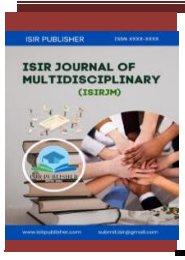
ISIR Journal of Multidisciplinary (ISIRJM) ISSN: XXXX-XXXX (Online)

Published: 25-Jan-2025



ISIR Journal of Multidisciplinary (ISIRJM)

ISSN: **XXXX**-XXXX (Online)

Frequency: Bimonthly

Published By ISIR Publisher

Journal Homepage Link- https://isirpublisher.com/isirjm/

Volume 1, Issue 1, January-February, 2025

ISIR PUBLISHER
ESTD: 2024

MOLECULAR BASIS OF GAMETOGENESIS AND SEX DETERMINATION IN *Plasmodium falciparum*

By

Tsaiba Ali Auwal[1], Ismail Muhammad[1], Abubakar Ahmanullah Aminu[1] Joshua Habibah Danjuma[2]

[1]Department of Zoology and Wildlife Ecology, Gombe State University, Gombe, Nigeria.

[2]Department of Science Laboratory Technology, Federal Polytechnic Kaltingo, Gombe State, Nigeria.

Article History

Received: 05/01/2025
Accepted: 20/01/2025
Published: 25/01/2025

Vol – 1 Issue – 1

PP: -01-11

Abstract

Successful malaria transmission mainly depends on successful gametogenesis and gametocytogenesis, which lead to successful gametocyte production which eventually differentiate in to male and female gametocyte. Unlike what it known in other organism, in which sex chromosomes are responsible for sex determination, in Plasmodium species, this mediated by various complex process, involving both genetic and environmental factors. Therefore, the aim of this paper was to review the current state of knowledge in the role of various genes towards sex determination in Plasmodium. Various data bases were consulted in order to generate relevant literatures on the topic. Commitment to form gametocytes or asexual parasites occurs before the schizont stage, with each merozoite in a schizont destined to become either a gametocyte or an asexual parasite. Each merozoite forms either a male or female gametocyte, indicating that sex determination also occurs before the schizont stage. Cellular transformation necessary for early sexual differentiation is regulated by several genes, such as pfs16, pf14.744, pf14.748, pfpeg3/mdv1, and pfpeg4, which are expressed at the start of gametocytogenesis. Two transcription factors PfAP2-G and PfAP2-I play a vital role in gametocytogenisis, in which protein encoded by these factors regulate differentiation in to male and female respectively coupled with environmental factors, such as nutrient availability, immune responses, and host-induced stress.

Keywords: *Plasmodium falciparum*, Gametogenesis, Gametocytogenesis, Sex determination, Transcription factors

INTRODUCTION

Malaria, a potentially fatal disease, is spread by infected female Anopheles mosquitoes carrying *Plasmodium* parasites. Globally, it claims approximately 405,000 lives annually. Nigeria, among other sub-Saharan African nations, bears a significant burden, reporting over 7.8 million cases and 4,294 malaria-related fatalities each year (Schneider & Reece, 2021). Children the ages of five years and pregnant women are the most susceptible populations. While there are five *Plasmodium* species (*P. falciparum, P. malariae, P. ovale, P. vivax and P.knowlesi*) that cause human malaria, *Plasmodium falciparum* is the deadliest, causing severe disease and the majority of malaria deaths, particularly in sub-Saharan Africa (Sinden, 2016). This species predominates in tropical regions, including South Africa, Mozambique, Burkina Faso, and Nigeria (Sondo et al., 2021).

During the erythrocytic stage, *P. falciparum* exhibits a unique cell tropism, infecting both reticulocytes and mature red blood cells, leading to rapid parasite growth and increased parasitemia (Rajapandi, 2019). This results in a surge of parasite populations every 48 hours, causing the characteristic symptoms of malaria, including fever, chills, muscle pain, vomiting, and severe anemia. The primary pathophysiological mechanisms of *P. falciparum* malaria involve the adhesion of parasitized red blood cells to the endothelium, platelets, and uninfected erythrocytes, leading to microvasculature obstruction and severe complications like cerebral malaria and stroke. As the parasite burden grows, the parasites must decide whether to continue proliferating or invest in sexual transmission, which is crucial for their survival (Pelleau et al., 2015).

Malaria infection in humans begins when a female Anopheles

*Corresponding Author: **Ismail Muhammad.**

mosquito injects *Plasmodium* sporozoites into the skin during feeding. The sporozoites actively enter the bloodstream and travel to the liver, where they multiply within liver cells, producing merozoites that are released into the blood. These merozoites invade red blood cells and undergo several developmental stages, eventually producing new merozoites that infect additional red blood cells. A small proportion of parasites develop into gametocytes, which are ingested by another mosquito, leading to fertilization and the formation of infectious sporozoites in the mosquito's salivary glands. Unlike in other organisms that have sex cells, in Plasmodium sex chromosomes are completely wanting, therefore development of either male or female gametocyte and subsequent development of either male or female offspring is mediated by various complex process, involving both genetic and environmental factors. Therefore, the aim of this paper was to review the current state of knowledge in the role various genes towards sex determination in *Plasmodium*.

Gametocyte Development

As shown in figure I, the development of gametocytes in *P. falciparum* involves five distinct morphological stages (I-V), with stages I and II resembling early asexual parasites. Later stages (III-V) are characterized by elongation, spindle shapes, and eventually, a crescent shape with minimal host cell surface visibility. In contrast, other Plasmodium species, like *P. vivax*, have similar asexual and sexual cycle lengths, with gametocytes developing within 48 hours and disappearing from circulation within three days of maturation (Sinden, 2016).

During the seventh day of development, male and female gametocytes can be distinguished based on their morphology, classified as stage IV gametocytes (Dagnogo et al., 2017). Female gametocytes are characterized by condensed hemozoin, accumulated osmiophilic bodies, high ribosome density, and a small nucleus with a nucleolus, enabling intense protein synthesis for the emerging macrogamete and zygote (Pandey et al., 2020). In contrast, male gametocytes exhibit scattered hemozoin, a larger nucleus without a nucleolus, and the presence of kinetochores and microtubule organizing centers, preparing for rapid mitotic division upon activation in the mosquito. These distinct features reflect the specialized roles of male and female gametocytes in the malaria parasite's life cycle (Jeninga et al., 2023).

Plasmodium parasites, alternate between human and insect hosts, requiring rapid adaptation mechanisms to coexist with each host (Simonetti, 2019). They switch between tissue-specific multiplication cycles and sexual reproduction, which facilitates transmission from human to mosquito. The sexual phase begins with gametocyte differentiation in human erythrocytes, followed by uptake in the mosquito's blood meal, gamete formation, and fertilization. A small proportion of asexual parasites develop into gametocytes, which are transmitted to the mosquito vector but don't contribute to disease pathology (Miao et al., 2017). The parasite has a haploid genome, and sex is determined by the expression of specific genes.

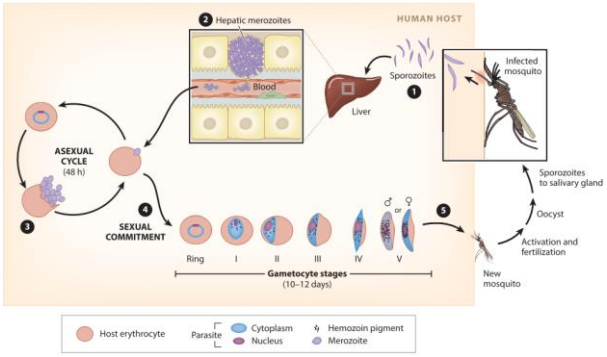


Fig 1.0: Lifecycle of *Plasmodium* parasite sexual commitment

Gametocyte maturation time varies among Plasmodium species, with *P. falciparum* gametocytes taking 10-12 days to mature. Gametocytogenesis involves three steps: commitment to sexual differentiation, prestage I development from sexual schizogony to identifiable stage I gametocytes, and poststage I development from stage I to mature stage V gametocytes (Mcrobert et al., 2018). Understanding sexual development in Plasmodium has advanced significantly with genome-wide analytical tools and genetic manipulation technology (Ikadai et al., 2013).

To complete their life cycle, gametocytes must be ingested by a female Anopheles mosquito during a blood meal. Within minutes of ingestion, gametocytes are activated in the mosquito's midgut and undergo gametogenesis, transforming into gametes. During this process, mature stage V gametocytes rapidly change from a banana shape to a rounded form, exit the host red blood cells, and are released as male and female gametes. The male gametes then develop flagella through exflagellation, enabling them to bind to female gametes and facilitate fertilization (Singh et al., 2019).

Genome of ***Plasmodium falciparum***

Genomic sequencing of *P. falciparum* was initially published in 2002 using the parasite clone 3D7. The genome is made up of a 6kb mitochondrial genome, a 35kb circular apicoplast plastid, and a ~23Mb nuclear genome arranged into 14 linear chromosomes that range in size from ~0.6 to 3.3Mb. The AT concentration of the nuclear genome, which averages 81% and peaks at about 90% in non-coding region, is thought to be the greatest of any sequenced genome. Gene density is relatively high, with up to about 50% of the genome sequence anticipated to be protein-coding, similar to many unicellular species. The average gene length is significantly greater than that of the other creatures, and over half of the approximately 5300 protein-coding genes have an intron. Due to in-depth research on *P. falciparum* genes that share no sequence homology with any known protein, the initial genome assembly revealed that up to 60% of the genes encoded proteins of unknown function. However, this ratio has now dropped to about 40%. However, the high AT-rich concentration can complicate gene prediction based on sequence homology. In 2020, Alphabet's/Google Deep Mind created the Alpha Fold program, which uses the latest developments in artificial intelligence (AI) and machine learning (ML) to predict protein structure from mRNA

*Corresponding Author: **Ismail Muhammad.**

sequences with great accuracy. This has made it easier to research a lot of these unidentified genes that code for proteins (Mohammed, 2023).

Gametogenesis in *Plasmodium falciparum*.

Plasmodium falciparum, the parasite responsible for malaria, undergoes a complex life cycle involving both asexual and sexual stages. Gametogenesis, the process of producing gametes (sperm and egg cells), is crucial for the transmission of the parasite. Understanding the molecular basis of gametogenesis and sex determination in *P. falciparum* can provide insights into the development of novel interventions against malaria. Research on *P. falciparum* suggests that the commitment to form gametocytes or asexual parasites occurs before the schizont stage, with each merozoite in a schizont destined to become either a gametocyte or an asexual parasite, but not both (Pathak et al., 2018). Similarly, each merozoite will form either a male or female gametocyte, indicating that sex determination also occurs before the schizont stage (Stewart et al., 2022). Only a subset of schizonts commits to gametocyte production during each 48-hour cycle, resulting in typically fewer than 10% of parasites producing gametocytes. This contrasts with Haemoproteus species, where nearly all merozoites invade red blood cells and convert to gametocytes (Osgood & Schall, 2014). Understanding how Plasmodium parasites regulate commitment to sexual differentiation is crucial for grasping parasite transmission. Recent in vitro studies have made significant progress in elucidating Plasmodium gametocytogenesis and commitment regulation, providing valuable insights into this critical aspect of the parasite's life cycle (Josling et al., 2018).

Molecular Insights into Gametocytogenesis

Gametocytogenesis is the process through which asexual *Plasmodium falciparum* parasites differentiate into sexual forms (gametocytes) capable of transmission to the mosquito vector, thus completing the malaria lifecycle. This transition from asexual to sexual development is a critical step in the parasite's lifecycle, and understanding its molecular regulation can provide insights into potential interventions aimed at interrupting malaria transmission. Gametocyte development is a tightly controlled, multistage process, regulated by a variety of transcriptional, post-transcriptional, and epigenetic mechanisms. Gametocytes contain over 900 proteins and 200–300 RNAs that are unique to each gametocyte (Gomes et al., 2022). Although gene expression is dynamic during gametocytogenesis, it does not appear to follow the asexual stages' cascade-like pattern. The cellular transformation necessary for early sexual differentiation may be regulated by several genes, such as pfs16, pf14.744, pf14.748, pfpeg3/mdv1, and pfpeg4, which are expressed at the start of gametocytogenesis. The earliest indicator of gametocytogenesis among them is Pfs16, whose expression is found 24 hours after merozoite invasion and persists throughout gametocyte development. Interestingly, all five of the aforementioned proteins are located in the Parasitophorous Vacuole Membrane (PVM) and are created at the beginning of sexual differentiation. Pfs16 disruption decreased gametocyte formation and mosquito transmissibility (Che Julius Ngwa et al., 2016).

Molecular Regulators of Gametocytogenesis

One of the key regulators of gametocytogenesis in *P. falciparum* is the transcription factor *PfAP2-G*, which is crucial for the initiation of the sexual differentiation process. The gene encoding *PfAP2-G* is activated in response to environmental cues, such as host immune pressure or nutrient stress, which trigger the commitment of asexual merozoites to undergo gametocytogenesis. This protein serves as a master regulator for male gametocyte development, with *PfAP2-G* expression inducing a cascade of genes responsible for the differentiation of male gametocytes (Josling et al., 2017). Additionally, *PfAP2-I* is another transcription factor involved in regulating the differentiation of female gametocytes. These two factors work in parallel, ensuring that both male and female gametocytes are generated in the appropriate proportions.

Further, *PfAP2-G* and *PfAP2-I* are part of the AP2 family of transcription factors, which are known to play essential roles in the regulation of various developmental processes in *Plasmodium* parasites. These transcription factors control a variety of downstream genes that govern the progression of gametocytogenesis, including those involved in gamete maturation and fertilization. Additionally, *PfAP2-G* and *PfAP2-I* are key targets for understanding how gametocytogenesis is regulated and how these processes might be manipulated to block malaria transmission (Josling et al., 2018).

Chromatin Remodeling and Epigenetic Regulation

In addition to transcriptional regulation, epigenetic modifications also play an essential role in the regulation of gametocytogenesis. Chromatin remodeling is an integral aspect of this process, as the parasite must switch from asexual replication to sexual differentiation, which involves substantial changes in gene expression. These changes are often accompanied by modifications to the histone proteins around which DNA is wrapped, as well as alterations in DNA methylation patterns.

One important player in chromatin remodeling is *PfSir2A*, a sirtuin protein that modifies histones and regulates chromatin structure. *PfSir2A* acts to silence genes related to asexual replication while promoting the activation of genes involved in sexual differentiation. This process is crucial for the parasite to successfully transition to gametocyte production. Moreover, sirtuins are involved in the regulation of stress responses, which may also play a role in the parasite's decision to commit to gametocytogenesis under conditions of environmental stress (Gomes et al., 2022).

Epigenetic regulation of gametocytogenesis has been shown to involve both the activation of sexual development genes and the repression of asexual genes. These epigenetic modifications are crucial for ensuring that the switch to sexual reproduction occurs in a controlled and timely manner. This regulation of chromatin during gametocytogenesis is an area of active research, as it provides potential targets for interventions that could block the production of gametocytes

*Corresponding Author: **Ismail Muhammad.**

and prevent transmission to the mosquito vector (Josling et al., 2017).

Environmental Cues and Hormonal Regulation

Environmental factors, such as nutrient availability, immune responses, and host-induced stress, are crucial for determining the timing and extent of gametocytogenesis. In *P. falciparum*, nutrient availability is one of the most important external factors that influence sexual differentiation. For instance, under conditions of limited resources or nutrient stress, the parasite may shift to sexual reproduction as a survival strategy. This shift is believed to enhance transmission potential, ensuring that *P. falciparum* can continue to propagate even when conditions are less favorable for asexual replication.

Hormonal signaling also plays a role in regulating gametocytogenesis in *P. falciparum*. In particular, changes in host immune responses and the presence of cytokines can trigger the transition to sexual reproduction. Recent studies suggest that *P. falciparum* may use host-derived signals to sense when conditions are optimal for transmission to the mosquito, such as when the host is becoming immune-compromised or when the parasite density is high enough for successful transmission to occur (Sinden, 2016; Osgood & Schall, 2014).

Role of the Gametocyte Proteome

As gametocytes mature, there are significant changes in their proteome. Proteomic analyses of male and female gametocytes have provided valuable insights into the molecular mechanisms underlying sexual differentiation. The gametocyte proteome reveals the specific proteins required for gamete formation, including proteins involved in the maturation of male and female gametes, as well as those involved in fertilization. One of the notable findings from proteomic studies is that male and female gametocytes have distinct proteomes, which suggests that the sexual differentiation of gametocytes involves complex molecular changes tailored to the specific roles of each sex in fertilization (Miao et al., 2017).

For instance, male gametocytes express specific enzymes that are involved in the fertilization process, such as proteases that aid in the breakdown of the mosquito midgut barrier. In contrast, female gametocytes express proteins necessary for the fertilization and early development of the zygote. These differences in the proteome between male and female gametocytes underscore the importance of sex-specific molecular pathways in ensuring the success of fertilization and transmission to the mosquito vector (Miao et al., 2017; Mcrobert et al., 2018).

Regulation of the Sex Ratio

One of the unique aspects of *P. falciparum* gametocytogenesis is the regulation of the sex ratio of the gametocytes. The sex ratio is the proportion of male to female gametocytes in a given population, and it is crucial for ensuring successful fertilization and transmission. In *P. falciparum*, the sex ratio of gametocytes can be influenced by a variety of factors, including host immune responses, nutrient availability, and genetic factors.

Recent studies have shown that the sex ratio of *P. falciparum* gametocytes is not fixed but can be adjusted under certain conditions. For example, when the parasite is under immune pressure or when the nutrient supply is limited, there may be a shift in the sex ratio, with a higher proportion of male gametocytes being produced. This adjustment in the sex ratio is believed to be a strategy to maximize fertilization rates when the production of female gametocytes is limited. The ability to regulate the sex ratio is another layer of complexity in the molecular control of gametocytogenesis and may offer potential avenues for intervention (Osgood & Schall, 2014; Miao et al., 2017).

Implications for Malaria Transmission

Understanding the molecular mechanisms that govern gametocytogenesis is crucial for the development of new strategies to interrupt malaria transmission. Targeting the early stages of gametocyte development, such as inhibiting the expression of *PfAP2-G* or *PfAP2-I*, could reduce the number of gametocytes produced and thus lower the chances of transmission to the mosquito vector. Additionally, interventions that disrupt the proteomic changes associated with gametocyte maturation could hinder the ability of the parasite to complete its lifecycle in the mosquito, effectively blocking transmission (Gomes et al., 2022; Josling et al., 2017).

Furthermore, a better understanding of how environmental factors and host immune responses influence gametocytogenesis could lead to the development of strategies that manipulate the conditions under which gametocytes are produced. For instance, altering the host's immune system to favor the production of male or female gametocytes, or targeting the epigenetic regulation of gametocytogenesis, may provide new methods for controlling malaria transmission at the level of the mosquito vector (Sinden, 2016).

Commitment to gametocytogenesis

The process of switching from the asexual blood stage to the gametocyte is referred to as gametocyte commitment. Gametocytes in Plasmodium typically form during each asexual erythrocytic cycle. However, in certain species like *P. vivax*, which infects humans, and *P. yoelii*, which infects rodents, gametocytes can also develop from merozoites emerging from pre-erythrocytic schizonts, providing an additional source of gametocytes beyond the traditional asexual erythrocytic cycles (Josling et al., 2018). In humans infected with *P. falciparum* the pfs16 mRNA gene associated with gametocytogenesis is detectable within 48 hours of infection, indicating early commitment to gametocyte formation. This early gametocytogenesis in P. vivax facilitates parasite transmission but complicates malaria control. During erythrocyte development, parasites make two crucial decisions: choosing between asexual and sexual pathways, and, if sexual, determining male or female gametocyte fate. Research shows that all merozoites from a single schizont commit to either asexual or sexual pathways, and gametocytes from a committed schizont are either all male or female,

*Corresponding Author: **Ismail Muhammad.**

suggesting sex determination occurs during schizont maturation. The ability to produce gametocytes is a genetically inherited trait, and parasite isolates vary in gametocyte conversion rates. Continuous passage without selection pressure for mosquito transmission can lead to spontaneous loss of gametocyte production, often accompanied by chromosomal aberrations. Defective *P. falciparum* lines have been used to identify genetic determinants of sexual development, aiding our understanding of gametocytogenesis (Zhenyu Liu, Jun Miao, 2018). Studies have shown that the malaria parasite's sex ratio bias towards females is due to a higher percentage of schizonts being directed towards female gametocyte production, compared to those allocated to male gametocyte production (Schneider & Reece, 2021).

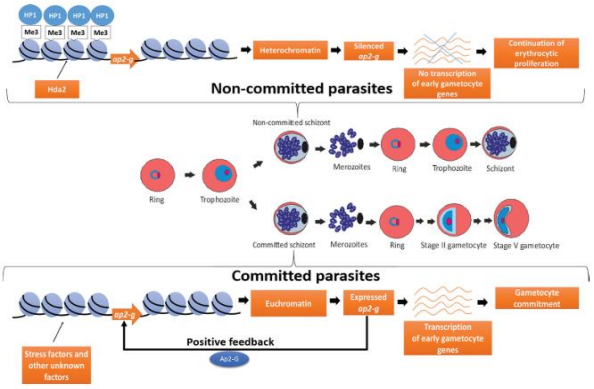


Fig 2.0: Committed and non-committed *plasmodium* parasites Reece et al. (2017)

Sex determination in *P. falciparum*.

Although the underlying molecular process for sex determination in *P. falciparum* is unknown (Gomes et al., 2022), it happens concurrently with or shortly after commitment to gametocyte development. Although the population of Plasmodium gametocytes is typically skewed towards females, host and environmental conditions can alter the sex ratio (Tao et al., 2014). The female gametocytes (FG) are ready for mitosis and the subsequent development of zygotes and ookinetes, while the male gametocytes (MG) are ready for rapid DNA replication and mitosis to produce eight motile microgametes once they are inside the mosquito midgut. The rodent parasite *P. berghei*'s MG- and FG-specific proteomes demonstrated unique sex-specific biology in line with this functional divide. The fact that 36% of the 650 MG proteome and 19% of the 541 FG proteome were found to be sex-specific highlights the parasite's sex selectivity. Surprisingly, only 69 proteins are shared by the sexes. The unique characteristics of *P. falciparum* gametocytes in terms of morphology, development, and sequestration imply notable differences in the induction, differentiation, and development of the two sexes in this parasite, even though conservation in gametocyte-specific gene sets across Plasmodium species predicts similarity in sex-specific biology in all malaria parasites. The inability to distinguish the two sexes plainly impedes attempts to analyse the molecular distinctions between MG and FG.

Because it mostly relied on species-species conservation and bioinformatic predictions, an attempt to determine the sex-partitioned proteomes of *P. falciparum* gametocytes may have overlooked crucial information explaining the actual biological distinctions between these species. The most recent report included a transcriptome analysis and a thorough proteome analysis of *P. falciparum* MG and FG that had been isolated. Significant differences between the sex-specific proteomes of MG and FG were observed, which is in line with their functional separation. The *P. falciparum* sex-specific proteomes showed significant inter-species changes that could explain the distinct biology of gametocytogenesis in these species, but they also showed an overall conservation of characteristics similar to those of *P. berghe* (Miao et al., 2017).

Genetic and Molecular Basis of Sex Determination

The first step in the sexual differentiation of *P. falciparum* is the commitment of a subset of merozoites to undergo gametocytogenesis, which is driven by transcription factors that switch the parasite from asexual replication to sexual development. The key regulators of this process include *PfAP2-G* and *PfAP2-I*, two transcription factors that play essential roles in controlling the expression of genes required for sexual commitment and gametocyte formation. These factors are activated by external environmental and host factors, such as immune system pressure or nutrient availability, which can modulate the parasite's decision to commit to sexual reproduction (Josling et al., 2017).

Once gametocytogenesis is initiated, the differentiation into male and female gametocytes is governed by further genetic regulation. One of the central genes involved in sex determination is *Pfap2-G*, a transcription factor that is specifically required for the differentiation of male gametocytes. *Pfap2-G* is expressed early in the process of gametocytogenesis and is essential for initiating the transcriptional program that leads to the development of male gametocytes. Conversely, *PfAP2-I* plays a critical role in the differentiation of female gametocytes. The expression of *PfAP2-I* leads to the activation of genes required for the maturation of *macrogametes*. These sex-differentiating transcription factors act as master switches, determining whether the gametocyte will develop into a male or female form (Gomes et al., 2022; Josling et al., 2017).

The Role of Chromatin and Epigenetic Regulation in Sex Determination

Sex determination in *P. falciparum* is not solely controlled by transcription factors; it also involves chromatin remodeling and epigenetic modifications that regulate gene expression. The regulation of these processes is essential to ensure that the genes involved in gametocyte development are properly expressed at the right time and place. Recent studies have revealed that chromatin changes are involved in the commitment of the parasite to sexual development. During the transition from asexual replication to sexual differentiation, there is a significant shift in histone modifications and DNA methylation, which enable the activation of sexual development-related genes and silence those associated with

*Corresponding Author: **Ismail Muhammad.**

asexual reproduction (Josling et al., 2018).

One notable protein involved in chromatin remodeling during sex determination is *PfSir2A*, a sirtuin protein that plays a role in regulating the chromatin structure and gene expression. *PfSir2A* acts by modifying histones, which leads to the repression of asexual genes and the activation of sexual development genes. The involvement of sirtuins and other chromatin-remodeling factors indicates that sex determination in *P. falciparum* is tightly controlled not only at the transcriptional level but also at the epigenetic level. These modifications ensure that the appropriate genes are expressed during gametocyte development and that the parasite can switch from asexual replication to sexual reproduction at the right time in its lifecycle (Gomes et al., 2022; Josling et al., 2018).

Environmental Regulation of Sex Determination

In addition to genetic and epigenetic regulation, environmental factors play a crucial role in sex determination. In *P. falciparum*, sex ratio regulation can be influenced by the conditions within the host, such as immune responses and levels of nutrients. For example, nutrient availability can affect the proportion of male and female gametocytes produced. Studies have shown that in the presence of certain stressors, such as low glucose or amino acid levels, *P. falciparum* may shift its gametocyte production toward male gametocytes in order to maximize the chances of fertilization when female gametocytes are scarce (Osgood & Schall, 2014). This environmental adaptability suggests that *P. falciparum* can modulate its sex ratio based on the available resources and the status of the infection, providing a strategic advantage in terms of transmission efficiency.

Furthermore, the sex ratio of *P. falciparum* gametocytes has been shown to be influenced by the genetic diversity of the parasite population. Experimental studies have demonstrated that in mixed-clone infections, the sex ratio can be skewed, with certain clones producing a higher proportion of male gametocytes. This shift in the sex ratio has been linked to competitive strategies within the parasite population, where clones with an advantage in producing male gametocytes may outcompete other clones, leading to an increased likelihood of fertilization and transmission to the mosquito vector (Osgood & Schall, 2014).

Genetic Factors Influencing the Sex Ratio

The regulation of the sex ratio is another important aspect of sex determination in *P. falciparum*. In *P. falciparum*, the sex ratio of gametocytes is not fixed; instead, it can vary depending on a range of factors, including genetic variation and environmental cues. The sex ratio is critical for ensuring that there is an appropriate balance between male and female gametocytes for fertilization to occur. Disruptions to this balance can lead to failed fertilization and a reduced likelihood of transmission to the mosquito vector.

Several genetic factors influence the sex ratio of *P. falciparum* gametocytes, including genes that regulate the expression of sex-determining transcription factors like *PfAP2-G* and *PfAP2-I*. Additionally, genes involved in gametogenesis, such as *PfGAM1*, which is required for gametocyte development, also play a role in shaping the sex ratio. By understanding these genetic factors, researchers can gain deeper insights into how *P. falciparum* modulates its sex ratio under different conditions, which could inform strategies for interrupting the transmission cycle of malaria (Mcrobert et al., 2018; Sinden, 2016).

Implications for Malaria Transmission and Control

Understanding the molecular and environmental regulation of sex determination in *P. falciparum* has significant implications for malaria transmission and control. The ability to influence the sex ratio of *P. falciparum* gametocytes could provide a potential strategy for reducing malaria transmission. For example, if the production of male gametocytes could be targeted, fertilization rates would be reduced, leading to fewer zygotes and thus fewer oocysts in the mosquito. This approach could effectively block transmission to the mosquito and, consequently, reduce the spread of malaria.

Several transmission-blocking interventions aim to target the process of sexual differentiation and gametocyte development. For instance, drugs that inhibit the production of *PfAP2-G* or *PfAP2-I* could potentially reduce the number of gametocytes produced, thereby reducing the likelihood of transmission to the mosquito vector. Additionally, genetic strategies aimed at altering the sex ratio could be used to skew the balance in favor of male gametocytes, limiting the chances of fertilization and the subsequent development of infective sporozoites in the mosquito (Stewart et al., 2022; Sinden, 2016).

Transcriptional and Post-transcriptional regulation during gametocytogenesis.

One important mechanism for regulating gene expression in malaria parasites has been proposed to be transcriptional regulation. Transcription-associated proteins (TAPs) are comparatively lacking in Plasmodium genomes when compared to those of free-living eukaryotes. The genome of *P. falciparum* contains 202 TAPs, which are classified as general, chromatin-related, and specialised transcription factors according to the most recent in silico and biological study. Thirteen of these TAPs are unique to stage V gametocytes, and at least 52 of them are expressed in these cells. The most prevalent TAPs in Plasmodium are zinc-finger proteins of the CCCH type (Eksi et al., 2012). Overall, chromatin-related factors involved in the epigenetic regulation of gene expression are highly represented, as are the general transcription factors linked to RNA polymerase II (Eksi et al., 2012). Overall, the general transcription factors associated with RNA polymerase II are well conserved, and chromatin-related factors involved in epigenetic regulation of gene expression are also highly represented.

Since it has addressed the lack of certain transcription factors in this group of parasites, the identification of a broad family of transcription factors in apicomplexan parasites that contain the plant Apetela2/ethylene response factor (AP2/ERF) domain is extremely noteworthy. The AP2 gene family in *P. falciparum* has 27 members. The importance of post-

*Corresponding Author: **Ismail Muhammad.**

transcriptional regulation in the expression of malaria parasite genes is becoming more and clearer. First, the average mRNA half-life rose dramatically during the late schizont stage, indicating variable post-transcriptional regulation of mRNA stability. Transcripts are also destroyed at varying rates during the Intraerythrocytic Developmental Cycle (IDC). Additionally, the translation efficiency may be impacted by the developmentally controlled and structurally distinct A and S kinds of malaria parasite ribosomal RNAs (rRNAs) (Josling et al., 2017).

S1-type rRNA in gametocytes and S2-type rRNA in mosquito stages take the role of the A-type rRNA pools that are predominant in asexual stage parasites. The abundance of most proteins peaks much later than the corresponding transcripts, suggesting that post-transcriptional control is in play. A comparison of the transcriptome and proteome data collected during the IDC shows a positive association between mRNA and protein abundance. Furthermore, the majority of the parasite's proteins undergo post-translational changes, the relevance of which has not yet been determined. For the "quiescent" mature gametocytes and sporozoites that await activation and the start of further developmental changes, translational regulation is particularly crucial.

Translationally suppressed variants of "maternal" transcripts that are required for later developmental stages are created and preserved throughout these phases. Although it has long been known that gametocytes are where the major ookinete surface antigen gene Pfs25 is transcribed, gametes and ookinetes are where most proteins are synthesised. A thorough worldwide examination of rodent parasites has found that a significant amount of the gametocyte transcriptome is not translated until gamete production and fertilisation in mosquitoes, suggesting that this occurrence is rather widespread (Liu et al., 2017)..

Commitment to Sexual Development

The transition from asexual replication to sexual reproduction in *P. falciparum* is triggered by a variety of host and parasite-derived signals. Sexual commitment is a critical decision point for the parasite, and it is regulated by several molecular and genetic mechanisms. One of the key regulators of this commitment is the transcription factor *PfAP2-G*, which is essential for the initiation of sexual differentiation. This protein controls the expression of numerous genes that are necessary for the formation of gametocytes, including those involved in both male and female differentiation (Josling et al., 2017). Without the action of *PfAP2-G*, the parasite cannot differentiate into sexual forms, and it remains in the asexual cycle.

Once *PfAP2-G* is activated, the parasite begins to express a distinct set of genes that guide the development of male and female gametocytes. In male gametocytes, this involves the activation of genes that are responsible for morphological changes and the accumulation of proteins specific to male gametocytes. The commitment to sexual reproduction is not only a genetic decision but is also influenced by environmental factors within the host, such as temperature and nutrient availability, which can modulate the rate and extent of gametocytogenesis (Miao et al., 2017).

Male Gametocyte Development

Male gametocytes undergo several stages of maturation before they are ready to be transmitted to the mosquito. These stages are characterized by distinct morphological and molecular changes that prepare the gametocytes for fertilization upon ingestion by the mosquito. In the early stages of gametocyte development, male gametocytes are small, morphologically similar to the asexual trophozoites, and possess few of the distinguishing features of the mature gametocytes. As they mature, they begin to accumulate specialized proteins and undergo a size increase.

The process of gametocytogenesis in *P. falciparum* involves several key steps. Early-stage male gametocytes, or *microgametocytes*, eventually undergo a transformation into the motile microgametes, which are capable of fertilizing female gametes in the mosquito's midgut. This process of maturation involves a series of intricate biochemical and molecular changes. For instance, the accumulation of *PfGAM1*, a key protein involved in gametogenesis, is critical for the maturation and functionality of male gametocytes. This protein is involved in processes that facilitate the parasite's transition from the gametocyte stage to the microgamete stage (Singh et al., 2019). Another essential molecular pathway involves the cGMP-dependent protein kinase (PKG), which plays a pivotal role in regulating gametogenesis. Upon ingestion of gametocytes by the mosquito, PKG activity is rapidly activated, leading to the development of motile microgametes. PKG is responsible for mediating the processes of exflagellation, where the microgametocyte divides into multiple microgametes, which are flagellated and capable of fertilizing the female gametes (Mcrobert et al., 2018). The motility of the microgametes is essential for successful fertilization in the mosquito's midgut.

Male gametocyte development is also tightly regulated by epigenetic modifications and chromatin remodeling. A study by Jeninga et al. (2023) demonstrated that the chromatin state of *P. falciparum* changes dramatically during sexual differentiation, with a global reshuffling of the genome that allows for the activation of gametocyte-specific genes. This chromatin remodeling process involves histone modifications that facilitate the transition to sexual stages, ensuring that the correct genes are expressed at the right time for successful gametocyte formation. These chromatin changes are particularly important for the expression of sex-specific genes that control male gametocyte differentiation and maturation.

The molecular regulation of male gametocyte development also involves a complex interplay between several signaling pathways. Transposon mutagenesis experiments have identified multiple genes essential for the development of male gametocytes. For example, *Pfgdv1*, a gene implicated in early gametocyte development, plays a significant role in the commitment of *P. falciparum* to sexual reproduction (Eksi et al., 2012). Disruption of genes like *Pfgdv1* has been shown to prevent proper gametocyte formation, highlighting the

*Corresponding Author: **Ismail Muhammad.**

importance of these genes in the successful development of male gametocytes.

Factors Affecting Male Gametocyte Development

Various factors can influence male gametocyte development, including genetic diversity and environmental stressors. The genetic diversity within *P. falciparum* populations can lead to variations in gametocyte production and sex ratios. A study by Osgood and Schall (2014) highlighted that genetic diversity within parasite populations could influence the ratio of male to female gametocytes, which has significant implications for transmission dynamics. A higher proportion of male gametocytes increases the likelihood of fertilization in the mosquito, thus enhancing transmission efficiency.

Environmental factors, such as drug pressure and host immune responses, also influence gametocyte development. The emergence of drug-resistant strains of *P. falciparum* has been associated with altered gametocyte production. For example, anti-malarial drug resistance can lead to upregulation of gametocytogenesis, as the parasite attempts to increase its transmission potential in response to selective pressure (Rajapandi, 2019). Similarly, changes in host immunity can affect the rate of gametocyte development, with immune responses potentially reducing gametocyte production or accelerating the transition to sexual stages.

Genetic and Epigenetic Regulation of Male Gametocyte Differentiation

The genetic and epigenetic regulation of male gametocyte differentiation is a complex process that involves multiple layers of control. In addition to the transcription factor *PfAP2-G*, which regulates the initial commitment to sexual reproduction, other transcription factors, such as *PfAP2-I*, have been shown to regulate the differentiation and maturation of male gametocytes. These transcription factors work in concert to ensure the proper timing and expression of gametocyte-specific genes (Josling et al., 2018). In addition to transcriptional regulation, epigenetic factors such as DNA methylation and histone modifications are essential for the regulation of gene expression during gametocytogenesis. These modifications play a critical role in controlling the switch from asexual to sexual development, ensuring that the genes required for male gametocyte development are activated at the right time and in the right sequence (Jeninga et al., 2023).

Role of Male Gametocytes in Malaria Transmission

The development of male gametocytes is a key determinant in the success of malaria transmission. Male gametocytes, once ingested by the mosquito, undergo exflagellation, a process where the microgametocyte divides into multiple motile microgametes. These microgametes swim toward and fertilize the female gametes, leading to the formation of a zygote, which eventually develops into an ookinete and penetrates the mosquito's midgut wall. The successful fertilization of the female gametes is essential for the continuation of the parasite lifecycle and the transmission of malaria to the next human host.

Transmission of malaria is heavily influenced by the number and viability of gametocytes in the human host. Higher gametocyte density, including a higher proportion of male gametocytes, increases the chances of successful fertilization and, consequently, malaria transmission (Sonden et al., 2021).

Female Gametocyte Development

The entire process of female gametocyte development, from commitment to fertilization, is tightly regulated by complex molecular mechanisms, including gene expression, protein synthesis, and signaling pathways. Gene expression plays a crucial role in female gametocyte development, with specific genes being expressed at distinct stages of development (Tao et al., 2014). The regulation of gene expression is mediated by transcriptional factors, such as AP2-G, which binds to specific DNA sequences to control gene expression. Protein synthesis is also essential, with proteins like Pfg377 being synthesized during female gametocyte development (Stewart et al., 2022). Signaling pathways, including the PI3K/Akt signaling pathway, also play a critical role in regulating female gametocyte development. These pathways transmit signals that control various cellular processes, including cell growth, differentiation, and survival. The tight regulation of these molecular mechanisms ensures the proper development of female gametocytes, which is essential for the transmission of the parasite (Chawla et al., 2021).

The development of female gametocytes in *Plasmodium falciparum* is a critical component of malaria transmission, enabling the parasite to propagate between the human host and the mosquito vector. Female gametocytes, upon maturation, differentiate into *macrogametes*, which, when fertilized by the male *microgametes* in the mosquito's midgut, lead to the formation of the zygote. This zygote will eventually develop into an ookinete and then an oocyst, completing the transmission cycle. The efficiency of female gametocyte development directly influences the success of malaria transmission, underscoring its importance in the lifecycle of the parasite (Gomes et al., 2022; Miao et al., 2017).

Commitment to Sexual Development

Female Gametocyte Development Stages

Once sexual differentiation is initiated, female gametocytes begin their progression through several maturation stages. Early-stage *macrogametes* resemble asexual parasites morphologically, but they gradually undergo significant changes that prepare them for fertilization in the mosquito's gut. These changes include an increase in size, structural reorganization, and the accumulation of proteins necessary for gamete function. The *macrogamete* eventually becomes distinct from the asexual form, with its cytoplasm and organelles reorganizing in preparation for fertilization by the male *microgamete* (Eksi et al., 2012).

One of the critical proteins involved in both male and female gametocyte development is *PfGAM1*. This protein is crucial for the differentiation and maturation of gametocytes and is expressed in both male and female forms. Studies have shown that *PfGAM1* is necessary for the correct structural development of gametocytes, including the formation of the

*Corresponding Author: **Ismail Muhammad.**

gametocyte cell membrane and cytoplasm. Although *PfGAM1* is shared between male and female gametocytes, its specific expression in female *macrogametes* helps to maintain the integrity and functionality of the gamete. The accumulation of such proteins ensures that the female gametocyte is fully prepared for fertilization when ingested by a mosquito (Miao et al., 2017; Josling et al., 2018).

As the female gametocyte matures, it becomes more specialized, and distinct sets of proteins are expressed. Proteomic studies have identified numerous proteins that are exclusive to female gametocytes, highlighting the uniqueness of the *macrogamete* and its readiness for fertilization. These proteins are involved in various processes, including cellular signaling, membrane fusion, and the stabilization of cellular components required for fertilization. Understanding the proteomics of female gametocytes has provided deeper insights into the specific molecular machinery involved in their maturation (Tao et al., 2014; Miao et al., 2017).

Molecular Regulation and Key Players

The molecular regulation of female gametocyte development is highly intricate, involving multiple transcription factors, kinases, and chromatin remodeling complexes. Apart from the aforementioned *PfAP2-G* and *PfAP2-I*, several other molecular elements, such as small RNAs and microRNAs, have been implicated in the fine-tuning of gene expression during gametocytogenesis. These regulatory molecules act by modulating the expression of genes that are required for the differentiation of both male and female gametocytes. For instance, specific microRNAs regulate the expression of genes involved in the cell cycle, apoptosis, and stress responses, which are crucial for maintaining the integrity of developing gametocytes (Stewart et al., 2022).

Furthermore, chromatin remodeling plays a critical role in the transition from asexual to sexual development. During gametocytogenesis, there is a notable shift in histone modifications and DNA methylation, which allows for the activation of genes required for sexual development. These epigenetic changes are facilitated by chromatin remodeling complexes, which adjust the chromatin structure to promote the expression of gametocyte-specific genes and silence those associated with asexual replication. This regulation ensures that the parasite can switch from asexual growth to sexual reproduction at the appropriate time, which is essential for maintaining the transmission cycle (Gomes et al., 2022; Josling et al., 2017).

The Role of Host Factors in Female Gametocyte Development

Host factors play an indispensable role in shaping the development of female gametocytes. During malaria infection, gametocytes are typically sequestered in the bone marrow, liver, and other protected tissues, allowing them to escape immune surveillance. This sequestration mechanism is crucial for the maturation of gametocytes, as it ensures that they are not destroyed by the host's immune system before reaching a mosquito vector. The release of mature female gametocytes into the bloodstream is often influenced by the immune environment within the host. For instance, malaria co-infections with other diseases such as HIV or tuberculosis can impact the immune response, potentially affecting the production and release of gametocytes (Sondo et al., 2021).

In addition to immune evasion, the nutritional environment of the host is another factor that influences gametocyte production. Nutrients such as glucose, amino acids, and lipids are essential for parasite growth and development, and their availability can modulate the rate at which gametocytes are produced. For example, low levels of glucose can suppress gametocyte formation, whereas the presence of certain amino acids can stimulate sexual differentiation. These host factors must be carefully coordinated to ensure that female gametocytes mature in time for transmission to the mosquito vector, and any disturbance in the host's physiological state can have direct consequences on transmission dynamics (Tao et al., 2014; Sondo et al., 2021).

Female Gametocytes and Malaria Transmission

The production of mature female gametocytes is vital for the transmission of malaria. Once ingested by a mosquito during a blood meal, the *macrogamete* undergoes fertilization by the male *microgametes*. This fertilization event leads to the formation of a zygote, which develops into an ookinete and later an oocyst in the mosquito's gut. The oocyst then releases sporozoites that migrate to the mosquito's salivary glands, completing the transmission cycle back to a new human host (McRobert et al., 2018).

The density of female gametocytes in the human host directly influences the transmission efficiency. A higher number of female gametocytes increases the likelihood of fertilization and successful transmission to the mosquito. As such, the ability of *P. falciparum* to efficiently produce viable female gametocytes is essential for maintaining malaria transmission in endemic areas. Strategies to control malaria transmission often target gametocyte development, and understanding the factors that influence female gametocyte production is key to these efforts. Factors such as drug resistance, immune responses, and host conditions all play a role in shaping the transmission dynamics of malaria. By gaining a deeper understanding of female gametocyte biology, researchers can develop more effective transmission-blocking interventions to combat malaria (Stewart et al., 2022; Sondo et al., 2021).

Reference

1. Chawla, J., Oberstaller, J., & Adams, J. H. (2021). Targeting gametocytes of the malaria parasite *Plasmodium falciparum* in a functional genomics era: Next steps. *Pathogens*, *10*(3), 1–22. https://doi.org/10.3390/pathogens10030346
2. Che Julius Ngwa, T. F. de A. R. and, Che Julius Ngwa, T. F. de A. R. and G. P., Pradel, G., & Additional. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Open Access*, *Chapter 7*, 13.
3. Dagnogo, O., Ako, A. B., Dago, N. D., Coulibaly, B., Toure, A. O., Djaman, J. A., Biosciences, U. F.

*Corresponding Author: **Ismail Muhammad.**

R., Boigny, U. F. H.-, Abidjan, B. P. V, & Ivoire, C. (2017). Comparative analysis of genomic DNA amplification yield for *Plasmodium falciparum* extracted from urine , saliva and blood. *Journal of Parasitology and Vector Biology*, *9*(July), 95–105. https://doi.org/10.5897/JPVB2017.0293

4. Eksi, S., Morahan, B. J., Haile, Y., Furuya, T., Jiang, H., Ali, O., Xu, H., Kiattibutr, K., Suri, A., Czesny, B., Adeyemo, A., Myers, T. G., Sattabongkot, J., Su, X., & Williamson, K. C. (2012). *Plasmodium falciparum* Gametocyte Development 1 (Pfgdv1) and Gametocytogenesis Early Gene Identification and Commitment to Sexual Development. *PLOS Pathogens*, *8*(10). https://doi.org/10.1371/journal.ppat.1002964
5. Gomes, A. R., Adjalley, S. H., Bardy, C., Cassan, C., Lee, M. C. S., & Talman, A. M. (2022). A transcriptional switch controls sex determination in *Plasmodium falciparum*. *Open Access*, *612*(December). https://doi.org/10.1038/s41586-022-05509-z
6. Ikadai, H., Shaw, K., Kanzok, S. M., Mclean, K. J., Tanaka, T. Q., & Cao, J. (2013). *Transposon mutagenesis identi fi es genes essential for Plasmodium falciparum gametocytogenesis*. *110*(18). https://doi.org/10.1073/pnas.1217712110
7. Jeninga, M. D., Tang, J., Selvarajah, S. A., Maier, A. G., Duffy, M. F., & Petter, M. (2023). *Plasmodium falciparum* gametocytes display global chromatin remodelling during sexual differentiation. *BMC Biology*, *21*(1), 1–29. https://doi.org/10.1186/s12915-023-01568-4
8. Josling, G. A., Russell, T. J., Venezia, J., Orchard, L., Painter, H. J., & Llinás, M. (2017). Dissecting the role of PfAP2-G in malaria gametocytogenesis. *Nature Communications*, *Nature com*(2020), 1–13. https://doi.org/10.1038/s41467-020-15026-0
9. Josling, G. A., Williamson, K. C., & Llinás, M. (2018). Regulation of Sexual Commitment and Gametocytogenesis in Malaria Parasites. *Annual Review of Microbiology*, *72*, 501–519. https://doi.org/10.1146/annurev-micro-090817-062712
10. Liu, of chromosomal aberrations [93–95]. M. *P. falciparum* lines that are defective in gametocytogenesis have been used to find the genetic of chromosomal aberrations [93–95]. M. *P. falciparum* lines that are defective in gametocytogenesis have been, Miao, J., & Cui, L. (2017). *Gametocytogenesis in malaria parasite: commitment, development and regulation*. *6*(11), 1351–1369. https://doi.org/10.2217/fmb.11.108.Gametocytogenesis
11. Mcrobert, L., Taylor, C. J., Deng, W., Fivelman, Q. L., Cummings, R. M., Polley, S. D., Billker, O., & Baker, D. A. (2018). Gametogenesis in Malaria Parasites Is Mediated by the cGMP-Dependent Protein Kinase. *PLoS Biol*, *6*(6). https://doi.org/10.1371/journal.pbio.0060139
12. Miao, J., Chen, Z., Wang, Z., Shrestha, S., Li, X., Li, R., & Cui, L. (2017). Sex-specific biology of the human malaria parasite revealed from the proteomes of mature male and female gametocytes. *Molecular and Cellular Proteomics*, *16*(4), 537–551. https://doi.org/10.1074/mcp.M116.061804
13. Mohammed, M. (2023). *Uncovering the genetics underlying host-parasite interactions during Plasmodium falciparum malaria transmission*.
14. Osgood, S. M., & Schall, J. J. (2014). Gametocyte sex ratio of a malaria parasite: Response to experimental manipulation of parasite clonal diversity. *Parasitology*, *128*(1), 23–29. https://doi.org/10.1017/S0031182003004207
15. Pandey, R., Abel, S., Boucher, M., Holder, A. A., Roch, K. G. Le, Tewari, R., Pandey, R., Abel, S., Boucher, M., Wall, R. J., Zeeshan, M., Rea, E., Batugedara, G., Hollin, T., Bottrill, A. R., Gupta, D., Holder, A. A., & Roch, K. G. Le. (2020). Mediate Atypical Mitosis and Are Essential for Parasite Proliferation and Transmission Plasmodium Condensin Core Subunits SMC2 / SMC4 Mediate Atypical Mitosis and Are Essential for Parasite Proliferation and Transmission. *CellReports*, *30*(6), 1883-1897.e6. https://doi.org/10.1016/j.celrep.2020.01.033
16. Pathak, A. K., Shiau, J. C., Thomas, M. B., & Murdock, C. C. (2018). Cryogenically preserved RBCs support gametocytogenesis of *Plasmodium falciparum* in vitro and gametogenesis in mosquitoes. *Malaria Journal*, *17*(:457), 1–15. https://doi.org/10.1186/s12936-018-2612-y
17. Pelleau, S., Moss, E. L., Dhingra, S. K., Volney, B., Casteras, J., & Gabryszewski, S. J. (2015). Adaptive evolution of malaria parasites in French Guiana : Reversal of chloroquine resistance by acquisition of a mutation in pfcrt. *Peer-Reviewed*, *Multidisciplinary Scientific Journal*, *112*(37). https://doi.org/10.1073/pnas.1507142112
18. Rajapandi, T. (2019). Upregulation of gametocytogenesis in anti-malarial drug-resistant *Plasmodium falciparum*. *Journal of Parasitic Diseases*, *43*(3), 458–463. https://doi.org/10.1007/s12639-019-01110-w
19. Schneider, P., & Reece, S. E. (2021). The private life of malaria parasites: Strategies for sexual reproduction. *Molecular and Biochemical Parasitology*, *244*(February), 111375. https://doi.org/10.1016/j.molbiopara.2021.111375
20. Simonetti, A. B. (2019). The Biology of Malarial Parasite in the Mosquito - A Review. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, *91*(May), 519–541.
21. Sinden, R. E. (2016). The biology of malaria transmission. *Recent Advances in Malaria*, 87–124. https://doi.org/10.1002/9781118493816.ch4
22. Singh, P., Alaganan, A., More, K. R., Lorthiois, A.,

*Corresponding Author: **Ismail Muhammad.**

Thiberge, S., & Gorgette, O. (2019). *Role of a patatin-like phospholipase in Plasmodium falciparum gametogenesis and malaria transmission*. 1–11. https://doi.org/10.1073/pnas.1900266116

23. Sondo, P., Bihoun, B., Tahita, M. C., Derra, K., Rouamba, T., Nakanabo Diallo, S., Kazienga, A., Ilboudo, H., Valea, I., Tarnagda, Z., Sorgho, H., Lefèvre, T., & Tinto, H. (2021). *Plasmodium falciparum* gametocyte carriage in symptomatic patients shows significant association with genetically diverse infections, anaemia, and asexual stage density. *Malaria Journal*, *20*(1). https://doi.org/10.1186/s12936-020-03559-0
24. Stewart, L. B., Freville, A., Voss, T. S., Baker, D. A., Awandare, G. A., & Conway, D. J. (2022). *Plasmodium falciparum* Sexual Commitment Rate Variation among Clinical Isolates and Diverse Laboratory-Adapted Lines. *Microbiology Spectrum*, *10*(6). https://doi.org/10.1128/spectrum.02234-22
25. Tao, D., Ubaida-Mohien, C., Mathias, D. K., King, J. G., Pastrana-Mena, R., Tripathi, A., Goldowitz, I., Graham, D. R., Moss, E., Marti, M., & Dinglasan, R. R. (2014). Sex-partitioning of the *Plasmodium falciparum* stage V gametocyte proteome provides insight into falciparum-specific cell biology. *Molecular and Cellular Proteomics*, *13*(10), 2705–2724. https://doi.org/10.1074/mcp.M114.040956
26. Zhenyu Liu, Jun Miao, and L. C. (2018). Gametocytogenesis in malaria parasite: commitment, development and regulation. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.4049/jimmunol.1801473.The

*Corresponding Author: **Ismail Muhammad.**