We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Animal Models of Zika Virus Sexual Transmission

Rafael K. Campos, Erin M. McDonald, Aaron C. Brault and Shannan L. Rossi

Abstract

ZIKV was first identified in the 1940s as a mosquito-borne virus; however, sexual transmission, which is uncommon for arboviruses, was demonstrated more than 60 years later. Tissue culture and animal models have allowed scientists to study how this transmission is possible. Immunocompromised mice infected with ZIKV had high viral loads in their testes, and infection of immunocompetent female mice was achieved following intravaginal inoculation or inoculation via mating with an infected male. These mouse studies lead researchers to investigate the individual components of the male reproductive system. In cell culture and mouse models, ZIKV can persist in Sertoli and germ cells of the testes and epithelial cells in the epididymis, which may lead to sexual transmission even after ZIKV has been cleared from other tissues. ZIKV has also been studied in nonhuman primates (NHPs), which appears to mimic the limited human epidemiological data, with low rates of symptomatic individuals and similar clinical signs. Although refinement is needed, these animal models have proven to be key in ZIKV research and continue to help uncovering the mechanisms of sexual transmission. This review will focus on the animal models used to elucidate the mechanisms of sexual transmission and persistence of flaviviruses.

Keywords: Zika virus, sexual transmission, animal models, human, primate, mouse, Sertoli cell, testes

1. Introduction

Zika virus (ZIKV) is a single-stranded, positive-sense virus of the genus *Flavivirus* of the family *Flaviviridae* that was discovered in the Ziika forest of Uganda in 1947 [1]. The virus was isolated during a surveillance campaign to attempt to identify yellow fever virus in the region. Sentinel rhesus macaques were placed in cages in the canopy layer of the trees and monitored daily for spikes in temperature. One of the rhesus macaques became febrile and was bled to isolate the causative agent of the fever. Serum from clarified blood caused illness when injected intra-cranially in white mice and the brain homogenates from these animals contained the first isolate of ZIKV, MR766. It is noteworthy that this strain, which is used in many contemporaneous studies, was passaged over 100 times in mice to increase its virulence in rodents. A second strain, ZIKV 758, was made from another rhesus macaque injected with homogenates of *Aedes africanus* mosquitoes collected in that same area. These data demonstrate that ZIKV caused febrile disease in NHPs

and could be isolated from mosquitoes, solidifying it as an arbovirus [1]. In the years since, many additional isolates have been made and the ones discussed in this review are listed in **Table 1**.

Prior to the major outbreaks in the 2000s, ZIKV had not been detected in the Americas and reported human cases of ZIKV-caused disease were scarce worldwide. Seroprevalence studies in Asia and Africa revealed human populations were exposed to the virus but disease was rarely reported, likely due to a high percentage of asymptomatic infection or because the signs and symptoms were indistinguishable from other known diseases [2]. Data collected during recent outbreaks in Yap Island and across the Americas revealed ZIKV is usually asymptomatic, with symptomatic cases being characterized by flu-like symptoms including self-limiting fever, headache, rash, and myalgia [3].

Worldwide outbreaks in Gabon, Micronesia and French Polynesia expanded the virus' known range and susceptible population, allowing prospective epidemiological studies. ZIKV likely arrived in the Americas in 2014, and in 2015 it quickly spread, starting its largest outbreaks to date [4, 5]. The large number of infected patients and heightened medical and scientific response allowed rare outcomes and transmission routes to be noticed. It was possible to identify that ZIKV causes congenital malformations [6]. Prior to this, cases of miscarriage, microcephaly and other congenital malformations, such as microcephaly or damage to brain and eye tissues, had been identified and were retrospectively observed to be correlated with infection [7, 8]. Infected travelers returning home initiated small traveler-associated transmission cycles, some of which were between sexual partners. Sexual transmission was proposed by Foy and colleagues, who described a probable case of ZIKV sexual transmission of a scientist that visited Senegal in 2008 [9]. Many more cases of sexual transmission were reported after the outbreaks in the Americas, confirming that sexual transmission played a role in the spread of ZIKV, although the full impact of sexual transmission may be underappreciated since it occurs concomitantly with the more efficient transmission by mosquito vectors [10–13].

ZIKV or viral RNA was found to persist in human semen, vaginal secretions and blood [14, 15]. Acquiring samples to study viral persistence in these tissues is difficult because ZIKV infection is frequently asymptomatic and identification of ZIKV-positive samples has to be done by serology or molecular biology, since other viruses that cause similar signs and symptoms are present in the same regions as

Strain	Year Isolation	Lineage	Location	Notes
MR766	1947	African	Ziika forest, Uganda	Contemporaneously available strains are heavily passaged in mice, altering their virulence
PRVABC59	2015	Asian (American)	Puerto Rico	Isolated from human serum during the American outbreak
FSS13025	2010	Asian	French Polynesia	Common isolate used collected prior to the American outbreak. Human isolate
IBH30656	1968	African	Nigeria	Isolated from human blood sample
H/PF/2013	2013	Asian	French Polynesia	Human isolate
BeH815744	2015	Asian (American)	Brazil	Human isolate

Table 1.
Commonly used ZIKV strains.

ZIKV. In Brazil, for example, there are several other flaviviruses and alphaviruses in circulation which produce clinical pictures that cannot be readily differentiated from the signs and symptoms associated with ZIKV. Therefore, molecular and serological tools are critical for the identification and classification of such samples [16]. In many locations, this requires a concerted collaborative effort between hospitals and laboratories, and many samples may need to be shipped before determining which patients came in contact with ZIKV. Additionally, sample collection is made difficult because once symptoms have rescinded, sample collections can no longer be made by hospitals and people may be opposed to having samples collected from them when they are no longer in need of medical care. In spite of these obstacles, several studies were able to assess ZIKV persistence in human samples. In semen, ZIKV RNA was detected for up to 6 months and ZIKV infectious particles could be isolated up to 1 month after primary infection [17]. Vaginal secretions were also found to be positive for RNA for up to 6 months after onset of symptoms [18]. These observations suggest sexual transmission could potentially happen even long after resolution of primary infection. Caution must be taken when trying to directly interpret the consequences of persistent viral RNA since this does not directly imply infectious viral particles. Regardless, transmission is possible, as demonstrated by a woman infecting her partner 44 days after onset of symptoms [13]. Although sexual transmission has most often been reported from males to females, there have been reports of suspected male-to-female [19], male-to-male [20] and female-to-male [21] transmissions. The actual rate of sexual transmission remains unknown.

Our current knowledge of the potential for sexual transmission among members of the *Flavivirus* genus is scarce but it appears that ZIKV is unique among this genus since most members are transmitted between hematophagous arthropods and vertebrate hosts. Although there is limited evidence that other flaviviruses could be sexually transmitted and persist in semen, the currently available body of data does not support this mode of transmission as significant for the spread of these viruses. West Nile virus (WNV) was proposed to have been possibly sexually transmitted in a clinical study of a woman who developed meningo-encephalitis by WNV 2 weeks after intercourse with an infected individual [22]. Other medically important flaviviruses, dengue virus (DENV) [23] and yellow fever virus (YFV) [24], have both been detected in human semen. DENV RNA was detected in a man returning from Thailand to Italy at 37 days post onset of symptoms, when virus RNA was not detectable in the serum or urine anymore [23]. Detection of DENV RNA in the seminal fluid is nevertheless not commonly reported, and another study that performed PCR in the semen of five man with acute DENV infection failed to detect any viral RNA [25]. YFV RNA was detected in urine and semen, but not in the serum, and the virus was isolated from urine on day 21 post onset the symptoms [24]. Another flavivirus, Japanese encephalitis virus (JEV), can be naturally found in porcine semen [26]. JEV was isolated from aborted fetus and semen of pigs which were naturally infected in China from 2004 to 2009 [27]. This raises the question of whether JEV also has the potential to reside in human testes and semen. Experimental approaches have also been used to determine the potential of flaviviruses to be sexually transmitted. Spondweni virus, a close relative to ZIKV, was detected in semen of mice lacking types I and III interferon (AG129) that were inoculated subcutaneously, although this was a rare occurrence when compared to ZIKV-infected AG129 mice [28]. Despite these clinical and experimental reports, the extent of the contribution of sexual transmission to the spread and maintenance of ZIKV in the human population remains unknown.

Examples of sexual transmission may be more common in the family *Flaviviridae* than in the genus *Flavivirus*, as exemplified by bovine viral diarrhea virus (pestivirus, BVDV) in cattle and hepatitis C virus (hepacivirus, HCV) in humans. Both of

these infections can cause persistent infections in their hosts under certain conditions and can be sexually transmitted. For BVDV, persistent infection was found in the testes, and bulls persistently shed viral particles in the seminal fluid which allows for sexual transmission [29–31]. For HCV, sexual transmission has been well-documented [32–35]; however, transmission is relatively inefficient [36] and increases with the number of partners, among human immunodeficient virus-positive (HIV+) individuals or among men who have sex with other men [37, 38]. Although there are similarities between ZIKV and these two viruses, caution must be exercised when drawing comparisons, especially when looking for viral determinants of persistence since there are multiple and substantial genomic differences between flaviviruses, pestiviruses and hepaciviruses.

The fact that ZIKV was shown to be sexually transmitted raised many questions, as many aspects of this mode of transmission are poorly understood for these viruses. Namely, the importance of sexual transmission for ZIKV in different regions of the world, the role of cells of the reproductive tract involved in ZIKV persistence and transmission, mutations in the virus which favor successful sexual transmission, interactions of ZIKV with the immune system in the reproductive tract and molecular interactions between ZIKV and host cells allowing for sexual transmission to take place, are all critical points of study. Although abundant data on human disease was generated during the recent outbreaks, animal experiments are an important tool to answer these questions because they allow controlled and rationally designed experiments which could ultimately lead to the development of new vaccines, therapeutics and prophylactic measures. This review will focus on what is currently known for ZIKV infection in the male reproductive tract.

2. Animal models of sexual transmission

Upon discovery of ZIKV, animal experiments were first conducted by Dick [39]. They found that white mice younger than 2 weeks were susceptible to ZIKV infections via intraperitoneal route, whereas mice older than 2 weeks were only susceptible to intracerebral inoculations [39]. Non-human primates inoculated subcutaneously developed neutralizing antibodies against ZIKV and only one animal presented a slight elevation in temperature [39, 40]. Several decades after this initial study, several other model species were used as animal models for ZIKV, but efforts were mostly focused on mice [41–43] and non-human primates (NHPs) [44–47], with most studies being conducted after the outbreak in the Americas in 2015–2016. The experimental design and analyses should carefully consider the anatomical and physiological differences from the species used to humans as well as confounding factors such as different viral strains and inoculation titer and route.

2.1 Mouse models

Mouse models of sexual transmission for ZIKV initially utilized either interferon receptor deficient mice lacking type I (A129) [41] and or type I and II (AG129) interferon receptors [42]. Other models included interferon responsive mouse strains for which a transient knock-down of interferon response was induced by exogenous treatment with antibody against murine type I interferon receptor of wild-type mice or $Rag1^{-/-}$ mice (lacking both B and T lymphocytes) [48]. Several of these models have demonstrated sexual transmission from needle-inoculated male mice to naive female mice following mating. These studies, coupled with the use of surrogate breeding females from which uteri were gavaged, allowed direct assessment of virus and viral RNA shedding efficiency over time. AG129 males infected

with ZIKV strain PRVABC59 were shown to shed infectious virus from 7 to 21 days post infection (dpi). Vasectomized males also were shown to shed virus; however, the magnitude was shown to be significantly lower compared to non-vasectomized males [42]. This finding was consistent with a previous study reporting ZIKV RNA shedding in symptomatic men infected with ZIKV in which vasectomized men shed significantly lower levels of ZIKV RNA [17].

The necessity for the use of mice lacking the ability to respond to interferon is due to the inherent resistance of murine STAT2 from being inhibited by ZIKV NS5 [49]. In contrast, human STAT2 has been demonstrated to be highly susceptible to antagonism by ZIKV NS5 [50]. As such, the subsequent development of humanized STAT2 mice have proven useful as a fully interferon responsive model for which ZIKV can replicate and induce pathogenic responses [51].

2.1.1 Sexual transmission murine models

A number of different models have been developed in order to directly assess sexual transmission Sexual transmission has been modeled via direct intravaginal inoculation. In one model, AG129 male mice euthanized and caudal epididymal lumen (containing sperm) was collected. This suspension was used to inoculate female AG129 mice. In this study, the antioxidant ebselen was used to alleviate testicular pathology caused by ZIKV. Although intravaginally inoculated female AG129 mice with sperm from male mice treated with ebselen demonstrated reduced mortality, sexual transmission was not prevented, as female organs (ovary/fallopian tubes, spleen and brain) were infected [52]. Other studies have used homogenized accessory gland fluid and epididymal lumen fluid from Ifnar1$^{-/-}$ male mice subcutaneously inoculated with PRVABC59 to intravaginally inoculated female AG129 mice. In this study, females became viremic and succumbed to infection. Furthermore, progesterone pre-treatment of female mice before intravaginal inoculation was shown to increase mortality of females [53]. IFNAR1$^{-/-}$ male mice inoculated subcutaneously with PRVABC59 and then at 14 dpi or 35 dpi, prostatic and seminal vesicular homogenates and epididymal flushes were collected. Female AG129 mice inoculated intravaginally with this insemination fluid failed to become viremic [54].

Intravaginal inoculation of di-estrus timed AG129 mice or LysMCre^{+}IFNAR$^{fl/fl}$ mice (lacking IFNAR in myeloid cells) was shown to result in viremia and virus replication in peripheral organs and in the vaginal tissues measured by viral assay by RT-PCR through 10 dpi [55]. In an alternative study, immunocompetent C57BL/6 N female mice intravaginally inoculated with PRVABC59 showed a slight increase in viral RNA in the lower female reproductive tract (LFRT) from dpi 1 to 2 and mRNA expression of type I and III IFNs, IRF3/7, RIG-I, and MDA-5 was comparable to uninfected controls, suggesting that a dampened antiviral immune response occurs in the LFRT in response to infection with ZIKV. When mice were treated with an enhancer of RIG-1 signaling, the increase in ZIKV RNA in LFRT was not observed [56]. After intravaginal inoculation during the diestrus phase, ZIKV was shown to replicate in vaginal mucosa of wild type (WT) C57BL/6 and Ifnar1$^{-/-}$ mice. Fetuses from pregnant WT C57BL/6 mice developed intrauterine growth restriction and fetal brains were infected following intravaginal ZIKV inoculation. Fetuses from pregnant IFNAR1$^{-/-}$ mice developed severe intrauterine growth restriction and fetal death was observed. The pregnant females were found to develop viremia. These data are suggestive of an ascending infection from the vaginal tissues to the uterus for fetal involvement [57].

In addition to sexual transmission models that have utilized intravaginal exposure to model sexual transmission potential, several studies have addressed transmission dynamics through direct coitus models. AG129 males were found to

sexually transmit to naïve AG129 females in 50% of all matings as measured by subsequent viremias in mated females. This initial study demonstrated *in utero* infection after sexual transmission [42]. A subsequent study compared sexual transmission with subcutaneous and intravaginal routes on female disease presentation, tropism and fetal infection. Sexual transmission of ZIKV to naïve female AG129 mice increased morbidity and mortality in these females as compared to female mice subcutaneously or intravaginally inoculated. Fetuses from females infected via sexual transmission had higher ZIKV titers compared to fetuses from pregnant females infected subcutaneously or intravaginally [58].

2.1.2 Potential sources of sexually transmitted virus/ZIKV infection of the murine testes and epididymides

The majority of ZIKV detected in the seminal fluid of infected AG129 mice during the peak timing of sexual transmission (10–12 dpi) was from the supernatant fraction, suggesting cell-free ZIKV may be largely responsible for sexual transmission. In this study, the testes and epididymides were determined to be infected concurrently and epididymal epithelial cells were identified as the predominant cell population infected in epididymides and shown to contain replicating ZIKV by *in situ* hybridization. In the testes, interstitial leukocytes and peritubular myoid cells were found to be infected initially, followed by extensive infection of all layers of the seminiferous tubule epithelium [59]. Similar results were also shown in IFNAR1$^{-/-}$ mice for which epithelial components of epididymides were identified to be infected and that testes and epididymides could be infected concurrently [60]. In another experiment with immune competent C57BL/6 mice treated with anti-IFNAR1 blocking antibody and subcutaneously inoculated with Asian and African genotype ZIKVs, sexual transmission potential was observed for all viruses with infectious virus identified in the epididymides from all groups even when infectious virus was absent from the testes and seminal vesicles. Infection of the epididymides was demonstrated to be critical for establishing sexual transmission potential, as infectious virus and viral RNA was detected in the epididymides and in semen days before infectious virus was detected in the seminal vesicles or testes [61].

Tissue restricted ZIKVs generated through the incorporation of microRNA target sequences within recombinant ZIKVs were utilized to assess the importance of different cell populations for sexual transmission potential. Testes-restricted ZIKVs could still infect the epididymides, demonstrating a hematogenous/lymphogenous route of infection. Epididymides-restricted ZIKV had high titers in epididymides by plaque assay, but immunohistochemical analysis confirmed epididymides-restricted ZIKV did not replicate in the epididymal epithelium, suggesting that ZIKV is transported from testes to epididymides via excurrent ducts in a cell-free form or transported in sloughed spermatids/infected luminal leukocytes, and ZIKV can infect the epididymides via hematogenous/lymphogenous route of infection [62].

2.1.3 Persistence and tropism of ZIKV on the male reproductive tract

Tropism and persistence of ZIKV in the male reproductive tract may be the key factor responsible for the presence of the virus in semen even long after initial infection. A model of the male mouse reproductive tract is shown in **Figure 1A**. Based on *in vivo* studies, ZIKV is thought to infect the testes, an immunologically privileged site, via a hematogenous route [62] and by infecting the Sertoli cells (SC, **Figure 1B**), an important cell population responsible for the formation of the blood-testes barrier, as shown using AG129 mouse models [63, 64]. SC are critical

for spermatogenesis, since they nourish germ cells and help them mature. Tight junctions formed by adjacent SC are functional components of the blood-testes barrier (**Figure 1C**), which prevents molecules from passing between the blood and the lumen of a seminiferous tubule. From the testes, ZIKV can then reach the epididymides by the excurrent testicular route either by infecting germ cells or as free virus particles [62]. ZIKV can also infect the epididymides directly from the hematogenous route [62] (**Figure 1**), and infection of epididymides was observed to happen concurrently with testes infection, indicating that epididymal infection could happen directly and through the testes, or independently of the testes. Infection of germ cells in the testes is not a requirement for sexual transmission as there are reported cases of ZIKV sexual transmission from vasectomized men [65] and in experiments with vasectomized AG129s [42]. Instead, AG129 mouse models indicate that infection of epididymal epithelial cells may the major factor leading to shedding of virus particles in the semen, with these cells being the predominant source of cell-free ZIKV in the seminal fluid [66].

Intracellular viral persistence is likely an important component for long-term sexual transmission. Although the cells that act as reservoirs of ZIKV in testes and the reproductive tract are unknown, possible reservoirs in the host are SC [67, 68] germ cells [69], Leydig cells [70] and epididymal epithelial cells [59, 62] which have been shown to support persistent infections of ZIKV (**Figure 1**). When primary SCs persistently infected with two strains of ZIKV (PRVABC59 or MR766) were monitored for a period of 6 weeks, it was found that 15% of the cells were still positive for both strains of ZIKV [67]. In this same study, Leydig cells were not observed to support persistence [67]. The mechanisms underlying persistence of ZIKV in testes are likely multifactorial and represent a complex phenomenon involving interactions between viral and host factors that needs to be studied in depth. The interactions that occur between ZIKV and host factors that are required for long-term infection of the testes/epididymides are poorly understood and viral persistence likely requires a balance between efficient viral replication and damage caused to host cells. The AXL receptor tyrosine kinase, which was previously shown to be required for entry of ZIKV and other flaviviruses into certain cell types, has been shown to be required for ZIKV entry in SCs [71], but may also be involved in negatively regulating SCs innate immune response [72]. ZIKV infection of SCs results in gene expression changes, with upregulation of antiviral pathways, dysregulation of junction and growth pathways [67, 73]. Although the immune

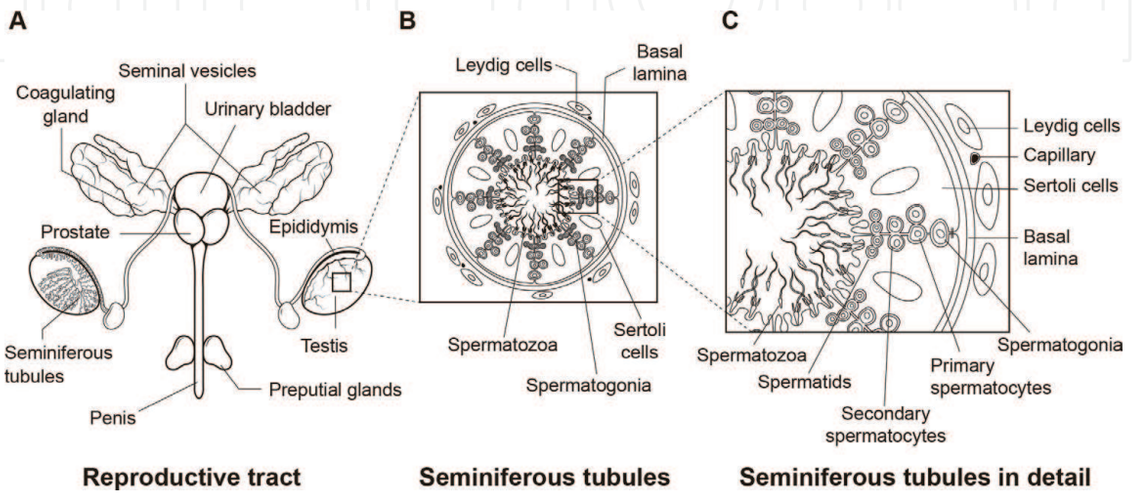

Figure 1.
The male reproductive tract. (A) Overall representation of the male reproductive tract. (B) Seminiferous tubules. (C) Seminiferous tubules in detail.

system of the reproductive tract in females is able to eventually clear ZIKV, largely due to interferon signaling [74, 75], such as the type III interferon lambda [76], the microenvironment of the male testes is an immune-privileged site that lacks adequate response for clearance [77].

2.1.4 Viral genetic changes associated with persistence

Mosquito-vectored flaviviruses that can result in persistent infections are usually associated with an accumulation of adaptive mutations, suggesting that viral genetics may also play an important role for establishing persistence. Examples of mutations in viruses causing persistence were shown with ZIKV, WNV [78–80], JEV [81], YFV [24, 82], and DENV [23]. These mutations happen in different viral proteins and appear to affect various viral functions, but in general attenuate the virus to cause less cytopathic effects, which is likely important for persistence. Identifying the mutations and cellular components that allow flaviviruses to cause long-term infections may elucidate mechanisms by which ZIKV is able to remain stable in seminal fluid long after infection. Although functional mutations in ZIKV may have been found in the male reproductive tract and ejaculates of mice [83], these were do not appear to be specific to these tissues as they were also found after passaging in Vero cells [83], and no mutation has been identified to be required for persistence and/or sexual transmission. Continuing research efforts using animal models are paramount to understand the mechanisms of ZIKV persistence, sexual transmission, reservoirs and interactions with cellular targets.

2.2 Non-human primate (NHP) models

NHP models are also frequently used to study ZIKV since their biology more closely relates to a human than does mouse biology. Several non-human primates were shown to be susceptible to ZIKV and used to study pathogenesis and infection of the reproductive tract, including cynomolgus macaques (*Macaca fascicularis*), rhesus macaques (*Macaca mulatta*), pigtail macaques (*Macaca nemestrina*) and olive baboon (*Papio anubis*), as well as neotropical non-human primates of the *Callithrix, Saimiri and Aotus* genera [46, 84]. These species presented viremia and variable degrees of clinical signs following ZIKV infection. Although many studies were conducted looking at ZIKV pathogenesis and the effects of infection on reproductive tissues, studies directly testing sexual transmission are lacking due to technical and logistical difficulties. Instead, vaginal or rectal inoculations have been used to simulate a sexual transmission. These and other insights into sexual transmission of non-human primates can also shed light on whether this type of transmission play a role in maintenance of ZIKV in nature, since primates are putative ZIKV reservoirs.

Because NHPs are evolutionarily close to humans, it is not surprising that these animals and their reproductive tract are anatomically and physiologically more similar to humans than other models such as mice; thus, NHPs are often considered to be one of the most relevant animal models [85, 86]. Unlike mice, immunocompetent NHPs are more suitable to study ZIKV infections, although the extent to which disease is mimicked is difficult to assess and varies with NHP species. Most NHP models were similar to humans in that most infections were asymptomatic, with clinical signs observed in some individuals [87, 88]. Considering that many human infections are asymptomatic, it is currently unclear if the clinical signs displayed by NHPs are a good model for disease rate and severity in humans. In a study using four cynomolgus macaques one individual was shown to present reduced body temperature [87]. Studying ZIKV pathogenesis in NHPs is important to understand disease

progression, clinical signs, negative effects to the reproductive tract and outcomes of pregnancy. These data also inform the design of sexual transmission studies as far as titers, timepoints and strain used for infection, tissues that are important for disease and persistence so sexual transmission can take place. It is worth noting that these experimental infections are performed using virus in a needle while most [89] natural infections occur by a mosquito bite; mosquito saliva has immunomodulatory properties that have been shown to enhance disease in other flaviviral infection [90–92] and could be another confounding factor in these studies.

2.2.1 Cynomolgus macaque

Cynomolgus macaques were shown to be suitable as models for ZIKV pathogenesis and sexual transmission [87, 88, 93]. Infection with 5 $\log_{10}$ of plaque forming units (PFU) of ZIKV from various geographical origins resulted in viremia which peaked at 2-4 dpi at 4-7 $\log_{10}$ ZIKV genome copies/ml [87, 88]. The outbreak strain PRVABC59 was more virulent than the Asian FSS13025 and the African IBH30656 ZIKV, with the animals infected with the PRVABC59 strain being viremic for extended periods of time. Bodily fluids checked did not include seminal fluid, and shedding of virus in the urine and saliva was not observed with either the FSS13025 [87] or IBH30656 strains. ZIKV FSS13025 was detected in testes [88], consistent with murine infections with this strain [41]. On the other hand, ZIKV PRVABC59 was detected in urine, saliva and testes [88]. The fact that virus was found in testes suggests this species of NHP could model virus persistence in testes well and may also be a positive feature of this model for sexual transmission in general. The cynomolgus macaque model has also been shown to likely support sexual transmission, as macaques inoculated with 7 $\log_{10}$ PFU of virus intravaginally and intrarectally became viremic 50% and 100% of the time, respectively [93]. To understand the implications of these findings would be important to know the ranges of ZIKV titers in the semen. Although it is not clear what the viral titers are in most human semen samples, studies detected up to 9 $\log_{10}$ RNA copies/ml of virus in semen of patients [17, 89].

2.2.2 Olive baboon

As with cynomolgus macaques, infection of olive baboons with different strains of ZIKV did not result in overt clinical signs. Following subcutaneous inoculation of a French Polynesian ZIKV (H/PF/2013), the baboons presented viremia that peaked at 3 and 4 dpi [45]. Around 40 days post infection, tissues were collected and virus was found in lymph nodes and epididymides, suggesting these are the places where the virus can persist even after viral clearance from the blood [45]. This suggests the testes were likely infected at some point and that the epididymides may also play a role in virus persistence [45]. Olive baboons were also used to model ZIKV infection during pregnancy. Infection of 4 $\log_{10}$ of ZIKV H/PF/2013 resulted in vertical transmission in 3 out of 4 pregnant NHPs [94]. Unlike the non-pregnant animals, all dams presented rash and conjunctivitis [94]. Fetal death and defects in the frontal cortex of the fetus were observed [94].

2.2.3 Rhesus macaque

The rhesus macaque is perhaps the most utilized NHP model to study ZIKV infection. Many studies of ZIKV were done using this species, including pathogenesis analyses in pregnant and non-pregnant animals, immunological and serological studies, and testing of anti-ZIKV vaccines or drugs [44, 95–106]. A study in which ZIKV was inoculated intravaginally in rhesus macaques to mimic sexual

transmission found ZIKV RNA in the reproductive tract of all 6 animals infected, thus raising the question of whether fetal disease could be more pronounced after sexual transmission when compared to vectored transmission [103]. This study lacked subcutaneous inoculation controls and further studies need to be conducted to confirm these findings. Another study comparing the intravaginal and subcutaneous routes using ZIKV PRVABC59 found peak viremia at 5–8 dpi that were variable in titer (3–7.5 $\log_{10}$ PFUs). Although the types of tissue found to be positive differed between subcutaneous and intravaginal inoculated animals, there was no obvious preference for reproductive tract tissues in the intravaginal route [107]. The data showed that intravaginal infection resulted in less $CD11C^{hi}$ myeloid cells, reduced expression of programmed cell death protein 1 (PD-1) in natural killer cells (NK) and more Ki67+ CD8+ central memory cells, indicating the route of infection may play a role in shaping the immune response [107].

Persistent ZIKV in reproductive tissues may play a role in sexual transmission long after primary infection. After intravenous inoculation of 5 $\log_{10}$ of a Brazilian ZIKV (BeH815744) in female rhesus macaques, ZIKV RNA was detected in multiple tissues of the NHPs, including reproductive tissues 14 dpi [108]. Lymphoid tissues had the highest detectable amount of viral RNA, suggesting these organs, which span many parts of the body, may act as possible viral reservoir [108].

2.3 Other animal models

Several other animal models have been used to study ZIKV but most are not focused on sexual transmission. These models include guinea pigs [109–111], hamsters [112], bats [113], chick embryos, piglets [114, 115] and boars [116]. Porcine fetuses were shown to present mild to severe neuropathology upon ZIKV infection [114, 115]. Boar semen was inoculated with ZIKV, but it was concluded that ZIKV does not appear to cause cell damage and cannot replicate efficiently or persist in the semen of this species [116]. With respect to the neotropical chiropteran model, viral RNA was found in different tissues of fruit bats 28 dpi, suggesting ZIKV can infect bats which may serve as virus reservoirs. Bats did not show any signs of disease [113]. Stat-2 knockout hamsters infected with ZIKV have shown presence on infected cells with morphology of SCs and spermatogonia, suggesting this could be a suitable model to study persistence of the virus in testes [112]. The guinea pig models report conflicting results. One study with ZIKV challenge mid-gestation showed no evidence of infection [109]; however, Kumar et al. show guinea pigs inoculated subcutaneously with PRVABC59 had viremia and presented signs such as fever, hunched posture and detectable viral RNA in the blood [110]. Deng et al. showed that intranasally-infected guinea pigs have virus in the sera, saliva and tears [111].

3. Conclusions

ZIKV has emerged explosively since 2007, causing an epidemic in the Americas in 2015/16 and become a matter of global health importance. Although data from epidemiologic analyses and case studies helped shed light on the diseases caused by ZIKV, animal models will be important to substantiate and extend these findings under controlled experimental settings. Animal models can be—and have already proven to be—useful as a tool to understand ZIKV transmission and ZIKV-caused illnesses. Animal models do not fully recapitulate diseases as seen in humans; therefore, it is critical to consider the advantages and drawbacks of each model when designing and executing the experiments as well as interpreting the data. Models

to study sexual transmission are currently scarce and need further development. Challenges include scarcity of good models to study ZIKV sexual transmission, low number of animals used and the requirement to improve reproducibility of the findings from animal models, which is caused by differences in experimental conditions and the number of animals used. As in certain cases increasing the number of animals used is not possible due to it being prohibitively expensive or posing ethical issues, future experiments assessing sexual transmission of ZIKV should focus on optimizing experimental design and analysis, when possible standardizing experimental conditions so they can be compared between studies.

Acknowledgements

We thank Drs. Sasha Azar and Nikos Vasilakis for their critical reading of the chapter and helpful discussions.

Conflict of interest

We have no conflicts of interest to declare.

Author details

Rafael K. Campos[1], Erin M. McDonald[2], Aaron C. Brault[2*] and Shannan L. Rossi[1*]

1 Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, USA

2 Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention (CDC), USA

*Address all correspondence to: zlu5@cdc.gov and slrossi@utmb.edu

References

[1] Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1952;**46**(5):509-520

[2] Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: Geographic expansion of the Asian lineage. PLoS Neglected Tropical Diseases. 2012;**6**(2):e1477

[3] Musso D, Gubler DJ. Zika virus. Clinical Microbiology Reviews. 2016;**29**(3):487-524

[4] Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: History, emergence, biology, and prospects for control. Antiviral Research. 2016;**130**:69-80

[5] Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerging Infectious Diseases. 2015;**21**(10):1885-1886

[6] Ventura CV, Maia M, Bravo-Filho V, Gois AL, Belfort R Jr. Zika virus in Brazil and macular atrophy in a child with microcephaly. Lancet. 2016;**387**(10015):228

[7] van der Eijk AA, van Genderen PJ, Verdijk RM, Reusken CB, Mogling R, van Kampen JJ, et al. Miscarriage associated with Zika virus infection. The New England Journal of Medicine. 2016;**375**(10):1002-1004

[8] Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al. Association between Zika virus and microcephaly in French Polynesia, 2013-15: A retrospective study. Lancet. 2016;**387**(10033):2125-2132

[9] Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerging Infectious Diseases. 2011;**17**(5):880-882

[10] Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerging Infectious Diseases. 2015;**21**(2):359-361

[11] D'Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, et al. Evidence of sexual transmission of Zika virus. The New England Journal of Medicine. 2016;**374**(22):2195-2198

[12] Freour T, Mirallie S, Hubert B, Splingart C, Barriere P, Maquart M, et al. Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin. 2016;**21**(23):30254

[13] Turmel JM, Abgueguen P, Hubert B, Vandamme YM, Maquart M, Le Guillou-Guillemette H, et al. Late sexual transmission of Zika virus related to persistence in the semen. Lancet. 2016;**387**(10037):2501

[14] Aliota MT, Bassit L, Bradrick SS, Cox B, Garcia-Blanco MA, Gavegnano C, et al. Zika in the Americas, year 2: What have we learned? What gaps remain? A report from the Global Virus Network. Antiviral Research. 2017;**144**:223-246

[15] Prisant N, Bujan L, Benichou H, Hayot PH, Pavili L, Lurel S, et al. Zika virus in the female genital tract. The Lancet Infectious Diseases. 2016;**16**(9):1000-1001

[16] Waggoner JJ, Pinsky BA. Zika virus: Diagnostics for an emerging pandemic

threat. Journal of Clinical Microbiology. 2016;**54**(4):860-867

[17] Mead PS, Duggal NK, Hook SA, Delorey M, Fischer M, Olzenak McGuire D, et al. Zika virus shedding in semen of symptomatic infected men. The New England Journal of Medicine. 2018;**378**(15):1377-1385

[18] Reyes Y, Bowman NM, Becker-Dreps S, Centeno E, Collins MH, Liou GA, et al. Prolonged shedding of Zika virus RNA in vaginal secretions, Nicaragua. Emerging Infectious Diseases. 2019;**25**(4):808-810

[19] Russell K, Hills SL, Oster AM, Porse CC, Danyluk G, Cone M, et al. Male-to-female sexual transmission of Zika virus-United States, January-April 2016. Clinical Infectious Diseases. 2017;**64**(2):211-213

[20] Deckard DT, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, et al. Male-to-male sexual transmission of Zika virus—Texas, January 2016. MMWR. Morbidity and Mortality Weekly Report. 2016;**65**(14):372-374

[21] Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. Suspected female-to-male sexual transmission of Zika virus—New York City, 2016. MMWR. Morbidity and Mortality Weekly Report. 2016;**65**(28):716-717

[22] Kelley RE, Berger JR, Kelley BP. West Nile virus Meningo-encephalitis: Possible sexual transmission. Journal of the Louisiana State Medical Society. 2016;**168**(1):21-22

[23] Lalle E, Colavita F, Iannetta M, Gebremeskel Tekle S, Carletti F, Scorzolini L, et al. Prolonged detection of dengue virus RNA in the semen of a man returning from Thailand to Italy, January 2018. Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin. 2018;**23**(18):18-00197

[24] Barbosa CM, Di Paola N, Cunha MP, Rodrigues-Jesus MJ, Araujo DB, Silveira VB, et al. Yellow fever virus RNA in urine and semen of convalescent patient, Brazil. Emerging Infectious Diseases. 2018;**24**(1):176-178

[25] Molton JS, Low I, Choy MMJ, Aw PPK, Hibberd ML, Tambyah PA, et al. Dengue virus not detected in human semen. Journal of Travel Medicine. 2018;**25**(1):1-3

[26] Althouse GC, Rossow K. The potential risk of infectious disease dissemination via artificial insemination in swine. Reproduction in Domestic Animals. 2011;**46**(Suppl 2):64-67

[27] Liu WJ, Zhu M, Pei JJ, Dong XY, Liu W, Zhao MQ, et al. Molecular phylogenetic and positive selection analysis of Japanese encephalitis virus strains isolated from pigs in China. Virus Research. 2013;**178**(2):547-552

[28] McDonald EM, Duggal NK, Brault AC. Pathogenesis and sexual transmission of Spondweni and Zika viruses. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0005990

[29] Rikula U, Nuotio L, Laamanen UI, Sihvonen L. Transmission of bovine viral diarrhoea virus through the semen of acutely infected bulls under field conditions. The Veterinary Record. 2008;**162**(3):79-82

[30] Newcomer BW, Toohey-Kurth K, Zhang Y, Brodersen BW, Marley MS, Joiner KS, et al. Laboratory diagnosis and transmissibility of bovine viral diarrhea virus from a bull with a persistent testicular infection. Veterinary Microbiology. 2014;**170**(3-4):246-257

[31] Voges H, Horner GW, Rowe S, Wellenberg GJ. Persistent bovine

pestivirus infection localized in the testes of an immuno-competent, non-viraemic bull. Veterinary Microbiology. 1998;**61**(3):165-175

[32] Sanchez-Quijano A, Rey C, Aguado I, Pineda JA, Perez-Romero M, Torres Y, et al. Hepatitis C virus infection in sexually promiscuous groups. European Journal of Clinical Microbiology & Infectious Diseases. 1990;**9**(8):610-612

[33] Salvaggio A, Conti M, Albano A, Pianetti A, Muggiasca ML, Re M, et al. Sexual transmission of hepatitis C virus and HIV-1 infection in female intravenous drug users. European Journal of Epidemiology. 1993;**9**(3):279-284

[34] Leruez-Ville M, Kunstmann JM, De Almeida M, Rouzioux C, Chaix ML. Detection of hepatitis C virus in the semen of infected men. Lancet. 2000;**356**(9223):42-43

[35] Sanchez-Beiza L, Bravo R, Toribio R, Navarro S, Soriano V. Sexual transmission of two different HCV types causing acute hepatitis C. Vox Sanguinis. 1996;**71**(4):244-245

[36] Marincovich B, Castilla J, del Romero J, Garcia S, Hernando V, Raposo M, et al. Absence of hepatitis C virus transmission in a prospective cohort of heterosexual serodiscordant couples. Sexually Transmitted Infections. 2003;**79**(2):160-162

[37] van de Laar TJ, van der Bij AK, Prins M, Bruisten SM, Brinkman K, Ruys TA, et al. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. The Journal of Infectious Diseases. 2007;**196**(2):230-238

[38] Dienstag JL. Sexual and perinatal transmission of hepatitis C. Hepatology. 1997;**26**(3 Suppl 1):66S-70S

[39] Dick GW. Zika virus. II. Pathogenicity and physical properties. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1952;**46**(5):521-534

[40] Taylor RM. Studies on certain viruses isolated in the tropics of Africa and South America; their growth and behavior in the embryonated hen egg. Journal of Immunology. 1952;**68**(4):473-494

[41] Rossi SL, Tesh RB, Azar SR, Muruato AE, Hanley KA, Auguste AJ, et al. Characterization of a novel murine model to study Zika virus. The American Journal of Tropical Medicine and Hygiene. 2016;**94**(6):1362-1369

[42] Duggal NK, Ritter JM, Pestorius SE, Zaki SR, Davis BS, Chang GJ, et al. Frequent Zika virus sexual transmission and prolonged viral RNA shedding in an Immunodeficient mouse model. Cell Reports. 2017;**18**(7):1751-1760

[43] Lazear HM, Govero J, Smith AM, Platt DJ, Fernandez E, Miner JJ, et al. A mouse model of Zika virus pathogenesis. Cell Host & Microbe. 2016;**19**(5):720-730

[44] Dudley DM, Aliota MT, Mohr EL, Weiler AM, Lehrer-Brey G, Weisgrau KL, et al. A rhesus macaque model of Asian-lineage Zika virus infection. Nature Communications. 2016;**7**:12204

[45] Gurung S, Preno AN, Dubaut JP, Nadeau H, Hyatt K, Reuter N, et al. Translational model of Zika virus disease in baboons. Journal of Virology. 2018;**92**(16):e00186-18

[46] Vanchiere JA, Ruiz JC, Brady AG, Kuehl TJ, Williams LE, Baze WB, et al. Experimental Zika virus infection of neotropical primates. The American Journal of Tropical Medicine and Hygiene. 2018;**98**(1):173-177

[47] Osuna CE, Lim SY, Deleage C, Griffin BD, Stein D, Schroeder LT, et al. Zika viral dynamics and shedding in rhesus and cynomolgus macaques. Nature Medicine. 2016;**22**(12):1448-1455

[48] Winkler CW, Myers LM, Woods TA, Messer RJ, Carmody AB, McNally KL, et al. Adaptive immune responses to Zika virus are important for controlling virus infection and preventing infection in brain and testes. Journal of Immunology. 2017;**198**(9):3526-3535

[49] Tripathi S, Balasubramaniam VR, Brown JA, Mena I, Grant A, Bardina SV, et al. A novel Zika virus mouse model reveals strain specific differences in virus pathogenesis and host inflammatory immune responses. PLoS Pathogens. 2017;**13**(3):e1006258

[50] Grant A, Ponia SS, Tripathi S, Balasubramaniam V, Miorin L, Sourisseau M, et al. Zika virus targets human STAT2 to inhibit type I interferon signaling. Cell Host & Microbe. 2016;**19**(6):882-890

[51] Gorman MJ, Caine EA, Zaitsev K, Begley MC, Weger-Lucarelli J, Uccellini MB, et al. An immuno-competent mouse model of Zika virus infection. Cell Host & Microbe. 2018;**23**(5):672-685 e6

[52] Simanjuntak Y, Liang JJ, Chen SY, Li JK, Lee YL, Wu HC, et al. Ebselen alleviates testicular pathology in mice with Zika virus infection and prevents its sexual transmission. PLoS Pathogens. 2018;**14**(2):e1006854

[53] Clancy CS, Van Wettere AJ, Morrey JD, Julander JG. Coitus-free sexual transmission of Zika virus in a mouse model. Scientific Reports. 2018;**8**(1):15379

[54] Clancy CS, Van Wettere AJ, Morrey JD, Julander JG. Zika virus associated pathology and antigen presence in the testicle in the absence of sexual transmission during subacute to chronic infection in a mouse model. Scientific Reports. 2019;**9**(1):8325

[55] Tang WW, Young MP, Mamidi A, Regla-Nava JA, Kim K, Shresta S. A mouse model of Zika virus sexual transmission and vaginal viral replication. Cell Reports. 2016;**17**(12):3091-3098

[56] Khan S, Woodruff EM, Trapecar M, Fontaine KA, Ezaki A, Borbet TC, et al. Dampened antiviral immunity to intravaginal exposure to RNA viral pathogens allows enhanced viral replication. The Journal of Experimental Medicine. 2016;**213**(13):2913-2929

[57] Yockey LJ, Varela L, Rakib T, Khoury-Hanold W, Fink SL, Stutz B, et al. Vaginal exposure to Zika virus during pregnancy leads to fetal brain infection. Cell. 2016;**166**(5): 1247-1256 e4

[58] Duggal NK, McDonald EM, Ritter JM, Brault AC. Sexual transmission of Zika virus enhances in utero transmission in a mouse model. Scientific Reports. 2018;**8**(1):4510

[59] McDonald EM, Duggal NK, Ritter JM, Brault AC. Infection of epididymal epithelial cells and leukocytes drives seminal shedding of Zika virus in a mouse model. PLoS Neglected Tropical Diseases. 2018;**12**(8):e0006691

[60] Clancy CS, Van Wettere AJ, Siddharthan V, Morrey JD, Julander JG. Comparative histopathologic lesions of the male reproductive tract during acute infection of Zika virus in AG129 and Ifnar(−/−) mice. The American Journal of Pathology. 2018;**188**(4):904-915

[61] McDonald EM, Duggal NK, Delorey MJ, Oksanish J, Ritter JM, Brault AC. Duration of seminal Zika viral RNA shedding in immunocompetent mice inoculated with Asian and

African genotype viruses. Virology. 2019;**535**:1-10

[62] Tsetsarkin KA, Maximova OA, Liu G, Kenney H, Teterina N, Bloom ME, et al. Routes of Zika virus dissemination in the testis and epididymis of immunodeficient mice. Nature Communications. 2018;**9**(1):5350

[63] Siemann DN, Strange DP, Maharaj PN, Shi PY, Verma S. Zika virus infects human sertoli cells and modulates the integrity of the in vitro blood-testis barrier model. Journal of Virology. 2017;**91**(22):e00623-17

[64] Sheng ZY, Gao N, Wang ZY, Cui XY, Zhou DS, Fan DY, et al. Sertoli cells are susceptible to ZIKV infection in mouse testis. Frontiers in Cellular and Infection Microbiology. 2017;**7**:272

[65] Arsuaga M, Bujalance SG, Diaz-Menendez M, Vazquez A, Arribas JR. Probable sexual transmission of Zika virus from a vasectomised man. The Lancet Infectious Diseases. 2016;**16**(10):1107

[66] Ma W, Li S, Ma S, Jia L, Zhang F, Zhang Y, et al. Zika virus causes testis damage and leads to male infertility in mice. Cell. 2016;**167**(6):1511-1524 e10

[67] Kumar A, Jovel J, Lopez-Orozco J, Limonta D, Airo AM, Hou S, et al. Human Sertoli cells support high levels of Zika virus replication and persistence. Scientific Reports. 2018;**8**(1):5477

[68] Mlera L, Bloom ME. Differential Zika virus infection of testicular cell lines. Viruses. 2019;**11**(1):E42

[69] Robinson CL, Chong ACN, Ashbrook AW, Jeng G, Jin J, Chen H, et al. Male germ cells support long-term propagation of Zika virus. Nature Communications. 2018;**9**(1):2090

[70] Uraki R, Hwang J, Jurado KA, Householder S, Yockey LJ, Hastings AK, et al. Zika virus causes testicular atrophy. Science Advances. 2017;**3**(2):e1602899

[71] Strange DP, Jiyarom B, Pourhabibi Zarandi N, Xie X, Baker C, Sadri-Ardekani H, et al. Axl promotes Zika virus entry and modulates the antiviral state of human sertoli cells. MBio. 2019;**10**(4):e01372-19

[72] Sun B, Qi N, Shang T, Wu H, Deng T, Han D. Sertoli cell-initiated testicular innate immune response through toll-like receptor-3 activation is negatively regulated by Tyro3, Axl, and mer receptors. Endocrinology. 2010;**151**(6):2886-2897

[73] Strange DP, Green R, Siemann DN, Gale M Jr, Verma S. Immunoprofiles of human Sertoli cells infected with Zika virus reveals unique insights into host-pathogen crosstalk. Scientific Reports. 2018;**8**(1):8702

[74] Fung KY, Mangan NE, Cumming H, Horvat JC, Mayall JR, Stifter SA, et al. Interferon-epsilon protects the female reproductive tract from viral and bacterial infection. Science. 2013;**339**(6123):1088-1092

[75] Cumming HE, Bourke NM. Type I IFNs in the female reproductive tract: The first line of defense in an ever-changing battleground. Journal of Leukocyte Biology. 2019;**105**(2):353-361

[76] Caine EA, Scheaffer SM, Arora N, Zaitsev K, Artyomov MN, Coyne CB, et al. Interferon lambda protects the female reproductive tract against Zika virus infection. Nature Communications. 2019;**10**(1):280

[77] Arevalo Romero H, Vargas Pavia TA, Velazquez Cervantes MA, Flores Pliego A, Helguera Repetto AC, Leon Juarez M. The dual role of the immune

response in reproductive organs during Zika virus infection. Frontiers in Immunology. 2019;**10**:1617

[78] Rossi SL, Zhao Q, O'Donnell VK, Mason PW. Adaptation of West Nile virus replicons to cells in culture and use of replicon-bearing cells to probe antiviral action. Virology. 2005;**331**(2):457-470

[79] Rossi SL, Fayzulin R, Dewsbury N, Bourne N, Mason PW. Mutations in West Nile virus nonstructural proteins that facilitate replicon persistence in vitro attenuate virus replication in vitro and in vivo. Virology. 2007;**364**(1):184-195

[80] Lustig Y, Lanciotti RS, Hindiyeh M, Keller N, Milo R, Mayan S, et al. Mutation in West Nile virus structural protein prM during human infection. Emerging Infectious Diseases. 2016;**22**(9):1647-1649

[81] Lyoo HR, Park SY, Kim JY, Jeong YS. Constant up-regulation of BiP/GRP78 expression prevents virus-induced apoptosis in BHK-21 cells with Japanese encephalitis virus persistent infection. Virology Journal. 2015;**12**:32

[82] Vlaycheva L, Nickells M, Droll DA, Chambers TJ. Yellow fever 17D virus: Pseudo-revertant suppression of defective virus penetration and spread by mutations in domains II and III of the E protein. Virology. 2004;**327**(1):41-49

[83] Duggal NK, McDonald EM, Weger-Lucarelli J, Hawks SA, Ritter JM, Romo H, et al. Mutations present in a low-passage Zika virus isolate result in attenuated pathogenesis in mice. Virology. 2019;**530**:19-26

[84] Terzian ACB, Zini N, Sacchetto L, Rocha RF, Parra MCP, Del Sarto JL, et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. Scientific Reports. 2018;**8**(1):16034

[85] Kerr JB. Functional cytology of the human testis. Baillière's Clinical Endocrinology and Metabolism. 1992;**6**(2):235-250

[86] Fayomi AP, Orwig KE. Spermatogonial stem cells and spermatogenesis in mice, monkeys and men. Stem Cell Research. 2018;**29**:207-214

[87] Azar SR, Rossi SL, Haller SH, Yun R, Huang JH, Plante JA, et al. ZIKV demonstrates minimal pathologic effects and mosquito infectivity in viremic cynomolgus macaques. Viruses. 2018;**10**(11):E661

[88] Koide F, Goebel S, Snyder B, Walters KB, Gast A, Hagelin K, et al. Development of a Zika virus infection model in cynomolgus macaques. Frontiers in Microbiology. 2016;**7**:2028

[89] de Laval F, Matheus S, Labrousse T, Enfissi A, Rousset D, Briolant S. Kinetics of Zika viral load in semen. The New England Journal of Medicine. 2017;**377**(7):697-699

[90] Surasombatpattana P, Patramool S, Luplertlop N, Yssel H, Misse D. Aedes aegypti saliva enhances dengue virus infection of human keratinocytes by suppressing innate immune responses. The Journal of Investigative Dermatology. 2012;**132**(8):2103-2105

[91] Styer LM, Lim PY, Louie KL, Albright RG, Kramer LD, Bernard KA. Mosquito saliva causes enhancement of West Nile virus infection in mice. Journal of Virology. 2011;**85**(4):1517-1527

[92] Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;**102**(5):400-408

[93] Haddow AD, Nalca A, Rossi FD, Miller LJ, Wiley MR, Perez-Sautu U, et al. High infection rates for adult macaques after intravaginal or intrarectal inoculation with Zika virus. Emerging Infectious Diseases. 2017;**23**(8):1274-1281

[94] Gurung S, Reuter N, Preno A, Dubaut J, Nadeau H, Hyatt K, et al. Zika virus infection at mid-gestation results in fetal cerebral cortical injury and fetal death in the olive baboon. PLoS Pathogens. 2019;**15**(1):e1007507

[95] Maness NJ, Schouest B, Singapuri A, Dennis M, Gilbert MH, Bohm RP, et al. Postnatal Zika virus infection of nonhuman primate infants born to mothers infected with homologous Brazilian Zika virus. Scientific Reports. 2019;**9**(1):12802

[96] Valiant WG, Mattapallil MJ, Higgs S, Huang YS, Vanlandingham DL, Lewis MG, et al. Simultaneous coinfection of macaques with Zika and dengue viruses does not enhance acute plasma viremia but leads to activation of monocyte subsets and biphasic release of pro-inflammatory cytokines. Scientific Reports. 2019;**9**(1):7877

[97] Bidokhti MRM, Dutta D, Madduri LSV, Woollard SM, Norgren R Jr, Giavedoni L, et al. SIV/SHIV-Zika co-infection does not alter disease pathogenesis in adult non-pregnant rhesus macaque model. PLoS Neglected Tropical Diseases. 2018;**12**(10):e0006811

[98] Coffey LL, Keesler RI, Pesavento PA, Woolard K, Singapuri A, Watanabe J, et al. Intraamniotic Zika virus inoculation of pregnant rhesus macaques produces fetal neurologic disease. Nature Communications. 2018;**9**(1):2414

[99] Mavigner M, Raper J, Kovacs-Balint Z, Gumber S, O'Neal JT, Bhaumik SK, et al. Postnatal Zika virus infection is associated with persistent abnormalities in brain structure, function, and behavior in infant macaques. Science Translational Medicine. 2018;**10**(435):eaao6975

[100] Martinot AJ, Abbink P, Afacan O, Prohl AK, Bronson R, Hecht JL, et al. Fetal neuropathology in Zika virus-infected pregnant female rhesus monkeys. Cell. 2018;**173**(5):1111-1122 e10

[101] Hirsch AJ, Roberts VHJ, Grigsby PL, Haese N, Schabel MC, Wang X, et al. Zika virus infection in pregnant rhesus macaques causes placental dysfunction and immunopathology. Nature Communications. 2018;**9**(1):263

[102] Abbink P, Larocca RA, Visitsunthorn K, Boyd M, De La Barrera RA, Gromowski GD, et al. Durability and correlates of vaccine protection against Zika virus in rhesus monkeys. Science Translational Medicine. 2017;**9**(420):eaao4163

[103] Carroll T, Lo M, Lanteri M, Dutra J, Zarbock K, Silveira P, et al. Zika virus preferentially replicates in the female reproductive tract after vaginal inoculation of rhesus macaques. PLoS Pathogens. 2017;**13**(7):e1006537

[104] Nguyen SM, Antony KM, Dudley DM, Kohn S, Simmons HA, Wolfe B, et al. Highly efficient maternal-fetal Zika virus transmission in pregnant rhesus macaques. PLoS Pathogens. 2017;**13**(5):e1006378

[105] Aliota MT, Dudley DM, Newman CM, Mohr EL, Gellerup DD, Breitbach ME, et al. Heterologous protection against Asian Zika virus challenge in rhesus macaques. PLoS Neglected Tropical Diseases. 2016;**10**(12):e0005168

[106] Best K, Guedj J, Madelain V, de Lamballerie X, Lim SY, Osuna CE, et al.

Zika plasma viral dynamics in nonhuman primates provides insights into early infection and antiviral strategies. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(33):8847-8852

[107] Woollard SM, Olwenyi OA, Dutta D, Dave RS, Mathews S, Gorantla S, et al. Preliminary studies on immune response and viral pathogenesis of Zika virus in rhesus macaques. Pathogens. 2018;**7**(3):E70

[108] Coffey LL, Pesavento PA, Keesler RI, Singapuri A, Watanabe J, Watanabe R, et al. Zika virus tissue and blood compartmentalization in acute infection of rhesus macaques. PLoS One. 2017;**12**(1):e0171148

[109] Bierle CJ, Fernandez-Alarcon C, Hernandez-Alvarado N, Zabeli JC, Janus BC, Putri DS, et al. Assessing Zika virus replication and the development of Zika-specific antibodies after a mid-gestation viral challenge in Guinea pigs. PLoS One. 2017;**12**(11):e0187720

[110] Kumar M, Krause KK, Azouz F, Nakano E, Nerurkar VR. A Guinea pig model of Zika virus infection. Virology Journal. 2017;**14**(1):75

[111] Deng YQ, Zhang NN, Li XF, Wang YQ, Tian M, Qiu YF, et al. Intranasal infection and contact transmission of Zika virus in Guinea pigs. Nature Communications. 2017;**8**(1):1648

[112] Siddharthan V, Van Wettere AJ, Li R, Miao J, Wang Z, Morrey JD, et al. Zika virus infection of adult and fetal STAT2 knock-out hamsters. Virology. 2017;**507**:89-95

[113] Malmlov A, Bantle C, Aboellail T, Wagner K, Campbell CL, Eckley M, et al. Experimental Zika virus infection of Jamaican fruit bats (*Artibeus jamaicensis*) and possible entry of virus into brain via activated microglial cells. PLoS Neglected Tropical Diseases. 2019;**13**(2):e0007071

[114] Wichgers Schreur PJ, van Keulen L, Anjema D, Kant J, Kortekaas J. Microencephaly in fetal piglets following in utero inoculation of Zika virus. Emerging Microbes and Infections. 2018;**7**(1):42

[115] Darbellay J, Cox B, Lai K, Delgado-Ortega M, Wheler C, Wilson D, et al. Zika virus causes persistent infection in porcine conceptuses and may impair health in offspring. eBioMedicine. 2017;**25**:73-86

[116] Luplertlop N, Suwanmanee S, Ampawong S, Vongpunsawad S, Poovorawan Y. In vitro study of Zika virus infection in boar semen. Archives of Virology. 2017;**162**(10):3209-3213