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Prenatal physical exercise and Zika virus infection have no effect on maternal behavior

Exercício físico pré-natal e infecção pelo vírus Zika não afetam o comportamento materno

El ejercicio físico prenatal y la infección por el virus Zika no tienen efecto sobre el comportamiento materno

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Abstract
Some of the worst effects associated with the Zika virus infection during gestation include microcephaly, and central nervous system damage. Pregnancy-related physical exercise is recognized to improve both the mother's and her unborn child's health. It is widely known that male newborns of late-pregnancy mother infection tend to exhibit more depressive and anxious behaviors as they age. However, nothing is known about how the Zika virus could affect a mother's behavior in the first few days after giving birth. The objective of this study was to evaluate if 4 weeks of moderate-intensity swimming during pregnancy prevents negative outcomes of prenatal Zika infection in the behavior of the dams. Dams were randomly selected and divided into three groups: Mock (n= 8) - untrained group, intraperitoneally injected with saline; Zika (n = 8) - untrained group, intraperitoneally injected with Zika; and Zika/swim (n = 8) - trained group, intraperitoneally injected with Zika. The swimming sessions were initiated before mating, which occurred between the 5th and 7th day of the 1st week of the swimming training, according to the estrous cycle and lasted until the parturition date. Prenatal Zika virus infection did not change maternal body weight or maternal behavior significantly independently of performing or not physical exercise.
Keywords: Swimming; Behavior; Brain; Infection.

Resumo
Alguns dos piores efeitos associados à infecção pelo vírus Zika durante a gestação incluem microcefalia e danos ao sistema nervoso central. É reconhecido que o exercício físico relacionado com a gravidez melhora a saúde da mãe e do feto. É amplamente conhecido que os recém-nascidos do sexo masculino com infecção materna no final da gravidez tendem a apresentar comportamentos mais depressivos e ansiosos à medida que envelhecem. No entanto, nada se sabe sobre como o vírus Zika pode afetar o comportamento da mãe nos primeiros dias após o parto. O

objetive deste estudo foi avaliar se 4 semanas de natação de intensidade moderada durante a gravidez previnem resultados negativos da infecção pré-natal pelo Zika no comportamento das mães. As mães foram selecionadas aleatoriamente e divididas em três grupos: Mock (n= 8) - grupo não treinado, injetado intraperitonealmente com solução salina; Zika (n = 8) - grupo não treinado, com injeção intraperitoneal de Zika; e Zika/natação (n = 8) - grupo treinado, injetado intraperitonealmente com Zika. As sessões de natação foram iniciadas antes do acasalamento, que ocorreu entre o 5º e o 7º dia da 1ª semana de treinamento de natação, de acordo com o ciclo estral e durou até a data do parto. A infecção pré-natal pelo vírus Zika não alterou significativamente o peso corporal ou o comportamento materno, independentemente da realização ou não de exercícios físicos.
Palavras-chave: Natação; Comportamento; Cérebro; Infecção.

Resumen

Algunos de los peores efectos asociados con la infección por el virus Zika durante la gestación incluyen microcefalia y daño al sistema nervioso central. Se reconoce que el ejercicio físico relacionado con el embarazo mejora la salud tanto de la madre como del feto. Es ampliamente conocido que los recién nacidos varones de madres infectadas al final del embarazo tienden a exhibir comportamientos más depresivos y ansiosos a medida que envejecen. Sin embargo, no se sabe nada sobre cómo el virus Zika podría afectar el comportamiento de la madre en los primeros días después del parto. El objetivo de este estudio fue evaluar si 4 semanas de natación de intensidad moderada durante el embarazo previenen resultados negativos de la infección prenatal por Zika en el comportamiento de las madres. Las madres fueron seleccionadas al azar y divididas en tres grupos: Mock (n= 8): grupo no entrenado, inyectado intraperitonealmente con solución salina; Zika (n = 8): grupo no entrenado, al que se le inyectó Zika por vía intraperitoneal; y Zika/nadar (n = 8): grupo entrenado, inyectado con Zika por vía intraperitoneal. Las sesiones de natación se iniciaron antes del apareamiento, que ocurrió entre el día 5 y 7 de la 1.ª semana del entrenamiento de natación, de acuerdo con el ciclo estral y se prolongó hasta la fecha del parto. La infección prenatal por el virus del Zika no cambió significativamente el peso corporal materno ni el comportamiento materno, independientemente de si realizaba o no ejercicio físico.
Palabras clave: Nadar; Comportamiento; Cerebro; Infección.

1. Introduction

The Zika virus pandemic expanded in 66 nations in 2015, and it was soon established that pregnant women who contracted the virus could have abnormalities in the developing fetus' brains (Shao et al., 2016). The outbreak was first identified in South and Central America. The severe defects in newborns born to moms infected with the Zika virus, including microcephaly, damage to the central nervous system, and fetal development limitation, are some of the severe consequences related with the virus (Cugola et al., 2016). Recent data points to a causal link between microcephaly and Zika virus infection (Li et al., 2016). The Zika virus outbreak and its detection in the amniotic fluid of pregnant women who have been infected, as well as in the microcephalic brain tissues, are time-correlated with fetal microcephaly (Schuler-Faccini et al., 2016).

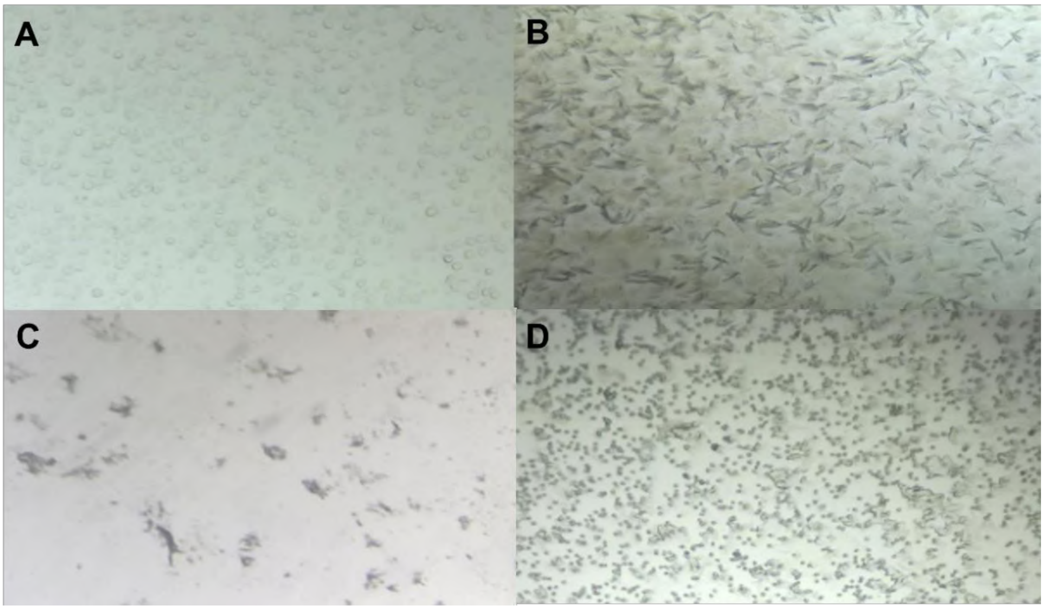
In the Americas, the Zika virus is endemic and is mostly transmitted via mosquitoes and sexual contact (Gurung et al., 2019). In the past, isolated cases of Zika virus infections were reported from Asia and Africa, with little to no morbidity other than a slight fever (Caine et al., 2019). The Zika virus, which was first identified in 1947 from an afebrile sentinel rhesus monkey in the Ugandan Zika forest, is a member of the Flaviviridae family and genus Flavivirus, which also contains dengue, West Nile, yellow fever, and Japanese encephalitis virus (Gurung et al., 2019). It is well established that late-pregnancy maternal infection causes an increase in anxiety- and depression-like behaviors in male offspring as they get older (Enayati et al., 2012). On the other hand, not much is known about how the Zika virus may impact a mother's behavior in the initial days following childbirth.

In both humans and mice, physical exercise during pregnancy has a positive impact on the health of the mother and her newborns (Ferrari et al., 2017). Physical exercise has been linked to several advantages for both human and mouse offspring, including decreased risk of excessive weight gain and enhanced glucose metabolism (De Sousa, Caria, et al., 2020; De Sousa, Hagenbeck, et al., 2020). It has been observed that physical exercise during an induced infected Zika virus gestation prevented changes in behavior in young mouse offspring. In the same study, the authors also showed that physical exercise prevented reduction of brain-derived neuro factor (BDNF) levels in the hippocampi of male and female mice pups while also inhibiting the overexpression of microglia and astrocytes (De Sousa, Peixoto, et al., 2020). The objective of this study was to evaluate if 4 weeks of moderate-intensity swimming during pregnancy prevents negative outcomes of prenatal Zika infection in the behavior of the dams.

2. Methodology

All animal care and experiments were conducted according to the Principles of Laboratory Animal Care (Brazilian Arouca Law and US National Institutes of Health) and were approved by the Ethics Committee on Animal Use of the *Universidade Federal dos Vales do Jequitinhonha e Mucuri* (022/2019). The study followed the recent proposed methodological principles (Merchán-Hamann & Tauil, 2021). Twelve weeks-old adult female (n=40) and male (n=20) Swiss mice were maintained in a 2:1 ratio (female:male) with free access to food and water, under a 12h light/dark cycle, with controlled room temperature (23±2ºC) and humidity. Dams were randomly selected and divided into three groups: Mock (n= 8) - untrained group, intraperitoneally injected with saline; Zika (n = 8) - untrained group, intraperitoneally injected with Zika; and Zika/swim (n = 8) - trained group, intraperitoneally injected with Zika. The swimming sessions were initiated before mating, which occurred between the 5th and 7th day of the 1st week of the swimming training, according to the estrous cycle (Figure 1).

Figure 1 - Representative photos of the estrous cycle.



Source: Self-made.

The estrous cycle repeats every 4 days on average, and it is divided into 4 stages: (A) proestrus, (B) estrus, (C) metestrus, and (D) diestrus. Proestrus is characterized by nucleated epithelial cells. Estrus is the fertile stage, and it presents anucleate cornified cells. Metestrus is a mixture of the other stages presenting nucleated epithelial cells, cornified cells, and leucocytes. Diestrus presents predominantly leucocytes.

The formation of a vaginal plug and daily measures of body mass within the next seven days were used to confirm pregnancy. Pregnant mice of each group were individually housed in cages. Zika virus (10^6 PFUs/100 ul) or an equal volume of saline, was intraperitoneally injected in the pregnant mice at embryonic day10.5. The physical exercise protocol was performed during the pregnancy period. Litters were normalized at birth to 8 pups/litter to avoid bias.

2.1 Virus

Zika virus was isolated from a patient in the state of Pernambuco, Brazil (gene bank ref. number KX197192). Zika virus used in the experiments was produced and tittered in our laboratories as previously described (De Sousa, Peixoto, et al., 2020).

2.2 Physical exercise protocol

Animals were familiarized with the swimming exercise for 1 week. The 1st familiarization session lasted 10 min, and sessions duration was increased by 10 min/day until the 6th day. Training was initiated 48 h after the last familiarization session. Swimming sessions consisted of 60 min, with no overload, 5 days per week, for 4 weeks. Exercise sessions were conducted during the light cycle, and mice swam in groups of six animals in a plastic pool (60 cm depth × 150 cm diameter). The water temperature was maintained at ~24°C. Between the 5th and 7th training sessions, animals were mated for 24 h according to their estrus cycle period, and swimming sessions finished on the day of the parturition.

2.3 Body mass

Dam´s body mass was measured every 3 days during the pregnancy period and on the 1st day after parturition (P1).

2.4 Maternal behavior

The following aspects of maternal behavior were measured: number of events licking the pups, number of events out of the nest, and number of breastfeeding events. Each observation session lasted one hour, and four observation sessions were performed each day at 10:00 am, 1:00 pm, 4:00 pm, and 7:00 pm. Maternal behavior was evaluated from P2 to P8.

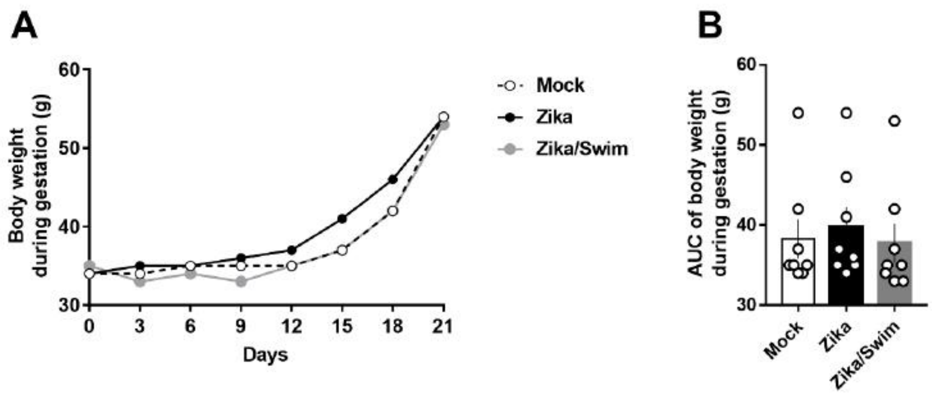
2.5 Statistical analysis

All data are expressed as means ± standard error mean (s.e.m). Significant differences were assessed by Two-way analyses of variance (ANOVA), followed by Tukey´s post hoc, using the GraphPad Prism 9.0 Software. The area under the curve (AUC) was used to evaluate dam's body mass during the pregnancy period. A p value $\leq$ 0.05 was considered significant.

3. Results

First, we evaluated the body weight of dams during the entire gestational period (Figure 2A-B).

Figure 2 - Prenatal Zika virus infection does not change maternal body weight independently of performing or not physical exercise.



Source: Self-made.

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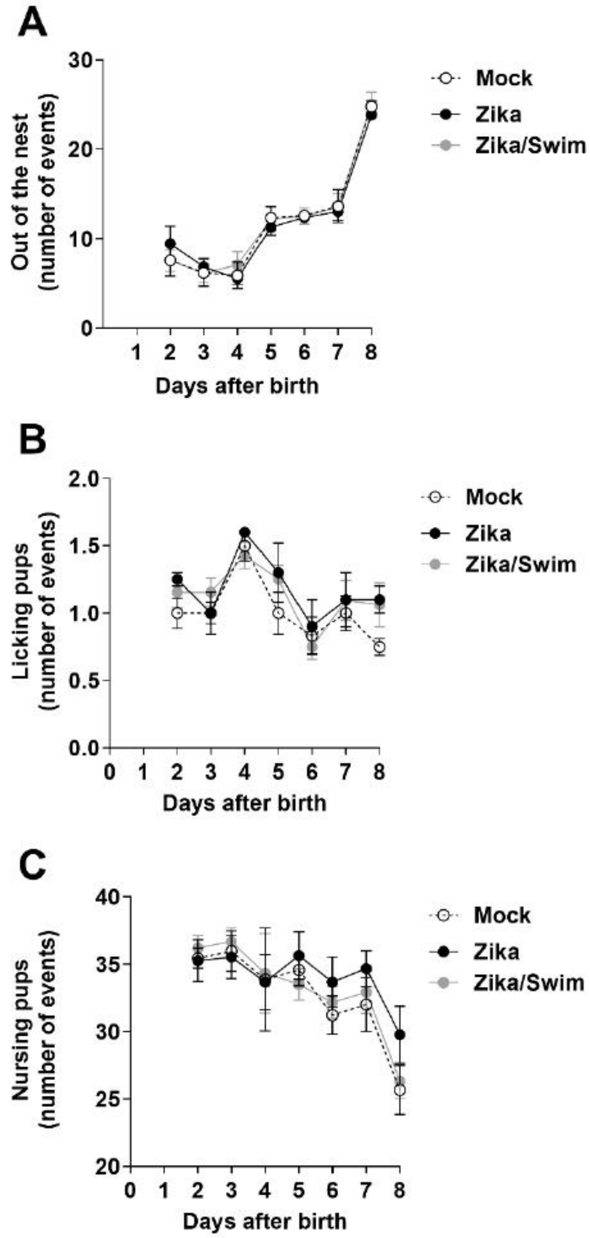
The body weight of dams was similar in all groups from the 1st day until 21 days of pregnancy (Figure 2A), with the total quantification for the entire period being also evaluated and not showing significant differences among the groups (Figure 2B). Thereafter, maternal behavior was measured from P2 to P8 (n = 8/group). Two-way ANOVA followed by Tukey was used and data are expressed as mean ± s.e.m (Figure 3A-C).

Figure 3 - Prenatal Zika virus infection does not change maternal behavior independently of performing or not physical exercise.



Source: Self-made.

No changes in maternal behavior were seen between Mock, Zika, and Zika/swim groups, as showed by the number of events out of the nest, licking, and nursing pups (Figure 3A-C, (A) Number of events out of the nest. (B) Number of events licking pups. (**C**) Number of events nursing pups). This result indicates that the maternal behavior was not significantly changed. Therefore, the virus and the physical exercise didn't affect the dam's behavior.

4. Discussion

Mammals need a caregiver's protection when they leave in the secure uterine environment and encounter several strange stimuli and hazards (Enayati et al., 2012). A mother and her pups build an extremely intimate link in the early stages of life, and this bond is greatly aided by the pups' ability to detect the scent of the nest, and to be breastfed (Reis et al., 2014). It is well recognized that maternal behavior has a significant influence over an infant's growth since she serves as an interface between the environment and the newly born mammal (Enayati et al., 2012; Reis et al., 2014). Several years of research have examined the effects of changes in maternal behavior on neonatal programming and its long-lasting effects on the brain (Bogoch et al., 2007; Castro et al., 2007; Gardener & Buka, 2013). Therefore, early life enviroment is a crucial time for the nervous system, during which the brain goes through functional organization, neuronal proliferation, migration and differentiation, gliogenesis, and myelination.

Thus, the gestational and perinatal stages represent a critical period to influence several consequences to the brain and behavior of the offspring (Sousa, 2018). When talking about drugs of abuse, for example, it has been shown that exposure to cigarette smoke during the prenatal and early postnatal phases raises the risk of developing a range of aberrant behaviors later in life (Xiao et al., 2016). In mice that are 18 and 32 days old, morphine given before, throughout, and following breastfeeding results in the death of pyramidal neurons (Ghafari & Golalipour, 2014). Not only the usage of drugs of abuse, but several other factors might influence significant changes to the brain and behavior of the offspring, and the prenatal and perinatal period can be determinant for these.

Prenatal viral-like immune activation appears to be a main cause of various neurodegenerative disorders, including schizophrenia-related metabolic and physiological abnormalities (Pacheco-López et al., 2013). There have also been reports of school-age children delivered from moms with gestational diabetes experiencing cognitive decline and neuropsychological impairment (Bolaños et al., 2015), and similar effects have been seen in several animal models presenting the same condition (De Sousa, 2021). Therefore, the intra-uterine and perinatal period can be a critical determinant of developing several conditions and neurodegenerative diseases during the perinatal period or later in life.

Pregnancy-related Zika virus infection is known to be associated with increased inflammatory levels (Trus et al., 2019). A pregnancy-related Zika virus infection is linked to microcephaly and neurobehavioral problems at birth (Oliveira et al., 2016), although prenatal exercise is thought to protect the developing brains of the unborn children (Bustamante et al., 2013). An inflammatory biomarker called C-reactive protein (CRP) has been linked in a recent study to unfavorable maternal outcomes like pre-eclampsia and gestational diabetes mellitus during pregnancy (Hawkins et al., 2015). The results of a randomized study conducted on a population of pregnant women from a variety of ethnic and socioeconomic backgrounds were presented by the authors, who indicated that while the effect of the exercise intervention on CRP levels was beneficial, it was not statistically significant. Nevertheless, diminishing the levels of a pro-inflammatory biomarker can bring positive consequences to the fetus, and during a gestation infected with Zika virus it could be crucial for avoiding negative outcomes like brain shrinkage and microcephaly.

5. Conclusion

In the present study, we didn't identify significant changes in maternal behavior that could have happened due to the Zika virus administration or the performance of the physical exercise protocol that could influence changes to the offspring. Therefore, regardless of whether the mother engaged in physical activity or not, the prenatal Zika virus infection did not significantly alter the maternal behavior and body weight.

Future studies should evaluate behavioral changes in the offspring at different time points, and test how these animals would respond to an external stimulus, as it is known that infections during the gestational period make the offspring more predisposal to develop several neurodegenerative diseases, such as obesity, type 2 diabetes, autism, dementia, among others.

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