Molecular Mechanisms of ZIKV-Induced Teratogenesis: A Systematic Review of Studies in Animal Models

Julia A. Gomes1,2 · Gabriela E. Wachholz3 · Juliano A. Boquett4,5 · Fernanda S. L. Vianna1,2,4,6 · Lavínia Schuler-Faccini1,4,6 · Lucas R. Fraga1,2,6,7

Received: 1 December 2021 / Accepted: 21 September 2022 / Published online: 10 October 2022 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Zika virus (ZIKV) is a teratogen that causes congenital anomalies, being linked to microcephaly in children exposed during pregnancy. Animal studies have been conducted to investigate the molecular mechanisms related to ZIKV teratogenesis. Although animal models can mimic the effects of ZIKV in human embryo development, few in vivo studies have addressed molecular changes following ZIKV infection in embryos. Moreover, few literature reviews have been conducted with these studies. The aim of this systematic review is to evaluate the molecular mechanisms of ZIKV teratogenesis determined from studies in animal models. PubMed/MEDLINE, EMBASE, Web of Science, and Scopus as well as grey literature were searched for studies that evaluated molecular alterations related to ZIKV teratogenesis which occurred during embryonic development. Nine studies were included: six with mice, one with mice and guinea pigs, one with pigs and one with chickens. In general, studies presented an unclear or high risk of bias for methodological criteria. Most of studies reported embryos exposed to ZIKV presenting microcephaly, reduced cortex thickness, and growth restriction. Different techniques were used to evaluated molecular changes in the animals following ZIKV infection: RNA sequencing, RT-qPCR, and in situ hybridization. It was found that common pathways are changed in most studies, being pathways related to immune response upregulated and those involved to neurodevelopment downregulated.

Keywords Zika virus infection · Congenital Zika syndrome · Congenital abnormalities · Gene expression · Molecular techniques · Molecular pathway · Nervous system development · Embryo

Introduction
Zika virus (ZIKV) was first isolated from Rhesus monkeys in the Zika Forest (Uganda) in 1947 and the first cases of human infection were reported in Nigeria in 1954 [1, 2]. The first major ZIKV outbreak was documented on Yap Island, Micronesia in 2007, followed by outbreaks in French Polynesia (2013) and Brazil (2015) [3–6]. Since then, ZIKV has spread throughout the Americas [7, 8]. Although the number of new cases has declined in the past few years, the persistent ZIKV transmission is still a risk for pregnant women. In addition, the vector *Aedes aegypti* adaptation to different
environments mostly related to the global warming can also represent a risk for possible new future outbreaks [9–12].

ZIKV is a teratogenic agent, and the infection during pregnancy can lead to a set of abnormalities in the exposed embryo or fetus. This set of abnormalities are mainly related to the impairment of central nervous system development and is named congenital Zika syndrome (CZS) [13, 14]. Two different linages of ZIKV are known, an Asian and an African [15]. The Asian lineage is the one related to the occurrence of CZS in humans [16]. The African lineage, on the other hand, has been showed, until the moment, to be related to congenital anomalies in experimental models [12, 17].

Although many studies have investigated how ZIKV leads to teratogenic outcomes, little is known about the precise molecular mechanisms related to CZS. To this end, several in vitro and in vivo studies have been conducted to investigate the teratogenic mechanisms of ZIKV [18–23]. The main molecular approaches of these studies include genomic, transcriptomic, and epigenomic evaluations after ZIKV infection [18–23]. Such methodologies seek to identify molecular targets and pathways related to ZIKV infection and teratogenesis.

Studies in animal models are important for developing hypotheses and strategies for both prevention of adverse outcomes related to ZIKV infection and health care for the damages that it causes in the individuals. Furthermore, animal models can well represent human infection, since several cell types are exposed and affected by the virus at the same time during the infection. However, to a lesser extent, in vivo studies have been used to investigate the molecular mechanisms of ZIKV teratogenesis [20, 24, 25].

To date, there have been few literature reviews of in-depth studies involving molecular methodologies to investigate ZIKV infection and teratogenesis, especially regarding in vivo studies. To evaluate the quality of these studies as well as to combine and compare them are essential to identify the main biological pathways related to ZIKV teratogenesis and other pathways that have yet to be investigated. Therefore, this systematic review evaluated and compared the main findings on molecular mechanisms of ZIKV teratogenesis in animal studies.

Methods

Protocol for This Systematic Review and Ethical Aspects

The protocol for this systematic review was developed by members of the Reproductive and Developmental Biology Laboratory. The study protocol followed SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) [26] recommendations. In addition, the protocol was registered with the International Prospective Registry of Systematic Reviews (PROSPERO) under registration ID CRD42019157316 [27] and published on Systematic Reviews [28].

Since the data analyzed in this review did not include patient data and no animal interventions were performed, ethical approval was not required. Nevertheless, it was registered with and approved by the Research and Ethics Committee of the Hospital de Clínicas de Porto Alegre (CAAE 23.337.119.6.0000.5327).

Research Question and PICO

This systematic review was based on the following research question: “What are the molecular mechanisms involved in teratogenesis of the Zika virus proposed in studies with animal models?”. Based on this research question, the study’s PICO (Population, Intervention, Comparison and Outcome) was established. The disease/health problem of interest was the CZS; the population/species studied were animal models with CZS, including all species previously studied in different types of biomedical and biological experimental studies; the intervention/exposure was exposure to ZIKV during embryonic/fetal development; and the control population was a group of animals in the same study that underwent the same conditions as treated/exposed animals but without exposure to ZIKV or other viruses. Outcomes related to morphological and molecular analysis were assessed.

Search Strategy

Electronic searches were performed in the PubMed/MEDLINE, EMBASE, Web of Science, and Scopus, and in the grey literature, through the following sources: bioRxiv, OpenGrey, and PQDT Open. The search strategy included terms related to (1) animal models, including several species used in biomedical studies, (2) ZIKV, and (3) human congenital anomalies described in CZS. The complete list of the search terms used for each database is available on [28]. The list of search terms was developed by all the members of the research group after consultation with a librarian at the Universidade Federal do Rio Grande do Sul, in Brazil, who has extensive experience in search strategies for systematic reviews. The search terms for animals were obtained from search filters developed by SYRCLE [29]. To achieve the broadest possible search for any study that has evaluated molecular changes (gene expression, proteomics, methylation pattern changes, or any other change in pattern structure or quantity of cellular macromolecules) caused by embryonic/fetal ZIKV exposure, we did not include search terms related to this outcome. The reference lists of available literature reviews were also searched for primary research.
articles not identified in the database search. There were no language or publication date restrictions.

**Study Selection**

Studies were screened for eligibility in two phases. In the first phase (screening), two independent reviewers screened the titles and abstracts of the studies. In case of uncertainties in this screening phase, the full manuscript was always evaluated. The second phase (full-text evaluation) consisted of full text evaluation of the studies selected in the first phase. In both screening phases, when discrepancies occurred between the two reviewers, a third reviewer was consulted. In addition, evaluation of agreement between reviewers was tested by using Cohen’s kappa coefficient test in SPSS v.20.0 software (SPSS Inc., Chicago, USA).

Studies were included if they evaluated at least one morphological parameter (anatomical, histological, etc.). Studies also had to show teratogenic effects of ZIKV (embryonic death, morphological alterations, etc.) in the group of embryos/fetuses exposed to ZIKV with a significantly higher prevalence than in mock control. Finally, all studies had to evaluate at least one molecular parameter in ZIKV-exposed animals and their controls (e.g., gene expression, protein expression, gene methylation). Conference abstracts were also included, and in the case of selection for posterior screening, the authors were contacted to obtain more data or the full manuscripts.

Review articles, editorials, case reports, human studies, studies performed exclusively in vitro, studies on vaccine development, studies lacking methodological information, and studies that do not fall into the PICO of this systematic review were excluded. Considering that we aimed to analyze molecular changes upon embryonic exposure to ZIKV, we did not include studies in which these analyses were performed postnatally. The absence of a proper and complete methodology description was also an exclusion criterion; hence, studies that did not describe the viral dosage or developmental period of exposure were excluded.

**Data Extraction and Quality Assessment**

Data extraction was also performed by two independent reviewers, and disagreements were resolved by consultation with a third reviewer. The authors of the selected studies were contacted when data were missing or unclear. The following data were extracted from each study: study ID (information of first author, title, DOI and year of publication), methodology (including type of study, sample size of each experimental group, animal housing, follow-up period, developmental age at which the animals were inoculated, the methods and techniques for outcome assessment and the guideline utilized by the study), animal model characteristics (species, lineage, age and genetic modification factors), intervention characteristics of virus application, virus dosage and period of exposure (method of ZIKV infection), and morphological and molecular findings (exposed animals compared to controls). Two outcome measures were assessed: morphological findings and molecular changes following ZIKV exposure. Data from different studies were described and compared when appropriate.

To evaluate the quality of the selected studies, a 10-point scoring system was applied that allowed assessment of the methodology and publication aspects. Scoring followed the SYRCLE Risk of Bias tool for animal intervention studies [29], which includes random sequence allocation, baseline characteristics, allocation concealment, random housing, blinding during the intervention, random outcome assessment, blinding during the outcome evaluation, incomplete outcome data, and selective outcome reporting. Three points considered important by the authors of this systematic review were also included: whether the publication had been peer-reviewed, reporting of sample size calculation, and reporting on the use of guidelines for the study’s planning, execution, and publication. The risk of bias was also evaluated by two independent reviewers; when discrepancies occurred, a third reviewer was also consulted.

**Data Synthesis**

A detailed description of each study was carried out, including general data, and morphological and molecular findings after ZIKV infection.

To summarize which post-exposure differentially expressed genes were shared between the studies that performed transcriptome analyses, Venn diagrams were produced using an online tool [30]. In this comparative analysis, it was considered, for each study, the list of genes described as having a statistically significant difference in gene expression before and after exposure to ZIKV. It is important to highlight each of these studies presented a single list of differentially expressed genes, but depending on the study they considered different p values as statistically significant, such as \( p < 0.05 \), or adjusted \( p < 0.05 \), or FDR (false discovery ratio) < 0.05. For this reason, we considered as differentially expressed genes in our comparative analysis only the genes described by the original authors of each study like that.

Finally, an enrichment analysis of gene ontologies was performed in R v.3.6.2, using the clusterProfiler package [31], to verify whether the differentially expressed genes shared between the studies were in common biological pathways.
Results

In total, 3446 records, published until March 15, 2022, were obtained: 3252 from database searching (933 – PubMed; 918 – EMBASE; 813 – Scopus; and 588 – Web of Science); 21 from grey literature; and 173 from other sources (e.g., reference list of important articles) (Fig. 1). After removing duplicate records \(n=1439\), 2007 unique studies were eligible for title and abstract screening. From this initial screening, 1953 studies were excluded, leaving 54 (2.6%) publications eligible for full-text assessment. Of these 54 studies, nine (16.7%) were selected for data extraction and were included in the qualitative synthesis. The main reasons for exclusion during the full-text evaluation phases were the following: inadequate study design/outcome (e.g., lack of morphological or molecular evaluation or control group; \(n=38\)), full text unavailable \((n=5)\), and publication type, such as correction \((n=1)\) or hypothesis \((n=1)\) studies (Supplementary Table 1). During title and abstract screening, the authors presented a slight agreement \(K=0.37\), according to the Cohen’s kappa coefficient test. During the screening of articles through full-text reading, the authors presented a substantive agreement \(K=0.63\).

Characteristics of the Included Studies

All the ten included studies were published between 2016 and 2021 (Table 1) and used the following animal models: mice \((n=7)\), guinea pigs \((1)\), pigs \((n=1)\), and chickens \((n=1)\). All studies involved experimental groups with ZIKV-infected animals and controls (mock infection). Westrich et al. used different viral inoculation doses to different experimental models, since the IFNAR1−/− mice and the guinea pigs were infected with \(10^5\), \(10^6\), and \(10^7\) PFU/mL of ZIKV and the immune-competent mice were infected only with the highest viral dose \((10^7\) PFU/mL\) [36]. Thawani et al. and Rathore et al., in addition to mock infection, included dengue virus (DENV) infection and animals injected with 4G2 [24, 35]. The developmental stage of virus infection, the viral inoculation dose, and the follow-up period varied widely among the studies (Table 1), even in studies on the same species. Only one study utilized an African ZIKV lineage to infect the animals [35]. Regarding the viral inoculation method, in all studies, the virus was injected into the embryo or the mother. However, in the embryo, the injections were to different developing organs (cerebroventricular cavity, lateral ventricles, or midbrain ventricles) or by different

![Fig. 1 Flow chart showing the search and screening strategy to identify publications eligible for investigating molecular mechanism of ZIKV teratogenesis from animal studies](image)
### Table 1 Main characteristics of the included studies

| Study ID       | Species                       | Experimental groups | Animal lineage | Genetic modification | Development age at inoculation | Follow-up period | ZIKV strain                                      | Viral inoculation dose | Viral inoculation method                                                                 |
|----------------|-------------------------------|---------------------|----------------|---------------------|--------------------------------|------------------|-----------------------------------------------|------------------------|------------------------------------------------------------------------------------------|
| Li et al., 2016 | Mice                          | ZIKV vs mock        | ICR            | No                  | E13.5                          | 5 dpi            | Asian lineage: SZ01 (GenBank: GEO: KU866423) | 1 μl (6.5 $10^5$ PFU/ml) | Injected into one side of the cerebroventricular space/lateral ventricle                  |
| Shao et al., 2016 | Mice                          | ZIKV vs mock        | C57BL/6 J or 129S1/SvImJ | No                  | E14.5                          | 3 dpi            | Asian lineage (Mexican isolate MEX1-44)       | ~1 μl (1.17 $10^6$ PFU/ml) | Injected into the lateral ventricles of embryo brains                                   |
| Vermillion et al., 2017 | Mice                          | ZIKV vs mock        | CD1            | No                  | E10 or E14                     | 2 or 4 dpi       | Asian and African lineages: IB H30,656 (Nigeria, 1968); PRV-ABC59 (Puerto Rico, 2015); FS13025 (Camodia, 2010) and Paraíba (Brazil, 2015) | 100 μL (0.69 $10^6$ PFU/ml) | Intraperitoneal/ intrauterine                                                             |
| Yockey et al., 2018 | Mice                          | ZIKV vs mock        | C57BL/6 (B6)   | Yes (IFNAR1−/− and IFNAR2−/−) | E5.5                          | 5 dpi            | Asian lineage: Cambodian FSS13025 and Brazilian PE243 | 1.5 $10^6$ PFU/ml (intravaginal); 100 μL diluted in PBS (3.4 $10^3$ or $10^3$ PFU/ml) (subcutaneous) | Intravaginal or subcutaneous                                                   |
| Khaiboullina et al., 2019 | Mice                          | ZIKV vs mock        | FVB/NJ         | No                  | GD-4.5                         | 13 dpi           | Asian lineage (HS-2015-BA-01)                 | 10 μL (10^5 PFU/ml) (intravaginal) | Intravaginal                                                                             |
| Rathore et al., 2019 | Mice                          | ZIKV vs mock        | C57Bl/6NTac and FcRN−/− | Yes (FcRN−/−) | E7                             | 1, 3 or 11 dpi   | Asian Lineage H/PF/2013 (European Virus Archive) | 100μL (10^6 PFU/ml) (intraperitoneal) | Intraperitoneal                                                                           |
| Westrich et al., 2021 | Mice and guinea pigs          | ZIKV vs mock        | C57BL/6 J and B6.129S2-Ifnar1tm1Agt/Mmjx (mice) and Hartley (guinea pigs) | Yes (IFNAR1−/−) | E12 (mice) and E21 (guinea pigs) | 14 dpi           | Asian lineage: PRVABC59 (ZIKV-PR; GenBank: KU501215) | $10^5$, $10^6$, and $10^7$ PFU/mL for IFNAR1−/− mice and guinea pigs; $10^7$ PFU/mL for C57BL/6 J | Subcutaneous or intravaginal                                                              |
routes (via intraperitoneal, intrauterine, intravaginal, or subcutaneous administration).

Regarding the animal lineage, mice studies used different breeds (C57BL/6 J or 129S1/SvlmJ, ICR, CD1, C57B6 and FVB/NJ, C57Bl/6NTac, C57BL/6). Only Yockey et al., Rathore et al., and Westrich et al. used genetically modified animals [25, 35, 36]. Yockey et al. and Westrich et al. used IFN-α/β receptor (IFNAR)-deficient mice, which, according to Yockey et al., are highly susceptible to ZIKV infection, while Rathore et al. used FcRN−/− animals to evaluate how FcRN contributes to fetal ZIKV infection in DENV-immune mice [25, 35, 36]. Li et al. provided no information about the breed they used [18]. With respect to the moment of ZIKV infection, seven studies considered embryonic development days as a unit, while one used gestational day. The embryonic age at inoculation ranged from E5.5 to E14.5 and GD4.5 (Table 1). The follow-up periods were similar in five studies, ranging from 2 to 5 days after viral infection [19, 25, 26, 34]. However, one study followed up the animals for 13 days, other for 58 days, and another performed the follow-up during three different days—1, 3, and 11 days after infection [24, 33, 36].

Alternative animal models were also investigated. Darbellay et al. used Landrace pigs, applying ZIKV via intraperitoneal, intra-amniotic, or intracerebral injection at GD50 [20]. In this study, different groups were exposed to different doses of ZIKV, and the post-infection follow-up was 28 days (Table 1). Furthermore, Thawani et al. used chicken (White Leghorn) embryos as an experimental model [24]. In this study, ZIKV infection occurred on day 2 of development (E2), the virus was inoculated through direct injection into the midbrain and the embryo development was followed-up for 3 days after infection. Westrich et al. also performed experiments using guinea pigs as experimental models in addition to mice, since they are also an immune-competent model [36].

**Morphological Outcomes Due to ZIKV Infection During Development**

The studies evaluated the morphological effects of ZIKV exposure during development in different ways (Table 2). Four studies evaluated the brain only, while the other six evaluated different organs such as placenta and the whole body. Different methods were utilized to evaluate the morphological outcomes of ZIKV infection, including histological analysis of the brain (different staining techniques, such as Nissl, immunohistochemistry) and morphometric evaluation (e.g., embryo/fetus weight and size/length, brain vesicles and placenta morphology and weight, and analysis of computed tomography and magnetic resonance imaging). Kaiboullina et al. and Westrich et al. only performed
### Table 2 Main characteristics related to morphological analysis

| Study ID | Sample size | Outcome evaluation method | Embryo age at morphological outcome evaluation | Main teratogenic finding | Evaluated organs |
|----------|-------------|---------------------------|-----------------------------------------------|--------------------------|------------------|
| Li et al., 2016 [18] | Not mentioned | Histological evaluation of brain slices, including Immunohistochemistry and Nissl staining and confocal imaging | 3dpi (E16.5) and 5dpi (E18.5) | Thinner cortex and microcephaly | Brain |
| Shao et al., 2016 [32] | 3 ZIKV/3 control | Histological processing, including TUNEL assay and Immunohistochemistry | P1 and P3 | Microcephaly, growth restriction, and abnormal vasculature | Brain |
| Vermillion et al., 2017 [33] | 4 ZIKV/5 control litters* | Histological evaluation of cortical thickness of neonates (Nissl-stained tissue sections), Immunohistochemistry, and Immunofluorescence imaging | P0 | Reduction on cortical thickness and reduced litter size | Brain |
| Yockey et al., 2018 [25] | Different number of infected and control animals according to period of exposure and described below* | Weight, size, gross anomalies, resorption, crown-rump measurement and placental morphology and histology, including Immunofluorescence and Immunohistochemistry | E9.5, E10.5, E11.5 and E12.5 | Ifnar1+/− fetuses present higher growth restriction, death, resorption, lower weight, and placental abnormalities | Whole body |
| Khaiboullina et al., 2019 [34] | 26 ZIKV/31 control | No morphometric evaluation, just fetal and placental weight | GD 17.5 | Reduction on fetal and placental weight | Whole body |
| Rathore et al., 2019 [35] | At least 5 individuals per each group and for each evaluation | Fetal mass, head circumference, cortical thickness, gene expression | E8, E10 or E18 | Reduction of fetal mass and head circumferences in all groups infected with ZIKV; stunned growth and cortical thickness was also moderately reduced in the fetuses of naïve ZIKV-infected | Whole embryo, brain, placenta |
| Westrich et al., 2021 [36] | At least 3 individuals per each group | Fetal and placental weight and fetal skull area | E19 (mice) and E58 (guinea pigs) | No differences in fetus and placental weight and in the skull area between ZIKV exposed fetus and mock for mice and guinea pigs | Whole fetus, placenta, maternal brain, uterus and spleen |
| Darbellay et al., 2017 [20] | 12 ZIKV/28 control | Computed tomography, magnetic resonance imaging, histology, and morphometry | 1 PN and 21 PN | Neonatal body length and weight in the IP+IA group was lower than in the control group; two piglets had possible neurological or developmental defects | Brain |
| Thawani et al., 2018 [24] | 3dpi: 8 ZIKV/10 controls; 7 dpi (brain wet weight): 11 ZIKV/10 controls; (TE epithelial thickness): 5 ZIKV/5 controls | Magnetic Resonance Imaging to brain size quantification, Histology and Morphometry | E5 and E9 | ZIKV treated embryos presented MB and FB smaller than controls | Whole body |

*Pup number not mentioned; dpi: days post infection

E: embryonic day; FB: forebrain; GD: gestational day; MB: midbrain; TE: telencephalon; P and PN: postnatal. Sample number of Yockey et al., 2018: E9.5 uninfected Ifnar1+/− n=7 and Ifnar1−/− n=12 from 2 litters infected Ifnar1−/− n=9 and Ifnar1+/− n=10 from 3 litters; E10.5 uninfected Ifnar1−/− n=15 and Ifnar1+/− n=5 from 3 litters, infected Ifnar1−/− n=24 and Ifnar1+/− n=17 from 6 litters; E11.5 uninfected Ifnar1−/− n=5 and Ifnar1+/− n=12 from 3 litters, infected Ifnar1−/− n=12 and Ifnar1+/− n=11 from 3 litters; E12.5 uninfected Ifnar1−/− n=11 and Ifnar1+/− n=11 from 3 litters, infected Ifnar1−/− n=19 and Ifnar1+/− n=19 from 5 litters
Molecular Neurobiology (2023) 60:68–83

anatomical evaluation (weighing fetuses and placentas) without further microscopy analyses [34, 36]. Studies carried out the morphological evaluation at different stages of development, but only three of them analyzed morphological changes during the postnatal period [20, 32, 33]. Shao et al. evaluated the newborn mice on postnatal days one and three. Darbellay et al. evaluated the newborn pigs on postnatal day one and 21, while Vermillion et al. performed the evaluation just after birth [20, 32, 33].

The mice model studies found similar morphological outcomes, including microcephaly, growth restriction and reduced cortical thickness [18, 25, 32, 33, 35]. The method of evaluating morphological outcomes was similar among these studies, including histological processing and morphometric evaluation as described in Table 2.

The morphological evaluation differed in the studies with porcine and avian models [20, 24]. Darbellay et al. used computed tomography, magnetic resonance imaging and histological analyses to evaluate morphological outcomes on postnatal days one and twenty-one [20]. They found differences between groups depending on the method of infection (Table 2), as well as neurological and developmental defects. Thawani et al. evaluated morphological outcomes by magnetic resonance imaging, morphometric measures, and histological processing on development days 5 and 9 [24]. They found that embryos exposed to ZIKV had fewer encephalic vesicles than controls, which could be interpreted as indicative of microcephaly.

Molecular Alterations Following ZIKV Infection

All of the studies evaluated the molecular mechanisms of ZIKV teratogenesis by analyzing differential gene expression (DGE) in embryos infected with ZIKV and controls (Table 3). Four of them analyzed DGE in the brain with transcriptome assays [18, 20, 32, 35]. Other five analyzed gene expression changes in placentas and brains or fetal tissue by real-time quantitative polymerase chain reaction (RT-qPCR) [25, 33–36], and one analyzed mRNA quantity in the developing brains by in situ hybridization [31].

Studies involving transcriptome analysis used different statistical criteria to assess the DGE between groups. Li et al. considered genes differentially expressed those which presented a p-value < 0.05; Shao et al. considered those with a p-value < 0.05 and a logFC < −1.5 or > 1.5; and Darbellay et al. considered those with a corrected p-value < 0.05, after control for the false discovery ratio (FDR) (Supplementary Table 2).

Overall, the results of DGE indicated that ZIKV causes an overexpression of genes involved in immune response, interferon production, antiviral response, and apoptosis pathways and causes reduced expression of microcephaly-associated, neurodevelopmental genes, and mitosis-associated genes (Table 3).

Studies that investigated the DGE through RT-qPCR or in situ hybridization evaluated previously selected targets (candidate gene approach) in placental, fetal brain, and fetal tissues. The genes investigated in these studies are involved in specific biological pathways: TAM receptors, interferon-inducible genes, hypoxia response, inflammatory response, genes related to cortical neurogenesis, and brain-related morphogens. All investigated genes were upregulated after ZIKV infection except cortical neurogenesis and brain-related morphogens, which were downregulated.

When all studies were compared, three genes (MX1, B2M, and C1QB) were differentially expressed in four of them, while thirty-four genes were differentially expressed in three studies (Supplementary Fig. 1). These genes are related especially to the antiviral response, such as MX1, and several are interferon-stimulated genes, such as STAT1, STAT2, and TLR3.

Studies that evaluated molecular alterations after ZIKV infection through transcriptome were compared in this review in order to describe whether there were genes and biological pathways similarly altered among them (Supplementary Table 2 and Fig. 2A). This comparison was purely a description of the similarities and differences regarding the changes caused by ZIKV, as these studies showed many differences in terms of groups, model, age of incubation, age of harvesting, etc. (Supplementary Table 3). Therefore, due to the nature of the approaches and data of these studies, it would not be possible to perform a meta-analysis of the data provided.

From our descriptive comparison, it was identified that 23 genes were found to be altered in all three studies (Fig. 2A). Enrichment analyses of gene ontologies related to these genes were performed and it was found that response to virus was the most common ontology related to them (n = 10 genes; p < 0.001), as well as ontologies associated with the immune response (Fig. 2B; Supplementary Table 4).

Risk of Bias/Quality

The risk of bias assessment is shown in Fig. 3. The risk of bias was unclear in most of the studies due to insufficient information. None of the studies had a low risk of selection and performance bias, one had a high risk of bias for adequate randomization, and two had a high risk of bias for blinded interventions. Regarding the risk of detection bias, most of the studies showed an unclear (> 70%) risk of bias. One study reported using a randomization method for outcome evaluation and two studies reported researcher blinding methods in outcome evaluation. Four studies had a low risk of attrition bias (incomplete outcome data), while three had an unclear risk and two had a high risk. The risk of
| Study ID | Sample size | Time-point of the molecular analysis | Molecular evaluation | Genes                                                                 | Method | Sample | Molecular alterations due ZIKV infection |
|---------|-------------|--------------------------------------|----------------------|----------------------------------------------------------------------|--------|--------|----------------------------------------|
| Li et al., 2016 [18] | 2/3 embryos for each dam, 1/3 embryos for each dam | 3 dpi (E16.5) | Transcriptome | – | RNA-seq | Whole brain | Upregulation of immune-response (especially genes related to cytokine production and the response to cytokines and apoptosis pathways (e.g., STAT1, STAT2, TRIM21, TRIM25, IFIT1, IFIT3, TLR3, TLR7, TLR9, CCL2, CCL3), antiviral response genes (e.g., TLR3, DDX58, IFIH1, OAS2, IRF7, ISG15, MX1, CCL5, CXCL10, IFNβ1) and flavivirus entry receptors (e.g., AXL); downregulation of microcephaly-associated genes (e.g., ASPM, CASC5, CENPF, MCHP1, RBBP9, STIL, TBR2)) | 510 genes (logFC > 0.26) | 95 genes (logFC < -0.26) |
| Shao et al., 2016 [32] | 3 embryos | 3 embryos | 3 dpi (E17.5) | Transcriptome | RNA-seq | Brain | Upregulation of immune response and apoptosis pathways (e.g., OASL2, USP18, IFIT1, MX1, OAS1B, IFIT3, IGf1, DDX50, IFI44, IFIT1, TLR3, CASP9) | 197 genes (logFC > 1.52) | 3 genes (logFC < -2.22) |
| Vermillion et al., 2017 [33] | 10 placenta (inoculated at E10) | 6 placenta (E10)+6 (E14) | 2 dpi (E12) | Candidate genes MERTK, AXL, and GAS6 | RT-qPCR | Placenta | TAM receptors and Interferon-inducible genes MERTK, AXL, and GAS6, ISG15, IFITM3, and OAS1B | – |
| Yockey et al., 2018 [25] | For ISG: 6 placenta and 6 fetuses from 1 infected litter | 4 placenta and 4 fetuses | 9 dpi (E17.5) | Candidate genes OASL2, MX1, and USP18 | RT-qPCR | Placenta and embryos | Interferon-inducible genes (ISG) OASL2, MX1, and USP18 in placenta and fetuses | – |
| For HRG: 9 fetuses per genotype | 3 Ifnar1−/− and 5 Ifnar1+/− fetuses | 5 dpi (E10.5) | VEGFA, ADM, BNIP3, PFKFB3, and GLUT1 | Placenta | Hypoxia response genes (HRG) VEGFA, ADM, BNIP3, PFKFB3, and GLUT1 in fetuses | – |
| Study ID               | Sample size | Time-point | Molecular evaluation | Genes     | Method     | Sample                      | Molecular alterations due ZIKV infection | Pathway/biological function | Upregulated genes | Downregulated genes |
|-----------------------|-------------|------------|----------------------|-----------|------------|-----------------------------|------------------------------------------|-------------------------------|-------------------|---------------------|
| Khaiboullina et al., 2019 [34] | 6 embryos ZIKV + in the brain | 13 dpi (GD 17.5) | Candidate genes | IL-1, IL-6, IL-18, CCL2, CXCL1, CXCL10, MX1, TLR7, and TNFα | RT-qPCR | Brain and placenta of embryos | Inflammatory factors | MX1, CCL2, CXCL1, and CXCL10 and modest increase of TLR7, IL-1β, IL-6, IL-18, and TNFα (ZIKV + in the brain) (without p-value) | TLR7, CCL2, CXCL1, IL-18, and IL-6 and modest increase of MX1, CXCL10, IL-1β, and TNFα (ZIKV + placentas) (without p-value) |
| Rathore et al., 2019 [35] | 5 mouse brains | 11 days (E18) | Candidate genes | BRN1, BRN2, OTX1, OTX2, EMX2, PAX6, and FOXG1 | RT-qPCR | Brains | Cortical neurogenesis | – | BGN1, BRN2, OTX1, OTX2, FOXG1, and PAX6 |
| Westrich et al., 2021 [36] | 3–5 (mouse) 3–7 (guinea pig) | 3–7 (mouse) 3–7 (guinea pig) | Candidate genes | Cxcl10 | RT-qPCR | Placenta and fetal tissue | Inflammatory response | Cxcl10 | – |
| Darbellay et al., 2017 [20] | 5 embryos 6 embryos | 28 dpi (GD 78) | Transcriptome | – | RNA-seq | Cerebral and cerebellar cortex | Upregulation of antiviral response and interferon production pathways (e.g., OAS1, STAT1, IGS12A, IFTTM1, IFIH1, IRF9, DDS60, IFT13, OAS2, MX1); Downregulation of mitosis associated genes (e.g., CCNB1, CDC1, TP53) | 323 genes = logFC > 0.35 | 33 gene = logFC < -0.29 |
| Thawani et al., 2018 [34] | 10 embryos 10 embryos | 3 dpi (E5) | Candidate genes | BMP7, FGF8B, SHH, and PTCH1 | in situ hybridization | Brain | Brain-related morphogens | – | BMP7, FGF8B, SHH, and PTCH1 |

dpi: days post infection; E: embryonic day; GD: gestational day; RNA-seq: transcriptome; RT-qPCR: reverse transcriptase-quantitative Polymerase Chain Reaction; logFC: logarithm fold change
Fig. 2 Genes with a differential gene expression, according to the authors, in the three studies that performed the transcriptome analysis to assess the gene expression after ZIKV exposure (all upregulated). A Venn diagram highlighting the number of statistically significant differentially expressed genes after ZIKV exposure, according to the authors of each study, shared between the three studies; B enrichment analyses of gene ontologies related to these differentially expressed genes shared in all studies that performed the transcriptome analysis.

Fig. 3 Risk of bias of the studies included in this systematic review. The proportion of studies classified as having low (green), unclear (yellow) or high (red) risk of bias according to different methodological variables.
Molecular Neurobiology (2023) 60:68–83

studies evaluated brain tissue only, which is due to the fact cal approaches to morphometric and weight measures. Most varied widely among the studies, ranging from histologi -

feron signaling systems facilitate the ZIKV infection, since ZIKV [25, 35, 36, 47, 48] and animals with deficient inter -
tial for an antiviral response and host resistance against (IFNAR)-deficient mice, since type I interferons are essen -

The most common animal model evaluated in the included studies was mice and this could be explained by their easy handling, husbandry, and low cost compared to other mammals [44, 45]. On the other hand, rodents are not naturally susceptible to ZIKV infection [46], so there is an implicit risk of bias, since some strategy must be used to make the infection possible, including high doses of the virus, immunosuppressive drugs, or genetically modified animals [44, 45]. Only Yockey et al., Rathore et al., and Westrich et al. used genetically modified interferon receptor (IFNAR)-deficient mice, since type I interferons are essential for an antiviral response and host resistance against ZIKV [25, 35, 36, 47, 48] and animals with deficient interferon signaling systems facilitate the ZIKV infection, since the deficiency makes them very susceptible [46, 49].

Teratogenic and morphological evaluation techniques varied widely among the studies, ranging from histological approaches to morphometric and weight measures. Most studies evaluated brain tissue only, which is due to the fact that the organs most affected by exposure to ZIKV infection are in the nervous system [15, 19, 21, 32, 33, 35]. Kaibou -lina et al. and Westrich et al. evaluated fetal and placental weight measures only and did not perform histological analyses, which could be considered a study limitation [34, 36]. Westrich et al. was the only study evaluating the skull area of the fetuses [36]. The main morphological outcomes of exposure to ZIKV during development were similar in the nine included studies [19, 21, 25, 26, 35, 34, 33, 32], ranging from microcephaly and growth restriction to reduced placental weight, which corroborates the phenotype observed in cases of CZS [14]. No differences in terms of placental and fetuses weight and fetuses skull area was identified by Westrich et al. [36].

Three studies evaluated morphological outcomes during the postnatal period [21, 32, 33]. The evaluation several days after birth is important because other phenotypes can be observed and described, such as cognitive alterations caused by exposure to ZIKV, which could only be identified during early life [50].

Regarding the molecular evaluations, in this systematic review, the most commonly investigated candidate genes were those related to the immune response pathways, especially associated with viral receptors, inflammatory response and interferon induction [25, 33, 34, 36]. Interestingly, studies with transcriptome approaches, when all genes are investigated a priori (i.e., without previous hypothesis), also found that genes related to immune response pathways were upregulated in animals exposed to ZIKV during development, especially pathways involved in antiviral response, interferon-inducible genes, and production/response to cytokines genes [18, 20, 32]. In addition, transcriptome studies also found the apoptosis pathway upregulated by ZIKV infection [18, 33]. Although immune response is essential for defense against infections, an exacerbated inflammatory profile has been shown to be harmful in a developmental context, since it leads to cell death and impaired cell proliferation and differentiation, which could result in neuronal cell division and, thus, impaired brain development [51, 52]. The immune response against ZIKV infection usually begins when the pathogen is recognized by cellular receptors, such as Toll-like receptor 3 (TLR3) [53, 54]. In this systematic review, we found that TLR3 was upregulated by ZIKV infection in the three studies that performed transcriptome analyses [18, 20, 32]. TLR3 acts by recognizing pathogen-associated molecular patterns and it is the most prevalent Toll-like receptor expressed in brain cells [55]. Genetic variants in TLR3 genes in mothers of children affected by the CZS have even been associated with higher susceptibility to ZIKV teratogenesis [56].

Following viral recognition and interferon production, a complex including STAT1, STAT2, and IRF9 is formed to activate interferon-inducible genes [46]. The STAT1, STAT2, and IRF9 were also upregulated genes in the same three
compared to in vitro studies, relatively few studies have investigated the role of these genes in the context of ZIKV teratogenesis. The activation of interferon-inducible genes is a later step in antiviral response, in which the proteins act to reduce viral replication and propagation actually begins [48]. Many interferon-inducible genes were found to be upregulated by ZIKV, both in the candidate gene and transcriptome studies included in this systematic review [18, 20, 25, 33, 57].

Regarding the function of these genes and their proteins, it should be pointed out the blocking virus entry (IFITM proteins), inhibition of viral protein translation (IFIT and OAS proteins), and induction of viral protein ubiquitination (ISG15, USP18, TRIM proteins) [58]. Interestingly, MX1 — an interferon-inducible gene — was upregulated in two candidate gene studies [33, 34] and exhibited a DGE in two studies that performed transcriptome analysis [18, 20]. Genetic variants that could impact the expression of this gene or its protein function have already been associated with the severity and progression of viral infections [58, 59]. Likewise, increased expression of this gene in the brain during ZIKV infection could affect the progression of the infection and the severity of the disease and, therefore, it deserves further investigation.

Having considered the immune and antiviral response pathways, other important pathways and genes were investigated by some studies. Thawani et al. and Rathore et al., who used a candidate gene approach, and Li et al., who used a transcriptome approach, showed that several genes involved in brain development had reduced expression during ZIKV infection [18, 24, 35]. Studies evaluating the gene expression, methylation, and proteomics have reported that genes and proteins related to brain development and neurological diseases or malformations are significantly reduced during ZIKV infection in the brain [21, 60, 61]. In many situations, due to genetic or environmental factors, including infectious agents, for example, the expression of genes and proteins that act on brain development may be reduced, leading to the development of a defective phenotype [62–65].

Yockey found an overexpression of genes related to the hypoxia pathway in the placentas of animals exposed to ZIKV [25]. It is known that viral infections, such as cytomegalovirus, which is also a teratogenic virus that leads to microcephaly, can change oxygen consumption and lead to cell hypoxia [66, 67]. Changes in brain development have already been associated with prenatal hypoxia [68]. Thus, given the effect of infections in this pathway, as well as their harmful role in development, further investigations are needed about the role of these genes in the context of ZIKV teratogenesis.

This systematic review has some limitations. First, compared to in vitro studies, relatively few studies have investigated the molecular mechanisms of ZIKV teratogenesis in animal models. Thus, it was not possible to perform a more robust comparison of the studies’ data. Regarding the investigated species by the studies, most of them investigated mice; pigs and chickens were evaluated in only one study each, while no study used non-human primates, which prevented better interspecies comparison. Considering the experiments and molecular results of the studies included in this review, a great variation in the methodology of their experiments (dose of exposure to ZIKV, period of exposure, among other factors) and the statistical criteria for considering the results significant was noticed. Therefore, the comparison of such studies has become much more complex, and the consistency of the comparisons is limited. This brought some caveats to comparing common genes and pathways affected by ZIKV in these studies, which was one of the goals of the systematic review. Since a meta-analysis could not be conducted, we performed such comparison through a descriptive form, which gave us some interesting clues on how ZIKV affects embryo development and leads to the observed malformations. Still, even though we could not run a more robust comparison, this systematic review evaluated and compared the main findings on molecular mechanisms related to ZIKV teratogenesis from animal studies. Finally, it was found that studies in animal models focused on differential gene expression due to ZIKV infection, in contrast to in vitro studies, which involved a variety of approaches, including methylation and proteomic analyses [21, 22, 61].

Despite these limitations, this systematic review was able to provide an overview of the main biological pathways that have been investigated and affected in the context of ZIKV infection and teratogenesis in animal models, as well as an assessment of the methodological quality of studies in this area. Future animal model studies should provide better reporting of methodological aspects and more standardized reporting of their results to increase their quality. Furthermore, other animal models and different molecular approaches, such as methylation and proteomics, should be addressed. Finally, other biological pathways can also be considered, since many studies have focused on pathways and genes related to the immune response, but few have focused on development, cell cycle and signaling.

ZIKV is a human and animal teratogen whose mechanisms of action are poorly understood. Animal models are a very important source of information about how teratogenesis occurs in humans and how the effects of ZIKV exposure could be prevented or even treated. In this systematic review, ten studies that investigated the molecular mechanisms of ZIKV teratogenesis were described and compared. These studies found that the most commonly
upregulated genes were related to viral response pathways, especially interferon-inducible genes, while genes related to brain development were reported as downregulated. Since these pathways were investigated only through DGE, genomic and proteomic investigations could clarify their role and importance in ZIKV teratogenesis. Thus, it would be possible to better understand the molecular action mechanisms of the virus and recognize susceptibility factors to ZIKV teratogenesis, as well as targets for treating its teratogenic effects. On the other hand, investigating biological pathways that have not yet been addressed but are commonly affected in teratogenic processes would also seem to be of great relevance, since they might clarify the relationship between ZIKV infection and other biological and developmental processes in the embryo or fetus [69].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-022-03046-4.

Acknowledgements The authors thank Viviane Carrión Castanho, librarian at the Universidade Federal do Rio Grande do Sul for technical assistance in determining the list of search terms.

Author Contribution JAG, GEW, JAB, and LRF participated of the study conception and design; JAG and LRF performed the literature search; JAG, GEW, JAB, and LRF performed data extraction; JAG and GEW performed data analysis; JAG and GEW wrote the manuscript; JAB, FSLV, LSF, and LRF reviewed the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant call Universal MCTIC/CNPq 2018 (project 424362/2018–0), as well as by the Hospital de Clínicas de Porto Alegre Fundo de Incentivo a Pesquisa e Eventos [grant number 2019–0649].

Data Availability Data presented in this study have been published by the authors of the cited studies and are openly available. Original data produced in this article are presented in our Supplementary Table 3.

Declarations

Ethics Approval This study was approved by the Research and Ethics Committee of the Hospital de Clínicas de Porto Alegre – Brazil (CAAE 23337119.6.0000.5327).

Consent to Participate Not applicable.

Consent to Publish All authors have read the manuscript and approved the final version of the manuscript.

Competing Interests The authors declare no competing interests.

References

1. Dick G, Kitchen S, Hadow A (1952) Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 46:509–520. https://doi.org/10.1016/0035-9203(52)90042-4
2. Macnamara F (1954) Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg 48:139–145. https://doi.org/10.1016/0035-9203(54)90006-1
3. Lanciotti R, Kosoy O, Laven J et al (2008) Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 14:1232–1239. https://doi.org/10.3201/EID1408.080287
4. Duffy M, Chen T, Hancock W et al (2009) Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 360:2536–2543. https://doi.org/10.1056/NEJMoa0805715
5. Oehler E, Wainin L, Larre P et al (2014) Zika virus infection complicated by Guillain-Barre syndrome—case report, French Polynesia, December 2013. Euro Surveill 19:20720. https://doi.org/10.2807/1560-7917.ES2014.19.9.20720
6. Campos GS, Bandeira AC, Sardi SI (2015) Zika virus outbreak, Bahia. Brazil Emerg Infect Dis 21:1885–1886
7. Ali S, Guglielmini O, Harber S et al (2017) Environmental and social change drive the explosive emergence of Zika virus in the Americas. PLoS Negl Trop Dis 11:e0005135. https://doi.org/10.1371/JOURNAL.PNTD.0005135
8. PAHO/WHO (2017) Regional Zika epidemiological update (Americas). https://www3.paho.org/hq/index.php?option=com_content&view=article&id=11599:regional-zika-epidemiological-update-americas&Itemid=41691&lang=en. Accessed 4 Aug 2021
9. Reinhold JM, Lazzari CR, Lahondère C (2018) Effects of the environmental temperature on Aedes aegypti and Aedes albopictus mosquitoes: a review. Insects 9:158. https://doi.org/10.3390/INSECTS9040158
10. Brady OJ, Hay SI, Reiner RC, Marinhu F (2019) Mapping the association between Zika virus infection and microcephaly in Brazil. Am J Trop Med Hyg 101:601
11. Lwande OW, Obanda V, Lindström A, Ahlm C, Evanter M, Näslund J, Globe-Trotting BG (2020) Globe-Trotting Aedes aegypti and Aedes albopictus: risk factors for arbovirus pandemics. Vector Borne Zoonotic Dis 20:70–81. https://doi.org/10.1089/VBZ.2019.2486
12. de Matos SMS, Hennigen AF, Wachholz GE, Rengel BD, Schuler-Faccini L, Rothe PM, Varela APM, Fraga LR (2021) Possible emergence of Zika virus of African lineage in Brazil and the risk for new outbreaks. Front Cell Infect Microbiol 11. https://doi.org/10.3389/FICMB.2021.680025
13. Araújo T, Rodrigues L, de Alencar XR et al (2016) Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case-control study. Lancet Infect Dis 16:1356–1363. https://doi.org/10.1016/S1473-3099(16)30318-8
14. del Campo M, Feitosa IML, Ribeiro EM et al (2017) The phenotypic spectrum of congenital Zika syndrome. Am J Med Genet Part A 173:841–857. https://doi.org/10.1002/ajmg.a.38170
15. Beaver JT, Lelutiu N, Habib R, Skountzou I (2018) Evolution of two major Zika virus lineages: implications for pathology, immune response, and vaccine development. Front Immunol 9:1640. https://doi.org/10.3389/fimmu.2018.01640
16. Faria NR, Quick J, Claro IM et al (2017) Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 546:406–410. https://doi.org/10.1038/NATURE22401
17. Kasprzykowski JF, Fukutani KF, Fabio H et al (2020) A recursive sub-typing screening surveillance system detects the appearance of the ZIKV African lineage in Brazil: Is there a risk of a new epidemic? Int J Infect Dis 96:579–581. https://doi.org/10.1016/J.IJID.2020.05.090
18. Li C, Xu D, Ye Q et al (2016) Zika Virus disrupts neural progenitor development and leads to microcephaly in mice (vol 19, pg 120, 2016). Cell Stem Cell 19:672. https://doi.org/10.1016/J.STEM.2016.04.017
Zhang F, Hammack C, Ogden SC et al (2016) Molecular signatures associated with ZIKV exposure in human cortical neural progenitors. Nucleic Acids Res 44:8610–8620. https://doi.org/10.1093/NAR/GKW765

Darbellay J, Lai K, Babiuk S et al (2017) Neonatal pigs are susceptible to experimental ZIka virus infection. Emerg Microbes Infect 6:e6. https://doi.org/10.1093/EMIL2016.133

Garcez P, Nascimento J, de Vasconcelos J et al (2017) Zika virus disrupts molecular fingerprinting of human neurophores. Sci Rep 7:40780. https://doi.org/10.1038/SREP40780

Janssens S, Schotsaert M, Karnik R et al (2018) Zika virus alters DNA methylation in an organoid model of the developing human brain. mSystems 3:e00219-17. https://doi.org/10.1128/mSystems.00219-17

Aguiar RS, Pohl F, Morais GL et al (2020) Molecular alterations in the extracellular matrix in the brains of newborns with congenital Zika syndrome. Sci Signal 13:eaay6736. https://doi.org/10.1126/SCISIGNAL.AAY6736

Thawani A, Sirohi D, Kuhn RJ, Fekete DM (2018) Zika virus can strongly infect and disrupt secondary organizers in the ventricular zone of the embryonic chicken brain. Cell Rep 23:692–700

Yockey LJ, Jurado KA, Arora N et al (2018) Type I interferons instigate fetal demise after Zika virus infection. Reprod Sci 25:64A. https://doi.org/10.1126/SCIIMMUNOLOGA116080

de Vries RBM, Hooijmans CR, Langendam MW et al (2015) A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. Evidence-based Preclin Med 2:1–9. https://doi.org/10.1002/EBM2.7

Vermillion M, Lei J, Shabi Y et al (2017) Intrauterine Zika virus infection of pregnant immunocompetent mice models transplacental transmission and adverse perinatal outcomes. Nat Commun 8:14575. https://doi.org/10.1038/S41467-017-01126-5

Yi G, Wang L, Han Y, He Q (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 16:284–287. https://doi.org/10.1089/OMI.2011.0118

Shao Q, Herrlinger S, SL Y et al (2016) Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage. Development 143:4127–4136. https://doi.org/10.1242/dev.143768

Vermillion M, Lei J, Shabi Y et al (2017) Intrauterine Zika virus infection of pregnant immunocompetent mice models transplacental transmission and adverse perinatal outcomes. Nat Commun 8:14575. https://doi.org/10.1038/NCOMMUNIS14575

Khaiboullina S, Uppal T, Kletkenkov K et al (2019) Transcriptome profiling reveals pro-inflammatory cytokines and matrix metallo-proteinase activation in Zika virus infected human umbilical vein endothelial cells. Front Pharmacol 10. https://doi.org/10.3389/FPHAR.2019.00642

Rathore APS, Saron WAA, Lim T et al (2019) Maternal immunity and antibodies to dengue virus promote infection and Zika virus-induced microcephaly in fetuses. Sci Adv 5:eaaq3208

Westrich JA, McNulty EE, Edmonds MJ, Nalls AV, Miller MR, Foy BD, Rovnak J, Perera R et al (2021) Characterization of subclinical ZIKV infection in immune-competent guinea pigs and mice. J Gen Virol 102(8):001641. https://doi.org/10.1099/jgv.0.001641

Morrison T, Diamond M (2017) Animal models of Zika virus infection, pathogenesis, and immunity. J Virol 91. https://doi.org/10.1128/JVI.00009-17

Antoniou E, Orooue V, Sarella A et al (2020) Zika virus and the risk of developing microcephaly in infants: a systematic review. Int J Environ Res Public Health 17:3806. https://doi.org/10.3390/ijerph17113806

Ventrica C, Maia M, Ventura B et al (2016) Ophthalmological findings in infants with microcephaly and presumable intra-uterus Zika virus infection. Arq Bras Ortoal 79(1):1–3. https://doi.org/10.5935/0004-2749.20160002

van den Pol A (2009) Viral infection leading to brain dysfunction: more prevalent than appreciated? Neuron 64:17–20. https://doi.org/10.1016/J.NEURON.2009.09.023

Gratton R, Trircarico P, Agrelli A et al (2020) In vitro Zika virus infection of human neural progenitor cells: meta-analysis of RNA-Seq assays. Microorganisms 8. https://doi.org/10.3390/MICROBIO.8002070

van Luijk J, Bakker B, Rovers M et al (2014) Systematic reviews of animal studies; missing link in translational research? PLoS One 9:e89981. https://doi.org/10.1371/JOURNAL.PONE.00899.81

Kilkenny C, Browne W, Cuthill I et al (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8:e1000412. https://doi.org/10.1371/JOURNAL.PBIO.1000412

Pena L, Miranda Guairanes K, Duarte Silva A et al (2018) In vitro and in vivo models for studying Zika virus biology. J Gen Virol 99(12):1529–1550. https://doi.org/10.1099/JGV.0.011153

Nazerai L, Pravsgaard Christensen J, Randrup Thomsen A (2019) A “furry-tale” of Zika virus infection: what have we learned from animal models? Viruses 11. https://doi.org/10.3390/V110010029

Grant A, Ponia S, Tripathi S et al (2016) Zika virus targets human STAT2 to inhibit type I interferon signaling. Cell Host Microbe 19(6):882–890. https://doi.org/10.1016/J.CHOM.2016.05.009

Wu Y, Liu Q, Zhou J et al (2017) Zika virus evades interferon-mediated antiviral response through the co-operation of multiple nonstructural proteins in vitro. Cell Discov 3. https://doi.org/10.1038/CELLDISC.2017.6

McNab F, Mayer-Barber K, Sher A et al (2015) Type I interferons in infectious disease. Nat Rev Immunol 15(2):87–103. https://doi.org/10.1038/NRI3787

Smith D, Hollidge B, Daye S et al (2017) Neuropathogenesis of Zika Virus in a highly susceptible immunocompetent mouse model after antibody blockade of type I interferon. PLoS Negl Trop Dis 11. https://doi.org/10.1371/JOURNAL.PNTD.0005296

Moura da Silva A, Ganz J, Sousa P et al (2016) Early growth and neurologic outcomes of infants with probable congenital zika virus syndrome. Emerg Infect Dis 22(11):1953–1956. https://doi.org/10.3201/EID2211.160956

Jiang N, Cowan M, Moonah S, Petri W (2018) the impact of systemic inflammation on neurodevelopment. Trends Mol Med 24:794–804. https://doi.org/10.1016/j.TMM.2018.06.008

Vasistha N, Pardo-Navarro M, Gasthaus J et al (2020) Maternal infection, pathogenesis, and immunity. J Virol 91. https://doi.org/10.1128/JVI.00397-19

Dang JW, Tiwari SK, Qin Y, Rana TM (2019) Genome-wide identification research reporting: the ARRIVE guidelines for reporting animal research. J Gen Virol 91. https://doi.org/10.1099/JGV.0.01713-6
response in primary human astrocytes. PLoS One 14. https://doi.org/10.1371/JOURNAL.PONE.0208543

55. Carty M, Bowie A (2010) Recent insights into the role of Toll-like receptors in viral infection. Clin Exp Immunol 161(13):397–406. https://doi.org/10.1111/j.1365-2249.2010.04196.X

56. Santos CNO, Ribeiro DR, Cardoso Alves J et al (2019) Association between Zika Virus Microcephaly in Newborns with the rs3775291 Variant in Toll-Like Receptor 3 and rs1799964 Variant at Tumor Necrosis Factor-α Gene. J Infect Dis 220:1797–1801. https://doi.org/10.1093/infdis/jiz392

57. Goodfellow FT, Tesla B, Simchick G et al (2016) Zika virus induced mortality and microcephaly in chicken embryos. Stem Cells Dev 25:1691–1697. https://doi.org/10.1089/SCD.2016.0231

58. Bigham A, Buckingham K, Husain S et al (2011) Host genetic risk factors for West Nile virus infection and disease progression. PLoS One 6. https://doi.org/10.1371/JOURNAL.PONE.00247

59. García-Álvarez M, Berenguer J, Jiménez-Sousa M et al (2017) Mx1, OAS1 and OAS2 polymorphisms are associated with the severity of liver disease in HIV/HCV-coinfected patients: a cross-sectional study. Sci Rep 7:41516. https://doi.org/10.1038/SREP41516

60. Dang J, Tiwari SK, Lichinchi G et al (2016) Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell 19(2):258–265. https://doi.org/10.1016/J.STEM.2016.04.014

61. Kandilya D, Maskomani S, Shyamasundar S et al (2019) Zika virus alters DNA methylation status of genes involved in Hippo signaling pathway in human neural progenitor cells. Epigenomics 11:1143–1161. https://doi.org/10.2217/EPI-2018-0180

62. van den Pol A, Mao G, Yang Y et al (2017) Zika virus targeting in the developing brain. J Neurosci 37:2161–2175. https://doi.org/10.1523/JNEUROSCI.3124-16.2017

63. Cordeiro C, Tsimis M, Burd I (2015) Infections and brain development. Obstet Gynecol Surv 70:644–655. https://doi.org/10.1097/OGX.0000000000000236

64. Lim JS, Lee JH (2015) Molecular genetic decoding of malformations of cortical development. J Genet Med 12:12–18. https://doi.org/10.5734/JGM.2015.12.1.12

65. Parrini E, Conti V, Dobyns W, Guerrini R (2016) Genetic basis of brain malformations. Mol Syndromol 7:220–233. https://doi.org/10.1159/000448639

66. Herber S, Silva AA, Sanseverino MTV et al (2019) Prevalence and causes of congenital microcephaly in the absence of a Zika virus outbreak in southern Brazil. J Pediatr (Rio J) 95:600–606. https://doi.org/10.1016/j.jped.2018.05.013

67. Pereira L, Petitt M, Tabata T (2013) Cytomegalovirus infection and antibody protection of the developing placenta. Clin Infect Dis 57(Suppl 4). https://doi.org/10.1093/CID/CIT583

68. Nalivaeva N, Turner A, Zhuravin I (2018) Role of prenatal hypoxia in brain development, cognitive functions, and neurodegeneration. Front Neurosci 12:825. https://doi.org/10.3389/FNINS.2018.00825

69. Schneider W, Chevillotte M, Rice C (2014) Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol 32:513–545. https://doi.org/10.1146/ANNUREV-IMMUNOL-032713-120231

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.