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Impact of boron on testosterone and sperm functional parameters in the ovines: A computational and mechanistic perspective

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Abstract

Testosterone biosynthesis and sperm functional competence are critical determinants of male fertility and reproductive efficiency in ovines (*Ovis aries*). Boron (B), a trace element with emerging roles in endocrine modulation, has been reported to influence steroid hormone metabolism and oxidative balance; however, its direct molecular targets and mechanisms of action in male reproductive physiology remain largely undefined. Given the lack of experimental structural data on micronutrient-protein interactions in reproductive systems, molecular docking was employed as a hypothesis-driven, cost-effective, and mechanistically informative approach to predict potential boron-protein interactions at the atomic level prior to *in vivo* validation. The present study utilized an insilico strategy to identify potential boron-binding domains in key proteins associated with testosterone synthesis and sperm functional regulation, namely steroidogenic acute regulatory protein (StAR), 17 β -hydroxysteroid dehydrogenase (17 β -HSD), androgen receptor (AR), and superoxide dismutase (SOD). Protein structures obtained from AlphaFold were analyzed for ligand-binding pockets using fpocket and CASTp, followed by molecular docking of boric acid using AutoDock Vina. Distinct docking scores and residue-specific interaction patterns revealed differential boron-binding propensities among the proteins, with AR and StAR exhibiting the most favorable interaction environments. Collectively, these findings provide computational evidence that boron may modulate testosterone synthesis and sperm functional parameters in ovines through multi-target molecular interactions.

Keywords: Boron, testosterone, sperm function, androgen receptor, oxidative stress, *in silico* docking

Introduction

Efficient male reproductive function in ovines (*Ovis aries*) depends on the coordinated regulation of endocrine signaling, steroidogenesis, and oxidative balance. Testosterone, synthesized primarily in Leydig cells, plays a pivotal role in spermatogenesis, libido, and maintenance of male reproductive tissues. Disruption of testosterone biosynthesis or androgen signaling pathways can lead to impaired sperm production and reduced fertility (Nieschlag *et al.*, 2010) ^[1]. In recent years, increasing attention has been given to trace minerals that influence reproductive physiology in livestock species. Boron is one such micronutrient that has gained importance due to its role in steroid hormone metabolism, enzymatic regulation, and antioxidant defense (Devirian and Volpe, 2003; Nielsen, 2008) ^[4, 8]. Experimental evidence indicates that boron supplementation can increase serum testosterone levels and modulate sex hormone balance (Naghii *et al.*, 2011) ^[7]. Additionally, boron has been shown to influence oxidative stress pathways, which are critical determinants of sperm quality and function (Aitken and Baker, 2004) ^[1]. Goats receiving boron exhibited improvements in the structure of testis tubules, and the activity of Sertoli cells increased. (Abdel-Wahab *et al.* 2022) ^[3]. Despite these physiological observations, the molecular mechanisms underlying boron action remain largely unexplored, particularly in ovine systems. A recent computational study demonstrated that boron interacts with calcium homeostasis proteins through residue-specific binding, suggesting its role as a multi-target modulator. Extending this concept, it is hypothesized that boron may similarly interact with proteins involved in testosterone synthesis and sperm function in ovines. Therefore, the present study aimed to computationally identify boron-binding domains in selected proteins associated with male reproductive physiology.

Materials and Methods

Selection of target proteins

Proteins involved in testosterone biosynthesis and sperm functional regulation in ovines (*Ovis aries*) were selected based on their well-established physiological roles in endocrine function and oxidative stress modulation. The selected proteins included steroidogenic acute regulatory protein (StAR), 17 β -hydroxysteroid dehydrogenase (17 β -HSD), androgen receptor (AR), and superoxide dismutase (SOD). StAR plays a critical role in cholesterol transport into mitochondria, representing the rate-limiting step in steroidogenesis, while 17 β -HSD catalyzes the final conversion of androstenedione to testosterone. The androgen receptor mediates testosterone signaling at the cellular level, and SOD is a key antioxidant enzyme involved in maintaining sperm integrity by neutralizing reactive oxygen species. Together, these proteins represent both endocrine and oxidative components essential for male reproductive function in ovines (Stocco, 2001; Payne and Hales, 2004; O'Donnell *et al.*, 2001; Aitken and Clarkson, 1987) [14, 13, 12, 2].

Protein sequence retrieval and structural modeling

Protein sequences corresponding to ovine homologs were retrieved from the UniProt database to ensure accuracy and species relevance. Since experimentally resolved three-dimensional structures for ovine proteins are limited, predicted structures were obtained from the AlphaFold Protein Structure Database, which provides high-accuracy structural models generated using deep learning approaches (Jumper *et al.*, 2021) [6]. Only regions with high prediction confidence (predicted Local Distance Difference Test (pLDDT) score >70) were considered for further analysis to ensure structural reliability. This approach has been widely adopted for computational studies involving non-model livestock species where crystallographic data are scarce.

Protein structure preparation

Prior to docking analysis, all protein structures were subjected to standard preparation protocols to ensure structural stability and compatibility with molecular docking tools. Hydrogen atoms were added to the protein structures to account for protonation states under physiological conditions, and water molecules and other non-essential heteroatoms were removed to avoid interference in ligand binding. Energy minimization was performed to relieve steric clashes and optimize geometry using appropriate force fields within docking preparation tools. These steps ensured that the protein conformations were in energetically favorable states for accurate interaction analysis.

Binding pocket identification

Potential ligand-binding pockets were identified using computational tools such as fpocket and CASTp. These tools analyze protein surface topology to identify cavities and pockets that are likely to accommodate small molecules. Binding sites were selected based on pocket volume, depth, and accessibility, with particular attention given to regions containing polar and charged residues that are known to facilitate boron interactions. The identification of biologically relevant binding pockets ensured that docking simulations were focused on functionally significant regions of the proteins. Boron was modeled in the form of boric acid (H₃BO₃), which is the predominant physiological form of

boron in biological systems. The ligand structure was prepared using Auto Dock tools by assigning appropriate atom types, partial charges, and rotatable bonds. Boric acid is known to interact preferentially with hydroxyl-containing and electron-donating groups, particularly those present in amino acid residues such as serine, threonine, histidine, lysine, aspartate, and glutamate. These chemical characteristics formed the basis for evaluating boron-protein interactions in the present study (Hunt, 1998; Nielsen, 2014) [5, 9].

Molecular docking analysis

Molecular docking simulations were carried out using Auto Dock Vina, a widely used tool for predicting ligand-protein interactions with high computational efficiency (Trott and Olson, 2010) [15]. Grid boxes were defined around the predicted binding pockets to ensure comprehensive sampling of ligand conformations. Docking parameters were maintained at default settings to ensure reproducibility and comparability with previous studies. Multiple binding poses were generated for each protein-ligand interaction, and the best conformations were selected based on the lowest binding energy (kcal/mol), which reflects the most stable interaction. Post-docking analysis was performed to identify key interacting residues within a 4 Å radius of the ligand. Special emphasis was placed on residues such as serine, threonine, histidine, lysine, aspartate, and glutamate due to their known affinity for boron coordination through hydroxyl and charged side chains. Interaction patterns, including hydrogen bonding, electrostatic interactions, and polar contacts, were analyzed to understand the structural basis of boron binding. Visualization and interaction mapping were conducted using molecular visualization tools to confirm the spatial orientation of boron within the binding pockets.

Computational framework and data interpretation

The overall computational workflow was designed to integrate structural prediction, binding pocket identification, and molecular docking to provide a mechanistic understanding of boron interactions with key reproductive proteins in ovines. Docking scores were interpreted as indicators of relative binding affinity, with more negative values representing stronger interactions. However, given the micronutrient nature of boron, moderate binding affinities were considered biologically relevant, consistent with reversible and regulatory interactions reported in previous studies (Nielsen, 2014) [9]. The integration of structural and biochemical insights allowed for the identification of potential molecular targets through which boron may influence testosterone synthesis and sperm functional parameters.

Results and Discussion

To the best of our knowledge, this study represents the first systematic *in silico* investigation to identify and compare boron-binding propensities across multiple proteins associated with testosterone synthesis and sperm functional regulation in ovines (*Ovis aries*). Unlike previous nutritional and physiological studies that inferred boron effects indirectly, the present work provides direct computational docking evidence, including predicted binding energies, pocket localization, and residue-level interactions, thereby offering a molecular-level explanation for boron-mediated

modulation of male reproductive function in sheep. Earlier studies have demonstrated that boron plays a significant role in endocrine regulation and reproductive physiology, particularly in modulating steroid hormones (Naghii *et al.*, 2011; Nielsen, 2014)^[7, 9].

Distinct boron-binding environments were identified across the four proteins analyzed, namely steroidogenic acute regulatory protein (StAR), 17 β -hydroxysteroid dehydrogenase (17 β -HSD), androgen receptor (AR), and superoxide dismutase (SOD). The predicted docking scores ranged from -3.9 to -4.9 kcal/mol, indicating moderate but biologically plausible boron-protein interactions. Such interaction strengths are consistent with reversible micronutrient-protein associations reported in earlier biochemical studies (Nielsen, 2014)^[9]. Among the proteins studied, androgen receptor and StAR exhibited the most favorable binding affinities, suggesting a higher propensity for boron association in ovine reproductive tissues.

Among the proteins analyzed, androgen receptor (AR) exhibited the strongest boron-binding potential. In ovines, AR plays a central role in mediating testosterone action within Sertoli and Leydig cells, thereby regulating spermatogenesis and maintenance of reproductive function

(O'Donnell *et al.*, 2001)^[12]. The clustering of boron near key residues such as tyrosine, histidine, and lysine within the ligand-binding domain suggests that boron may stabilize receptor conformation or enhance ligand-binding efficiency. This interaction may contribute to improved androgen signaling, thereby supporting spermatogenic activity and reproductive performance. Previous studies have reported that boron supplementation significantly increases free testosterone levels in males, supporting the present computational findings (Naghii *et al.*, 2011)^[7].

Steroidogenic acute regulatory protein (StAR) also demonstrated high boron-binding affinity, particularly within regions associated with mitochondrial cholesterol transport. In ovine Leydig cells, StAR mediates the rate-limiting step of steroidogenesis by facilitating cholesterol transfer into mitochondria for testosterone synthesis (Stocco, 2001)^[14]. The interaction of boron with polar residues such as serine and threonine suggests the formation of reversible borate complexes that may enhance protein functionality. This provides a plausible molecular explanation for improved testosterone synthesis and endocrine efficiency observed in boron-supplemented models (Nielsen, 2014)^[9].

Table 1: Predicted boron-binding characteristics of testosterone-regulatory and sperm-functional proteins in *Ovis aries*

Protein	Physiological role	Major predicted boron-binding region	Dominant interacting residues	Predicted docking score (kcal/mol)	Predicted relative affinity	Structural basis
StAR	Cholesterol transport for steroidogenesis	Mitochondrial targeting domain	Ser, Thr, Lys	-4.7	High	Presence of polar residues enables reversible borate interactions facilitating cholesterol transport
17 β -HSD	Conversion of androstenedione to testosterone	Catalytic active site pocket	His, Asp	-4.2	Moderate	Interaction near catalytic residues may influence enzymatic conversion efficiency
AR	Testosterone receptor and gene regulation	Ligand-binding domain	Tyr, His, Lys	-4.9	High	Stabilized by hydrogen bonding and polar interactions within hormone-binding cavity
SOD	Antioxidant defense in sperm cells	Metal-binding region	His, Glu	-4.1	Moderate	Potential modulation of redox-active site improving oxidative stress resistance

Moderate boron-binding affinity was observed with 17 β -hydroxysteroid dehydrogenase (17 β -HSD), particularly near its catalytic pocket. This enzyme catalyzes the final step in testosterone biosynthesis by converting androstenedione to testosterone in ovine testes (Payne and Hales, 2004)^[13]. Although the interaction is comparatively weaker than that observed for AR and StAR, the proximity of boron to catalytically important residues such as histidine and aspartate suggests a potential modulatory effect on enzyme activity. Such interactions may contribute cumulatively to enhanced testosterone production in ovines. Superoxide dismutase (SOD), a key antioxidant enzyme, exhibited moderate boron-binding near its metal-binding region. In ovine spermatozoa, oxidative stress is a major factor affecting sperm motility, viability, and membrane integrity due to the high content of polyunsaturated fatty acids and limited cytoplasmic antioxidant defenses (Aitken and Clarkson, 1987)^[2]. The interaction of boron with residues involved in redox regulation suggests a potential role in stabilizing enzyme activity and enhancing antioxidant capacity. Studies have shown that boron supplementation improves antioxidant status and reduces oxidative stress markers, which supports these findings (Nielsen, 2018)^[10]. Collectively, these findings support the concept that boron acts as a multi-target modulator within the ovine

reproductive axis, influencing both endocrine regulation and oxidative stress pathways. The simultaneous interaction of boron with proteins involved in testosterone synthesis and antioxidant defense highlights its integrative role in improving reproductive efficiency in *Ovis aries*. These results provide a mechanistic framework linking boron supplementation to enhanced testosterone levels and improved sperm functional parameters, which are critical determinants of fertility in sheep. Further *in vitro* and *in vivo* validation studies are warranted to confirm these computational predictions and to establish optimal dietary boron supplementation strategies in ovine production systems.

Conclusion

The present *in silico* study demonstrates that boron interacts with key proteins involved in testosterone synthesis and sperm functional parameters, with androgen receptor and StAR emerging as primary targets. Secondary interactions with 17 β -HSD and SOD suggest additional roles in hormone biosynthesis and oxidative protection. These findings provide a mechanistic basis for the beneficial effects of boron on male fertility and support its potential application in nutritional strategies aimed at improving reproductive performance. Further experimental validation is required.

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