neill et al. 2016). The metabolic organization of the testis supports this hypothesis because Sertoli cells convert glucose to lactate and endocrine signals regulate transporters and metabolic enzymes required for germ-cell support (Boussouar and Benahmed 2004; Rato et al. 2012; Alves et al. 2013). Leydig and Sertoli cells also generate lipid mediators that can affect immune behavior (Frungieri et al. 2015). Regulatory T cells and other immune populations likewise have distinct metabolic requirements (Newton et al. 2016; Pearce and O'Neill 2016; Ye et al. 2021). For cattle, however, a metabolic switch in testicular macrophages remains a hypothesis, not an established mechanism. No study identified for this review has simultaneously demonstrated metabolic reprogramming of adult bovine testicular macrophages together with local cytokine changes and altered steroidogenic output. Stable-isotope tracing, extracellular-flux analysis and spatial metabolomics should therefore be used to test whether metabolic reprogramming precedes steroidogenic decline. Thus, the proposed immunometabolic transition should be regarded as a testable hypothesis extrapolated from broader immunological and experimental evidence, rather than as a demonstrated mechanism in cattle.

3.5. Single-cell and spatial omics

Bulk testicular transcriptomic analysis reflects the combined molecular signals of germ cells, Sertoli cells, Leydig cells, vascular populations, and leukocytes, which may limit the resolution of cell-specific responses. The cellular resolution has been improved further through single-cell sequencing that enables the identification of these populations in bovine tissue. In a study, a total of 26,888 cells from five adult Holstein bull testes were sorted into five germ-cell populations and eight somatic-cell populations, including Sertoli cells, Leydig cells, macrophages, and T cells (Wang et al. 2024). Cell-type annotations of the dataset were supported by unique profiles of cell markers. Macrophages were characterized by expression of C1QA, C1QB, C1QC, CD14, CD163, CD74, CSF1R, and LYZ markers. Sertoli and Leydig cell populations also had distinct cell signatures. The role of macrophages was highlighted by a functional enrichment analysis that pointed out the immune-response processes predominantly associated with those genes. On the other hand, the Sertoli-cell gene functions were mainly associated with mitochondrial functions (Wang et al. 2024). Single-cell transcriptomic approach in the human and mouse testes have revealed cell-specific transcriptomes at different stages of spermatogenesis and also helped identify different cell types (Guo et al. 2018; Green et al. 2018; Shami et al. 2020). These cross-species datasets provide a basis for identifying conserved cellular programs, but they do not by themselves establish equivalent functional mechanisms in adult bovine testes.

Additional bovine datasets have provided complementary information on testicular cellular organization. Developmental single-cell analysis of Angus testes resolved immune and somatic populations across the pubertal transition, while spatial transcriptomic analysis localized macrophages, T cells, Sertoli cells, Leydig cells, and different germ-cell stages within their native tissue environment (Gao et al. 2024; Jin et al. 2026). These findings indicate that the relevant cell populations are positioned within the bovine testis in a manner compatible with local intercellular communication, although their spatial proximity alone does not establish functional crosstalk. Further progress will therefore depend on validating candidate ligand–receptor interactions rather than relying on descriptive cell mapping alone. Computational approaches such as CellChat, NicheNet, and related frameworks may be useful for generating hypotheses about intercellular communication, but causal relationships will require experimental confirmation. Co-culture systems, organotypic testis models, spatial proteomics, single-cell chromatin profiling, and direct steroidogenic or barrier-function measurements could be integrated to determine whether predicted changes in cell–cell signaling are associated with measurable endocrine or structural outcomes. In this context, the omics datasets are best used to prioritize testable mechanisms, while direct functional experiments remain necessary to establish causality in adult cattle.

3.6. Heat stress as an immune–endocrine perturbation

Heat stress provides a more useful bovine model to test the proposed immune–endocrine rheostat. Bull testes depend on counter-current heat exchange within the pampiniform plexus and scrotal heat loss to remain cooler than body core temperature. Breed, season, and environment influence thermoregulation and semen phenotypes (Hansen 2004; Nichi et al. 2006; Klein et al. 2022). Scrotal insulation has therefore been widely used to model testicular hyperthermia in bulls (Fernandes et al. 2008; Kastelic et al. 2018). In a study on 20 Nelore bulls, allocated to control, 12-, 24-, and 48-h scrotal insulation groups, a clear endocrine response to thermal stress was observed (Rizzoto et al. 2020). Mean intratesticular temperature increased progressively from 35.28 ± 0.31 °C in the control group to 38.62 ± 0.57 °C after 48 h of insulation (Fig. 1D). Fig. 1D shows the progressive thermal exposure that preceded marked suppression of local steroidogenic function. Testicular testosterone was 3,119 ± 973.3 ng/g in controls and 295.5 ± 122.8 ng/g at 48 h, a reduction of more than 90%. STAR expression decreased from approximately 1.1 to 0.3 relative units. The same experiment documented simultaneous protective and injury-associated responses. GPX1 increased by approximately 70% at 48 h, whereas HSP70 increased approximately 34-fold at 12 h and remained above control values later; BCL2 decreased at 24 h and then rebounded, whereas HSF1, BAX, P53 and CASP8 did not change significantly (Rizzoto et al. 2020). These quantitative responses are summarized in Table 2. Table 2 also places this endocrine suppression beside bovine Sertoli-cell, infectious and omics evidence to distinguish direct from inferred mechanisms.

Prolonged scrotal insulation has also been associated with endocrine alterations that persist beyond the period of thermal exposure. Ferrer et al. (2022) reported changes in testosterone and anti-Mullerian hormone profiles following eight days of scrotal insulation, with reduced testosterone concentrations still evident during subsequent follow-up, suggesting that Leydig-cell function may remain impaired after the initial heat insult. This delayed endocrine response

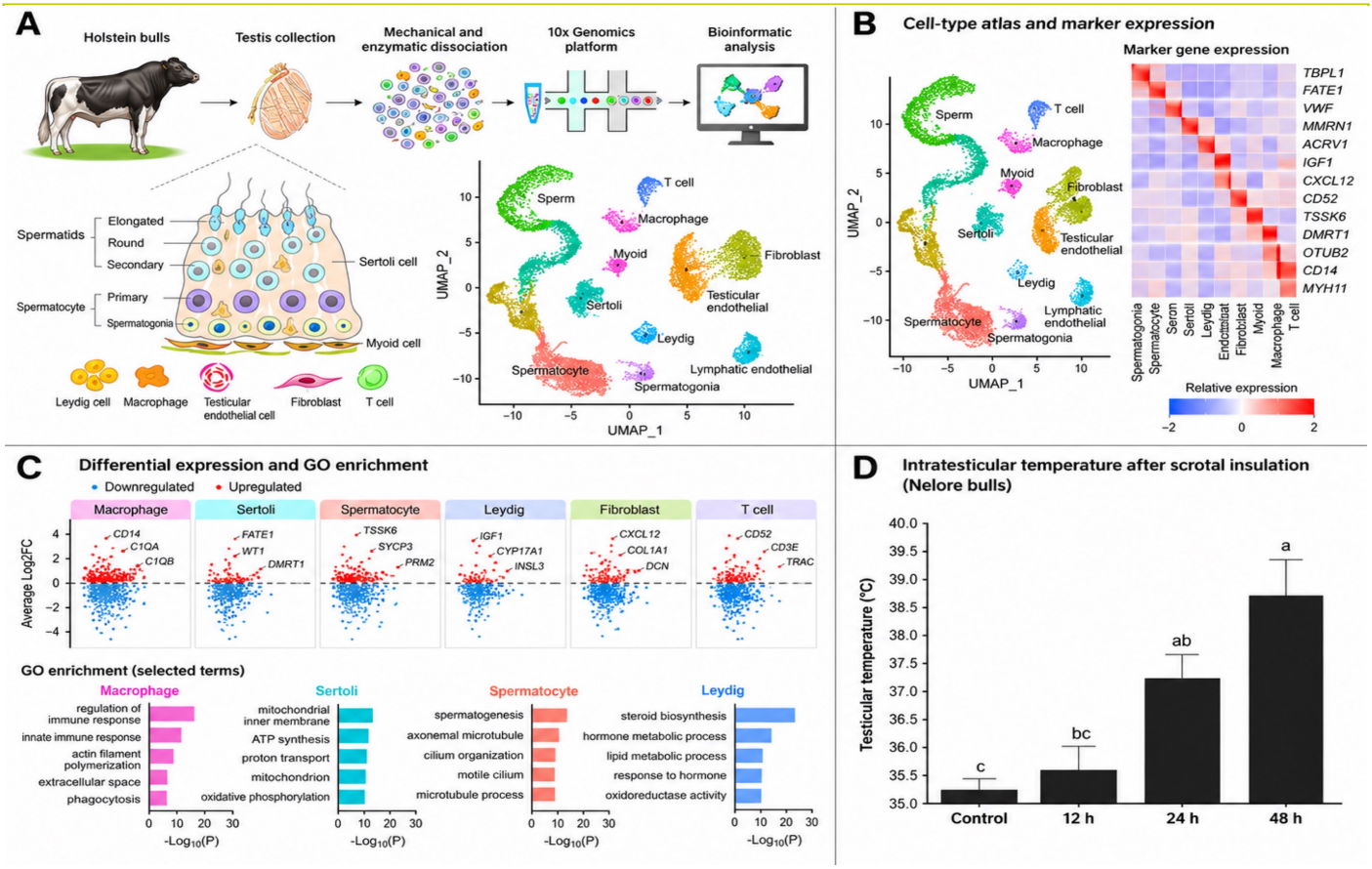


Fig. 1. *Integrated cellular architecture and thermal-stress response of the bovine testis.*
(A) Single-cell RNA-sequencing workflow and cellular landscape of adult Holstein bull testes. 26,888 high-quality cells obtained from five bulls and resolved five germ-cell populations and eight somatic-cell populations, including Sertoli cells, Leydig cells, macrophages, T cells, myoid cells, fibroblasts, and endothelial populations. (B) Transcriptomic annotation of major testicular cell populations based on representative cell-type markers, illustrating the molecular distinction among germ, endocrine, structural, and immune compartments. (C) Cell-type-specific transcriptional and functional enrichment patterns. Macrophage-associated genes were enriched in immune-response-related functions, whereas Sertoli-cell genes showed prominent enrichment in mitochondrial and energy-related processes, highlighting functional specialization among testicular somatic cells. (D) Intratesticular temperature in Nelore bulls subjected to scrotal insulation for 12–48 h. Temperature increased progressively with exposure duration, reaching 38.62 ± 0.57°C after 48 h compared with 35.28 ± 0.31°C in controls. Different superscript letters indicate significant differences ($P < 0.05$). Panels A–C are based on Wang et al. (2024) and panel D is based on Rizzoto et al. (2020). The datasets are independent and are combined here for conceptual illustration

indicates that the reproductive consequences of testicular heat stress may not be fully captured by a single measurement of semen quality or circulating hormone concentration. Heat-shock and oxidative pathways are highly conserved but should be interpreted with species context. Experimental hyperthermia in mice links germ-cell susceptibility to HSP responses and oxidative stress (Rockett et al. 2001; Paul et al. 2009). In cattle, heat-response genes and breed-associated thermotolerance have been described at the systemic level (Collier et al. 2008; Deb et al. 2014; Wang et al. 2026), and seasonal HSP expression differs under tropical conditions (Kumar et al. 2015). These studies support stress-response biology but do not directly establish immune-cell remodeling inside the bull testis. Heat stress in cattle also modifies systemic immune function, and combined thermal and inflammatory stress can impair bovine macrophage function (Bagath et al. 2019). Direct demonstration that local testicular macrophage reprogramming mediates the fall in steroidogenesis after heat exposure is still missing. This gap is experimentally important because sperm and semen molecular phenotypes can remain altered after thermal exposure (Celeghini et al. 2024), while environmental heat and reproductive development interact with broader endocrine and management factors (Dance et al. 2015).

As summarized in Fig. 1, recent single-cell and physiological studies in cattle provide complementary evidence that testicular homeostasis depends on coordinated interactions among germ cells, endocrine cells, immune populations, and somatic support cells. Single-cell RNA sequencing of 26,888 cells from five adult Holstein bulls resolved five germ-cell and eight somatic-cell populations, including Leydig cells, Sertoli cells, macrophages, T cells, myoid cells, fibroblasts, and endothelial populations, thereby establishing the cellular framework required for local immune–endocrine communication. Macrophages were characterized by immune-response-related transcriptional signatures, whereas Sertoli cells showed prominent enrichment in mitochondrial and energy-associated functions, consistent with their central role in metabolic support and blood–testis-barrier maintenance; Leydig cells formed a distinct endocrine population within the same tissue environment. This multicellular organization is particularly relevant under thermal stress. Fig. 1 combines independent cellular and thermal-stress datasets for conceptual illustration; it does not imply that these measurements were obtained from the same animals. The cellular and thermal-stress evidence presented in Fig. 1 is consistent with the view that bovine testicular homeostasis is maintained through coordinated endocrine,

Table 2. Key bovine studies informing the proposed immune–endocrine framework of testicular homeostasis

Perturbation / model	Bovine system	Key observation	Quantitative finding	Rheostat interpretation	Reference
Acute testicular hyperthermia	20 Nelore bulls; 0, 12, 24, 48 h insulation	Local steroidogenesis falls while stress-defense genes are induced	Temperature 35.28→38.62°C; testosterone 3,119→295.5 ng/g at 48 h; STAR ~1.1→0.3; GPX1 +~70%; HSP70 ~34-fold at 12 h	Concurrent compensation and injury; quantitative anchor for severe local steroidogenic suppression	(Rizzoto et al. 2020)
Eight-day scrotal insulation	10 bulls	Leydig- and Sertoli-associated endocrine markers remain altered after the heat insult	Testosterone decreased during later follow-up (days 33–96); AMH showed a prolonged biphasic response	Demonstrates endocrine memory beyond the exposure period	(Ferrer et al. 2022)
Microcystin-LR exposure	Primary bovine Sertoli cells	TLR4–NF-κB activation, cytokine induction and BTB-protein loss	40–80 μg/L produced early injury; later anti-inflammatory/barrier responses partially recovered	Direct bridge from danger sensing to inflammatory and barrier dysfunction	(Adegoke et al. 2018a)
TLR4 inhibition during toxicant exposure	Primary bovine Sertoli cells	Blocking TLR4 reduces inflammatory and mitochondrial/barrier injury	TLR4-IN-C34 attenuated cytokine induction, mitophagy and BTB-protein loss	Supports causal, not merely correlative, innate signaling	(Adegoke et al. 2019)
Selenium supplementation	Primary calf Sertoli cells	Anti-inflammatory and BTB-associated expression changes	0.25–0.50 mg/L increased IL-4, IL-10, TGF-β1 and barrier-associated proteins; higher levels were less favorable	Shows a dose-dependent redox/nutritional influence on barrier resilience	(Adegoke et al. 2018b)
Acute bovine besnoitiosis	7 naturally infected breeding bulls	Vasculitis, inflammatory infiltrates and severe germline degeneration coexist	Severe vascular lesions in all sampled bulls; germinal epithelium was lost to Sertoli-cell-only tubules in four bulls	In vivo example of coupled vascular, immune, thermal and tubular decompensation	(González-Barrio et al. 2020)
Experimental BVDV infection	Postpubertal bulls	Testicular/reproductive-tract viral persistence despite systemic immune response	Viral RNA remained detectable in semen for up to 7 months	Illustrates the pathogen-sanctuary cost of immune privilege	(Givens et al. 2003)
Adult testis scRNA-seq	5 Holstein bulls	Immune, endocrine and germ-cell populations resolved in the same tissue	26,888 cells; 5 germ-cell and 8 somatic-cell types, including macrophages, T cells, Leydig and Sertoli cells	Defines cellular substrate for crosstalk, not causal communication	(Wang et al. 2024)
Developmental scRNA-seq	Prepubertal and pubertal Angus testes	Developmental remodeling of germ and somatic compartments	11,083 cells profiled; macrophage, T-cell, Leydig and Sertoli populations resolved	Shows developmental plasticity of the bovine niche	(Gao et al. 2024)
Spatial transcriptomics	Calf and adult bovine testes	Cell populations localized within intact tissue architecture	Spatial maps resolved macrophages, T cells, Leydig, Sertoli, myoid and germ-cell populations	Enables spatial testing of local immune–endocrine interactions	(Jin et al. 2026)

Note: The table includes in vivo experimental/pathological, in vitro mechanistic, and descriptive omics evidence; these study types are complementary and are not treated as equivalent levels of causality

metabolic, immune, and spermatogenic processes. A testable interpretation is that environmental stress may alter the testicular microenvironment from a homeostatic state toward a compensated stress response, with more persistent or severe disturbances potentially contributing to inflammatory and endocrine dysfunction when adaptive capacity becomes insufficient.

3.7. Infection and failure of immune privilege

Infection exposes the central trade-off of testicular immune privilege: excessive inflammation can destroy reproductive tissue, whereas overly effective immune exclusion may permit pathogen persistence. Acute bovine besnoitiosis provides direct *in vivo* evidence of the destructive outcomes of severe testicular inflammation. González-Barrio et al.

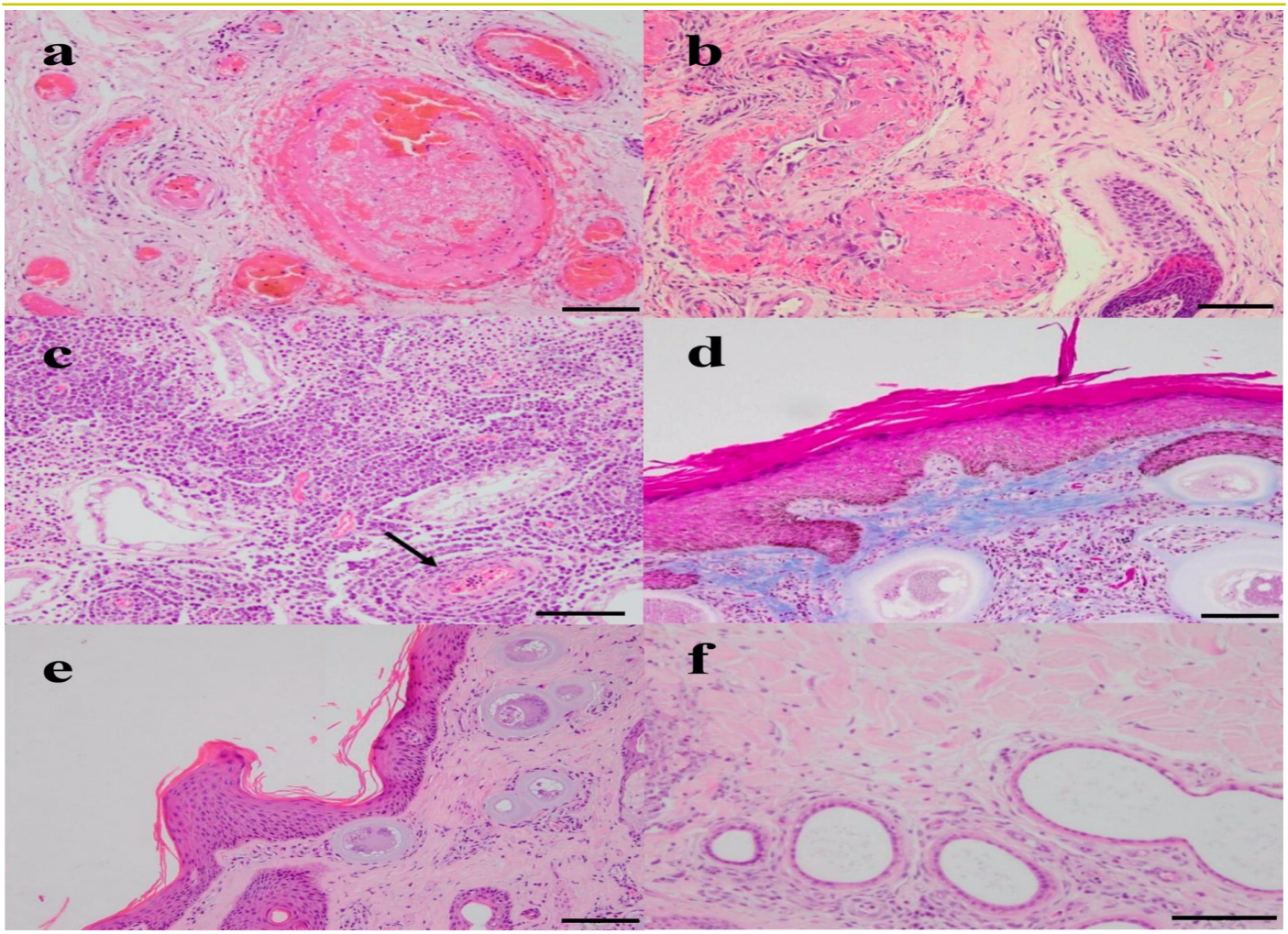


Fig. 2. *Vascular lesions and inflammation in scrotal skin, testicular parenchyma and pampiniform plexus during acute bovine besnoitiosis. The panels show thrombosis, edema, necrotizing vasculitis, lymphoplasmacytic inflammation, neovascularization and scrotal lesions (González-Barrio et al. 2020)*

(2020) examined seven naturally infected breeding bulls and found severe congestion, thrombosis, necrotizing vasculitis and angiogenesis in reproductive tissues, with intense inflammatory infiltrates and germline atrophy. Representative vascular and inflammatory lesions are shown in Fig. 2. In four bulls, the germinal epithelium was reported to have degenerated to the extent that only Sertoli cells remained in the affected tubules. Fig. 2 illustrates the accompanying vascular and inflammatory pathology and demonstrates that vascular, immune, and tubular injury coexist in the same disease state. Earlier and chronic besnoitiosis studies likewise document reproductive-tract involvement and compromised sexual function (Dubey et al. 2013; Esteban-Gil et al. 2016).

The pathogenic sequence proposed from the acute cases linked vascular injury to impaired testicular cooling, inflammatory infiltration to BTB damage, and these changes to testicular degeneration (González-Barrio et al. 2020). That sequence is reproduced in Fig. 3, which provides the clearest published bovine example of a systems-level transition in which thermoregulation, immune injury and barrier failure can reinforce one another. Bovine viral diarrhea virus (BVDV) illustrates the negative outcome potentially linked to testicular immune privilege. Studies of post-pubertal bulls showed that BVD viral RNA persisted for long in semen and testicular tissue after virus has been cleared from the body. Thus, these observations suggested that under certain situations the male reproductive tract may harbor persistent viral infections (Givens et al. 2003). Reduction in immune accessibility at immune-privileged tissues may enable the persistence of pathogens in such sites while protecting sperm-producing cells against any damage mediated by the immune system. Inflammatory conditions affecting the male reproductive tract should be interpreted according to their anatomical location. Orchitis, epididymitis, and accessory-gland disease involve distinct tissue compartments and may be associated with similar abnormalities in semen quality despite differences in the underlying pathology. Application of the proposed rheostat framework is therefore more appropriate when inflammatory changes are specifically identified within the testis and are accompanied by measurable disturbances in endocrine function, blood–testis barrier integrity, or other testicular parameters.

3.8. Toxicant and nutritional modifiers

Environmental toxicants may provide a second route for activation of innate immune-sensing pathways. In bovine Sertoli cells, microcystin-LR activates TLR4–NF-κB in parallel with mitochondrial injury and loss of barrier proteins (Adegoke et al. 2018a; Adegoke et al. 2019). Comparative studies show that toxicant-induced BTB disruption can coexist with inflammation and reduced testosterone, as demonstrated for deoxynivalenol in mice (Cao et al. 2020). These findings support a

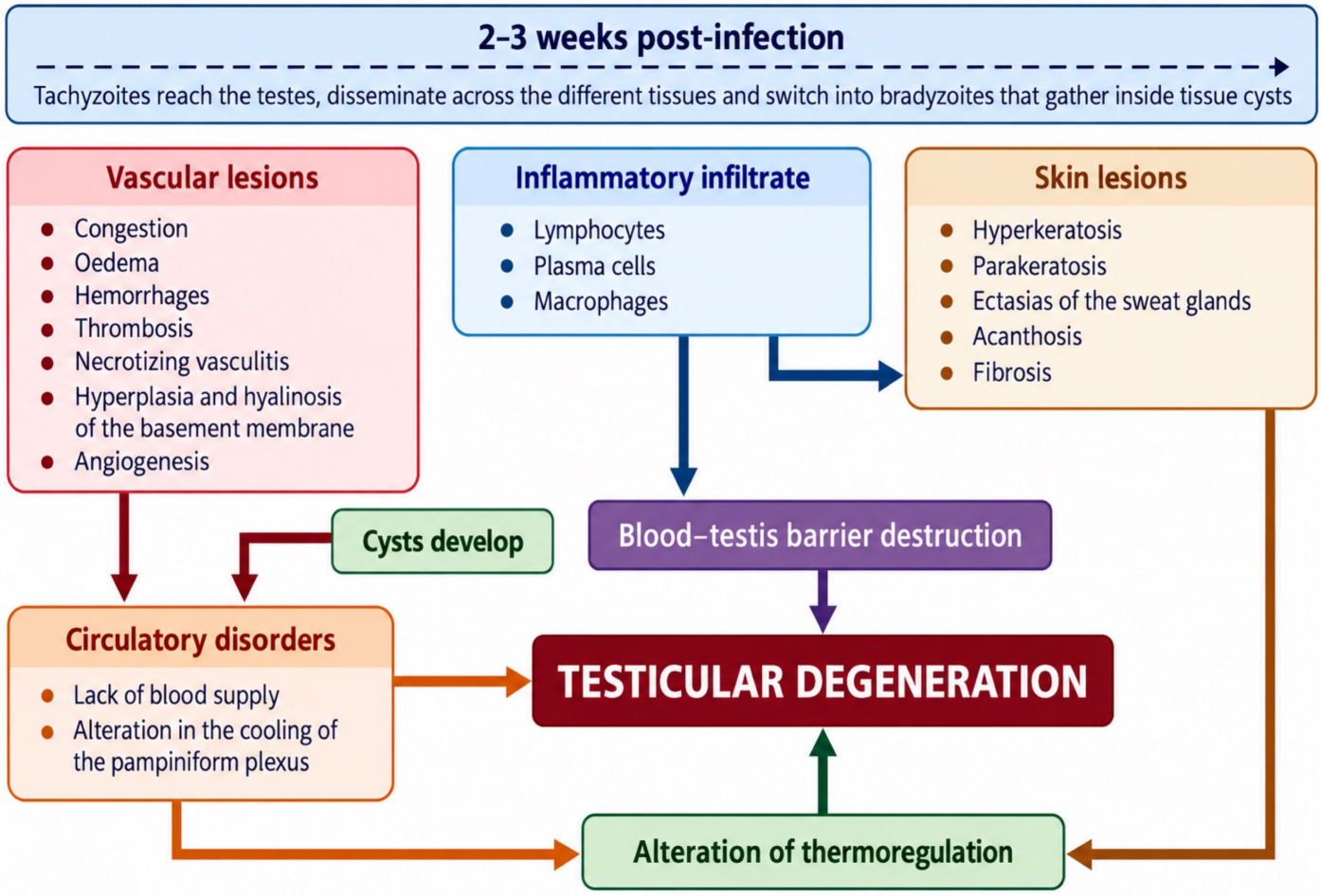


Fig. 3. *Proposed pathogenesis of testicular degeneration during acute bovine besnoitiosis, linking vascular lesions, inflammatory infiltration and skin injury with circulatory disturbance, BTB destruction and altered thermoregulation (González-Barrio et al. 2020)*

general danger-sensing mechanism but should not be interpreted as an evidence that every reproductive toxicant acts through the same pathway. Nutritional evidence is more limited: bovine Sertoli-cell studies show that selenium concentration can alter anti-inflammatory and blood–testis-barrier-associated gene expression, whereas broader nutritional effects on bull endocrine development do not establish local testicular immune mechanisms (Adegoke et al. 2018b). The most defensible conclusion is therefore conditional: toxicant and nutritional exposures can modify components of the proposed framework, but the extent to which local immune-cell metabolism mediates these effects *in vivo* remains unresolved. This distinction prevents a broad environmental narrative from being mistaken for causal bovine immunometabolism.

4. Discussion

4.1. Operational states within a continuous conceptual framework

The accumulated evidence is organized into three descriptive states that represent operational regions along a continuous response spectrum, not discrete biological categories or validated thresholds. State I, homeostatic tolerance, is defined by preserved intratesticular androgen action, an intact BTB, regulatory local immune signaling, adequate Sertoli-cell metabolic support and ongoing spermatogenesis. Physiological cytokine and ROS signaling can occur in this state without exceeding local regulatory capacity. State II, compensated stress, begins when a perturbation produces measurable stress responses while core reproductive functions remain partly preserved or recoverable. Candidate indicators include the induction of HSP70 and GPX1, transient inflammatory signaling, compensatory IL-10/TGF-β-related responses, and reversible barrier remodeling. The time course results reported by Rizzoto et al. (2020) supports the interpretation that compensatory and injury responses can overlap temporally. The bovine Sertoli-cell response to microcystin-LR similarly showed early inflammatory/barrier disturbance followed by partial recovery of anti-inflammatory and barrier-associated signals. State III, decompensated inflammation, is defined mechanistically rather than by a single threshold. Persistent suppression of steroidogenesis, sustained innate-inflammatory signaling, failure of barrier restoration, germ-cell loss or progressive vascular/tubular pathology would indicate that compensatory capacity has been exceeded. Acute besnoitiosis provides an in vivo example in which vascular injury, inflammation, thermoregulatory failure and germline degeneration coexist (González-Barrio et al. 2020). The quantitative threshold separating State II from State III is not yet known. The >90% reduction in intratesticular testosterone after 48 h of insulation is an anchor for severe local steroidogenic suppression, not a universal cutoff. Likewise, macrophage phenotype, seminal cytokines levels or serum testosterone should not be treated as stand-alone markers of these states. The model predicts that the most informative transition signature will combine local endocrine output, immune-cell state, BTB integrity and germ-cell

outcomes.

4.2. From testicular dysregulation to sperm phenotype

Changes in semen quality may appear after the initial testicular insult has resolved because germ-cell populations differ in their sensitivity to thermal and inflammatory stress. Semen collected during the recovery period can therefore reflect damage that occurred at earlier stages of spermatogenesis, sometimes several weeks before sampling (Fernandes et al. 2008; Kastelic et al. 2018). This temporal separation may complicate interpretation of associations between circulating cytokine concentrations measured at a single time point and subsequent semen characteristics. These observations should not be considered representative of all reproductive-tract infections. Since orchitis, epididymitis, and accessory-gland diseases affect anatomically and immunologically different compartments, abnormalities in semen alone cannot be taken as a sign of testicular inflammation. Therefore, the rheostat model fits well when the disease is restricted to the testis and is clinically associated with endocrine or barrier dysfunction.

Sperm function may be altered directly or indirectly by inflammatory processes. Excessive production of reactive oxygen species (ROS) has been linked to oxidative damage of cell membranes, mitochondrial dysfunctions, and DNA damages. However, by altering the activities of Sertoli and Leydig cells cytokines may disrupt cellular metabolism, decrease steroid production, and interfere with androgenic regulation of spermatogenesis (Ma et al. 2025; Fomichova et al. 2025). Breaking down of immune tolerance or disruption of the integrity of the blood-testis barrier may foster the conditions favorable for autoimmune or inflammatory damage occurring within the seminiferous tubules (Jacobo et al. 2011; Harakal et al. 2022). Conventional semen examination is still the main tool for evaluating reproductive performance clinically, whereas molecular profiling may give further insights into biological pathways leading to variations in infertility. Multicolor flow cytometry has been applied to define sperm features correlated with bull fertility, and combined methylomnic-transcriptomic methods have discovered potential molecular factors explaining reproductive performance of sires (Bucher et al. 2019; Gross et al. 2020). Proteomic analysis of semen from *Bos indicus* cattle has revealed molecular disparities, which remain undetected by ordinary assessments of sperm morphological and motility (Ramesha et al. 2020).

4.3. Translational implications for bull fertility assessment

The translational value of the proposed immune–endocrine framework lies in its potential of identifying bulls at risk before irreversible reproductive decline occurs. Breeding-soundness examination, scrotal circumference and semen quality remain indispensable because they are validated, practical endpoints (Kastelic 2014; Chenoweth and McPherson 2016; Butler et al. 2020). A mechanistic panel should complement rather than replace them. Candidate longitudinal measurements include intratesticular or serum testosterone interpreted with LH, FSH and AMH; scrotal or testicular temperature; sperm DNA integrity and mitochondrial function; selected semen RNAs or proteins; and, where validated, local inflammatory mediators. Distinction between serum and intratesticular testosterone is essential because local steroidogenic suppression can be substantial even when systemic endocrine measurements are less informative (Rizzoto et al. 2020; Ferrer et al. 2022). No single cytokine should be expected to diagnose testicular inflammation. A more defensible objective is to identify a multilevel signature of failed resilience—for example, persistent thermal load together with reduced steroidogenic output, impaired barrier markers and a deteriorating sperm functional profile. Such a panel remains conceptual and requires prospective validation against fertility outcomes.

The framework should not be interpreted as support for empirical testosterone supplementation. Exogenous androgen can suppress GnRH and gonadotropins through negative feedback and thereby impair spermatogenesis. An appropriate intervention target is restoration of physiological regulation through improved thermoregulation, disease control, appropriate nutrition, and, eventually, validated pathway-specific strategies rather than through nonspecific endocrine replacement.

5. Conclusions

Bovine testicular homeostasis may be more appropriately viewed as the coordinated outcome of endocrine, immune, barrier, vascular, and metabolic processes rather than as the consequence of a single regulatory pathway. Evidence from cattle indicates that disturbances in these compartments can occur concurrently. Acute hyperthermia has been associated with marked suppression of intratesticular steroidogenesis; bovine Sertoli-cell models have linked innate immune activation with mitochondrial injury and blood–testis barrier disruption; and acute besnoitiosis has been associated with vascular inflammation, impaired thermoregulation, and germ-cell degeneration. Several mechanistic relationships incorporated into the proposed rheostat remain insufficiently demonstrated in cattle. Causal macrophage–Leydig communication and macrophage metabolic reprogramming, in particular, are still supported mainly by comparative evidence. Recent bovine single-cell and spatial datasets have identified the relevant cell populations and their anatomical distribution, providing a useful basis for further investigation, although these findings should not be interpreted as direct evidence of functional signaling between cell types. The immune–endocrine rheostat may therefore be considered a testable framework for examining how testicular homeostasis is maintained or lost under stress. A resilient testicular environment would be expected to accommodate transient disturbances while maintaining steroidogenic activity, barrier integrity, and germ-cell support, whereas decompensation may become apparent when these functions are impaired concurrently. Longitudinal, cell-resolved studies in which local endocrine output, immune state, and subsequent sperm phenotype are measured within the same animals need to define these transitions more clearly and evaluate their potential relevance for predicting or reducing bull subfertility.

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