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# Molecular and developmental insights into gonadogenesis and primordial germ cell migration in pacific white shrimp (*Litopenaeus vannamei*): A comprehensive review

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## Abstract

The Pacific white shrimp, *Litopenaeus vannamei*, is a key species in global aquaculture, valued for its rapid growth and high market demand. Understanding the molecular mechanisms of gonadogenesis and primordial germ cell (PGC) migration in this species is vital for optimizing breeding programs. This review consolidates current knowledge on these processes, highlighting the role of the vasa gene as a germ cell marker. Vasa expression was tracked from fertilized eggs to juveniles aged four months, revealing the conserved DEAD-box helicase motif essential for germ cell development. Early detection of vasa messenger ribonucleic acid (mRNA) in developmental stages such as nauplius and zoea indicates its role in germ cell specification. By the post-larval stages, PGCs formed clusters associated with somatic gonadal precursor cells (SGPs), which support PGC migration and development. Quantitative polymerase chain reaction (qPCR) and reverse transcription PCR (RT-PCR) analyses demonstrated dynamic changes in vasa gene expression, suggesting critical windows for PGC proliferation and differentiation. Comparative studies with other species, such as *Macrobrachium rosenbergii* and *Fenneropenaeus chinensis*, emphasize the evolutionary conservation of germ cell development mechanisms. These insights are crucial for enhancing reproductive management and sustainability in shrimp farming, facilitating targeted interventions to improve breeding efficiency and hatchery success.

**Keywords:** *Litopenaeus vannamei*, primordial germ cell migration, vasa gene, gonadogenesis, crustaceans

## Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, is a vital species in global aquaculture due to its rapid growth, high market demand, and adaptability to varied farming conditions[1–3]. Understanding the molecular mechanisms governing gonadogenesis and primordial germ cell (PGC) migration in this species is crucial for optimizing breeding programs and ensuring sustainable shrimp production[4–7]. Gonadogenesis, the process of gonad formation and development, and PGC migration, the movement of germline precursors to the gonadal ridge, are fundamental to reproductive success and species propagation. The vasa gene, a well-known germline marker, plays a pivotal role in the development and differentiation of germ cells across various species. In *L. vannamei*, the vasa-like gene has been characterized and its expression during oogenesis documented, highlighting its significance in germline development[8]. Furthermore, gonadal development in this species has been shown to correlate with age and weight, providing insights into the reproductive maturation process[9].

Studies on other crustaceans and model organisms offer valuable comparative insights. For instance, the role of vasa in zebrafish (*Danio rerio*) gonad differentiation and the dependence of puberty onset on body growth rather than age underscores the potential parallels in *L. vannamei*[10]. Moreover, research on the peppermint shrimp (*Lysmata vittata*), another crustacean species, elucidates the ontogenetic development of gonads and external sexual characters, which may offer analogous mechanisms in *L. vannamei*[11]. Investigations into the primordial germ cell migration in various species, such as the mouse (*Mus musculus*) and the freshwater prawn (*M. rosenbergii*), provide a foundational understanding of PGC behavior and its regulatory factors. In mice, fine structural observations of PGC origin and associations reveal the intricate dynamics of germ cell migration[12]. Similarly, the morphology and regional distribution of PGCs in M. rosenbergii emphasize the conserved nature of germ cell development across species[13].

Recent advances in molecular techniques have enabled the characterization and expression analysis of vasa homologs in various species, enhancing our understanding of germline development. For instance, the vasa homolog in the striped catfish (*Pangasianodon hypophthalmus*) has been identified and its expression in gonads and PGCs documented, offering parallels for similar studies in *L. vannamei*[14]. In this review, we aim to consolidate current knowledge on gonadogenesis and PGC migration in *L. vannamei*, drawing comparisons with related studies in other species to provide a comprehensive overview. By elucidating these processes, we hope to contribute to the enhancement of breeding programs and the overall sustainability of the shrimp aquaculture industry.

## Material and methods

### Literature research and selection

A thorough and systematic literature review was undertaken to identify pertinent studies related to gonadogenesis and PGC migration in *L. vannamei* and related crustacean species. The search was conducted across multiple electronic databases, including PubMed, Google Scholar, and Web of Science, to ensure comprehensive coverage of relevant scientific literature. Keywords employed in the search strategy encompassed terms such as "*Litopenaeus vannamei*," "gonadogenesis," "primordial germ cell migration," "vasa gene," "germline development," and "shrimp reproductive biology."

### Article selection criteria

Articles were selected based on predetermined inclusion criteria, which encompassed relevance to the research topic, scientific rigor, and publication in peer-reviewed journals. Studies were included if they provided insights into the molecular mechanisms underlying gonadogenesis and PGC migration, particularly in *L. vannamei* or crustacean species. Furthermore, preference was given to articles that employed experimental approaches, such as gene expression analysis, histological examination, or molecular characterization, to elucidate the processes of interest.

### Data extraction and synthesis

Information extracted from selected articles included details regarding the methodology employed, key findings, and implications for understanding gonadogenesis and PGC migration. Data synthesis involved a comprehensive analysis of the collected information to identify common themes, trends, and gaps in current knowledge. Comparative analysis with findings from related species was conducted to provide broader insights into the developmental processes under investigation.

### Quality assessment

To ensure the reliability and validity of the synthesized information, the quality of selected studies was critically evaluated. Factors assessed included study design, sample size, experimental procedures, and data analysis methods. Studies meeting high methodological standards were accorded greater weightage in the synthesis process, while those with potential limitations or biases were carefully considered in the interpretation of results. Overall, the literature search and selection process aimed to gather a diverse range of studies that collectively contribute to a comprehensive understanding of gonadogenesis and PGC migration in *L. vannamei* and related species.

## Discussion

The study of PGCs and gonadal development in the white leg shrimp (*L. vannamei*) provides significant insights into their reproductive biology, which is crucial for aquaculture and species conservation efforts. The vasa gene, a known marker for germ cells, was utilized to track PGC migration and development across various developmental stages, from fertilized eggs to juveniles aged four months. This study revealed the intricate process of PGC migration, involving the conserved DEAD-box helicase motif in vasa, which is essential for germ cell development. The DEAD-box helicase motif is highly conserved across species, emphasizing its fundamental role in the regulation of RNA processes critical for germ cell formation and function[8,11]. The detailed tracking of vasa expression provided a comprehensive understanding of how PGCs migrate, proliferate, and differentiate into mature gonadal tissues, offering valuable data for enhancing reproductive efficiency in shrimp farming.

In the early developmental stages, vasa mRNA was detected in fertilized eggs and later stages, such as nauplius and zoea, where PGCs were identified as discrete spots along the midgut. The detection of vasa mRNA in these stages underscores its early and persistent role in germ cell specification and maintenance. As the shrimp developed through the mysis stage, vasa-positive signals were observed distributed along the midgut and pereopod, highlighting the migratory pattern of PGCs. This migratory behavior is critical for ensuring that PGCs reach their target destinations where they can initiate gonadal development[11,15]. This pattern was consistent with previous findings in other shrimp species, such as *F. chinensis*, demonstrating a conserved mechanism among crustaceans. The consistency of these findings across different species suggests that the mechanisms governing PGC migration are deeply rooted in the evolutionary history of crustaceans, reflecting a shared biological heritage that underpins their reproductive strategies[15].

By the post-larval stages (PL1-15), PGCs formed clusters under the heart and began mitotic division, increasing in number. This cluster was associated with somatic gonadal precursors (SGPs), small flat cells that play a crucial role in supporting PGC migration and development. The interaction between PGCs and SGPs is a fundamental aspect of gonadal formation, as SGPs provide both physical support and essential signaling cues that guide PGCs to their final destination within the developing gonad. The continuous observation of these clusters through PL15 indicated an essential phase for the establishment of the gonad. This period marks a critical window during which the foundational structures of the gonad are laid down, setting the stage for subsequent maturation and differentiation6,8. These findings underscore the importance of the cellular microenvironment and intercellular interactions in germ cell development, highlighting the complex interplay of cellular and molecular factors that orchestrate the formation of reproductive organs.

In juveniles, particularly by day 65, the PGC cluster expanded to form a gonad between the heart and hepatopancreas, known as the genital ridge. The genital ridge is a key anatomical feature that serves as the precursor to the mature gonads, housing the PGCs as they undergo further differentiation. The appearance of external sexual characteristics, such as the thelycum in females and petasma in males, was noted by day 114. This differentiation was corroborated by the presence of vasa mRNA in spermatogonia within the developing testes, underscoring the specificity of the vasa probe for detecting germ cells during gonad development. The detailed observation of these stages provides a comprehensive timeline of gonadal development, facilitating targeted interventions in shrimp breeding programs. By understanding the precise timing and sequence of these developmental events, aquaculturists can optimize breeding conditions and improve the efficiency of shrimp production[16,17].

Quantitative PCR (qPCR) and RT-PCR analyses showed dynamic changes in vasa gene expression, which were detectable early in PL7 and showed significant fluctuations through to PL30. This temporal expression pattern suggests critical windows for PGC proliferation and differentiation. The ability to track these changes in real time allows researchers to pinpoint the stages at which PGCs are most active, providing valuable insights into the regulatory mechanisms that control germ cell development. Notably, vasa transcripts were exclusively expressed in the ovaries and testes, further confirming its role as a germ cell marker. This specificity underscores the reliability of vasa as a molecular tool for studying germ cell biology in crustaceans[8,18]. Understanding these expression patterns allows for more precise manipulation of reproductive timing and conditions in aquaculture settings, enabling the development of strategies to enhance fertility and hatchery success.

Comparisons with other species, such as the giant freshwater prawn *M. rosenbergii* and the Chinese shrimp *F. chinensis*, reveal similarities in the developmental processes and vasa expression patterns. This cross-species analysis highlights the evolutionary conservation of germ cell development mechanisms in crustaceans. The conserved nature of these processes suggests that findings in *L. vannamei* could be applicable to other crustacean species, broadening the impact of this research. The ability to apply insights gained from one species to others within the same taxonomic group enhances the generalizability of the research findings and provides a framework for developing universal aquaculture practices that can be adapted to a variety of crustacean species[13,15].

Furthermore, the study highlights the role of somatic gonadal precursor cells in creating a conducive environment for PGCs through chemokine signaling and physical support, akin to the mechanisms observed in vertebrates. This intricate interplay between PGCs and SGPs underscores the complexity of gonadal development and the critical role of the microenvironment in germ cell migration and differentiation. The chemokine signaling pathways involved in this process are highly conserved and play a pivotal role in directing PGC migration by creating gradients that guide the cells to their target locations. Such insights are essential for developing strategies to enhance reproductive success and sustainability in shrimp farming. By manipulating the signaling pathways and microenvironmental conditions that influence PGC behavior, aquaculturists can improve the efficiency of gonad development and increase the yield of viable offspring[12,19,20].

## Conclusions

The study of PGCs and gonadal development in whiteleg shrimp (*L. vannamei*) reveals the complex processes of PGC migration and differentiation, which are crucial for aquaculture and species conservation efforts. Utilizing the vasa gene as a germ cell marker allowed for tracking PGC migration from fertilized eggs to four-month-old juveniles. This process involves the essential DEAD-box helicase motif for germ cell development. The detection of vasa mRNA from early developmental stages to post-larvae indicates a consistent pattern of PGC migration, similar to other shrimp species, underscoring a conserved mechanism among crustaceans. The formation of PGC clusters near the heart and gonadal development highlights the critical role of SGPs in supporting PGC migration and development. qPCR and RT-PCR analyses revealed dynamic changes in vasa gene expression, emphasizing critical windows for PGC proliferation and differentiation. The similarities in germ cell development mechanisms between L. vannamei and other crustacean species underline the evolutionary conservation of these processes, broadening the impact of this research on reproductive management and sustainability in shrimp farming.

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## Conflicts of Interest

The authors declare no conflict of interest

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