Effects of prior Zika infection on male fertility outcomes

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Abstract

Zika virus (ZIKV), a mosquito-borne, fever-causing pathogen, swept through Latin America in 2015 and 2016. Previously thought to be a mild febrile illness of little clinical importance, unusual characteristics of ZIKV were newly identified during the novel American pandemic – prolonged shedding in semen, sexual transmissibility, and congenital anomalies. One study examined ZIKV's effect on semen measures, demonstrating a decline in sperm count and testosterone after acute ZIKV infection that recovered after several weeks. Fever itself can impair sperm production, confounding interpretation of these results. To investigate the long-term effect of ZIKV infection on human male fertility, we conducted a cohort study of young healthy men in two cities that experienced intense ZIKV transmission 1-2 years prior to the study; namely, León, Nicaragua, and Iquitos, Peru.

Healthy men aged 18-40 years were enrolled between July 2018 and March 2019, after the ZIKV epidemic had subsided at each site. A baseline questionnaire recorded demographic, clinical, and epidemiological data; these data and symptoms of infection were updated at quarterly visits. The participating men provided semen and blood samples quarterly for 12 months in León, and 9 months in Iquitos, although loss to follow up was high (>40%). Fresh semen and blood analysis were performed on-site. Semen and serum markers of fertility were compared between ZIKV-seropositive and seronegative men, averaging each man's results across their visits. Data were analyzed using Student's t-test for continuous data comparisons of means between groups of interest. We enrolled 110 men (50 in Peru, 60 in Nicaragua). Median age was 23 years (IQR 19-27) and 55% were students. More than 90% had evidence of prior dengue infection by IgG, and 20% reported prior chikungunya infection. 39% were seropositive for ZIKV infection at enrollment. There was no association between age and odds of ZIKV positivity. None were febrile at enrollment, though 6 reported a fever within the previous week. 26% of men reported having impregnated a partner, with no difference by ZIKV status. There were no differences at enrollment or averaged across the study duration by ZIKV exposure in semen pH, total sperm count, ejaculate volume, percent progressive motile sperm, vitality, or round cell count. We observed lower average FSH levels in participants seropositive for Zika ($p=0.04$). These preliminary data suggest that any effect of Zika virus infection on male fertility is likely short-lived.

Introduction

Zika virus (ZIKV) is a member of the *Flaviviridae* family and is closely related to other mosquito-spread pathogens such as dengue, yellow fever, and West Nile viruses. Spread by *Aedes* mosquitos, it is endemic to its vector's habitats throughout the tropical regions of the world. Symptoms of ZIKV infection include fever, rash, joint pain, and conjunctivitis. In infected pregnant women, it can cause fetal microcephaly, a birth defect in which the baby's head is significantly smaller than normal, and other severe fetal brain defects[1]. Studies have shown that Zika virus may infect and replicate in the male reproductive tract, including the testes, seminal vesicles, and prostate gland[2]. In mouse models, ZIKV infection caused a profound decrease in murine testicular size and sperm production, resulting in significantly impaired fertility[3]. It remains unknown if ZIKV has a similar effect in humans. Zika virus has also been detected in semen for extended periods, with some cases reporting detection up to 6 months after the initial infection[4]. Additionally, there have been cases of Zika virus being transmitted through sexual contact, including from infected men to their sexual partners. This further highlights the importance of preventing and treating Zika virus infections in men. Our study aimed to further understand how Zika infection impacted a variety of markers of male fertility, including ejaculate volume, semen pH, and sperm motility. Data on several secondary outcomes and potential confounders, such as medical history and ongoing medical complications were also collected.

Methods

This study was an observational cohort study that followed 110 men aged 18-40 years. 50 resided in Iquitos, Peru and 60 in León, Nicaragua from 2018-2019. Many participants (55%) were local university students, other participants included industrial workers, taxi drivers, engineers, teachers, and scientists, among other professions. Partner researchers at the Universidad Nacional de las Amazonas Peruanas in Iquitos and Universidad Nacional Autónoma de Nicaragua-León were responsible for taking informed consent, enrolling participants and collecting data. All participants first filled out questionnaires for eligibility; inclusion criteria were sex, age, residence in either Iquitos or León, and willingness to provide semen and blood samples in 3-month intervals for a year. Participants were excluded if they have had any

significant injury to their testes, vasectomies, or other markers of male infertility. Starting at enrollment, and returning for follow up visits every 3 months for 9 months (in Iquitos) or 12 months (in León), participants responded to follow-up surveys on novel diagnoses of any medical complications and provided fresh semen and blood samples. Semen samples were analyzed microscopically to collect data on motility, vitality (survival), and activity of sperm within a few hours of sample collection. Blood was tested for ZIKV EDIII IgG, dengue IgG and IgM, and follicle stimulating hormone (FSH). FSH was considered an important potential covariate as FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes, and is critical for the initiation of spermatogenesis.

Data integration and analysis occurred at the University of North Carolina at Chapel Hill from 2022-2023. As the data came from two separate institutions, the separate databases were cleaned and integrated in Microsoft Excel. Average values for our measures of interest across visit dates were computed in Excel, and analyzed using SAS Studio 9.4. SAS was used for visualizing cohort demographics and calculating differences in means of our measures of interest between participants who were seropositive and seronegative for ZIKV. Confounding variables such as novel STI diagnoses or chikungunya infection were also considered via exclusions during secondary analyses.

Results

Our cohorts consisted of 110 men aged 18-40, with a median age of 23 (IQR=8). 50 resided in Iquitos, Peru, and 60 in León, Nicaragua. The mean ages of participants from Iquitos (23.7 years) and León (24.3 years) did not differ significantly ($p=0.501$). 43 of the 110 participants (39.1%) were seropositive for ZIKV, and the remaining 67 were seronegative. 104 of the 110 participants (94.5%) were seropositive for Dengue, another mosquito-borne virus endemic to tropical regions but present in the Americas for much longer compared to ZIKV. A minority (31.8%) of participants had received vaccination against yellow fever. An even smaller proportion (5.5%) had received any vaccination against Dengue. 20% of participants self-reported having chikungunya prior to the onset of the study. 43.6% of participants were regular smokers, and 69.1% consumed alcohol on at least a weekly basis. 10.9% of the participants self-reported having had a STI diagnosis in the past. 26.4% had impregnated a woman at least once.

Table 1: Summary of Cohort Demographics

	León		Iquitos		Overall	
	Number	**% of total**	**Number**	**% of total**	**Number**	**% of total**
Participants	60	0.54	50	0.46	**110**	**1.00**
Age						
<24	29	0.26	27	0.25	**56**	**0.51**
≥24	31	0.28	23	0.21	**54**	**0.49**
ZIKV						
Negative	38	0.35	29	0.26	**67**	**0.61**
Positive	22	0.20	21	0.19	**43**	**0.39**
Dengue						
Positive	56	0.51	48	0.44	**104**	**0.95**
Negative	4	0.04	2	0.01	**6**	**0.05**
Chikungunya						
Positive	22	0.20	0	0.00	**22**	**0.20**
Negative	38	0.35	50	0.45	**88**	**0.80**
STI						
Yes	4	0.04	11	0.10	**15**	**0.14**
No	56	0.51	39	0.35	**95**	**0.86**

This study had a high loss to follow up, perhaps due to lack of ample reward for completing all visits, or a discomfort with providing blood or semen samples so regularly. There was also a discrepancy in the length of the study; while data was collected through 12 months in León, only up to 9 months were collected in Iquitos. Additionally, there can be a large variability day-to-day in an individual's sperm count, ejaculate volume, etc. dependent on a variety of factors. Due to these reasons, the primary analysis variables were calculated by averaging values from the first three visits (Enrollment, 3 months, and 6 months) together.

Table 2: Loss to follow up (by % of original cohort size)

	Enrollment (%)	*3 months (%)*	*6 months (%)*	*9 months (%)*	*12 months (%)*
Iquitos	50 (100%)	45 (90%)	45 (90%)	23 (46%)	0 (0%)
León	60 (100%)	49 (82%)	43 (72%)	37 (62%)	37 (62%)

The average pH of semen samples over the first 6 months of the were not significantly different across the ZIKV positive (7.45) and negative groups (7.44) (p=0.819). Similarly, the average volume in milliliters of ejaculate in samples was not significantly different across the two groups. Lack of significant differences for sperm count, percentage of motile progressive sperm, and vitality can additionally be observed in Table 3. However, average follicle stimulating hormone (FSH) presence in the blood samples was significantly lower in ZIKV-positive men at an alpha level of 0.05: 3.28 mIU/mL in the ZIKV negative group and 1.83 mIU/mL in the ZIKV positive group (p=0.044). Adjusting via exclusion of three known confounders: STI, chikungunya diagnoses and high fevers prior to visit increased the p-value to 0.0733.

Table 3: T-test comparison of means between ZIKV seropositive and seronegative adult men

Zika Serology				
6-month average value	**Positive (95% CI)**	**Negative (95% CI)**	**Difference (95% CI)**	**p-value (Satterthwaite)**
pH	7.45 (7.37-7.53)	7.44 (7.36-7.51)	-0.01 (-0.12-0.10)	0.819
Volume (mL)	2.56 (2.17-2.94)	2.60 (2.25-2.96)	0.04 (-0.05-0.56)	0.863
Sperm Count	209.6 (166.9-252.4)	199.2 (173.0-225.5)	-10.39 (-59.95-39.16)	0.676
Progressive Motile Sperm (%)	48.45 (43.27-53.63)	50.98 (46.71-55.25)	2.53 (-4.08-9.14)	0.448
Vitality (%)	64.97 (61.09-68.85)	66.55 (63.55-69.54)	1.58 (-3.26-6.41)	0.517
FSH (mIU/mL)	1.83 (1.41-2.25)	3.28 (1.92-4.63)	1.45 (0.04-2.86)	0.044

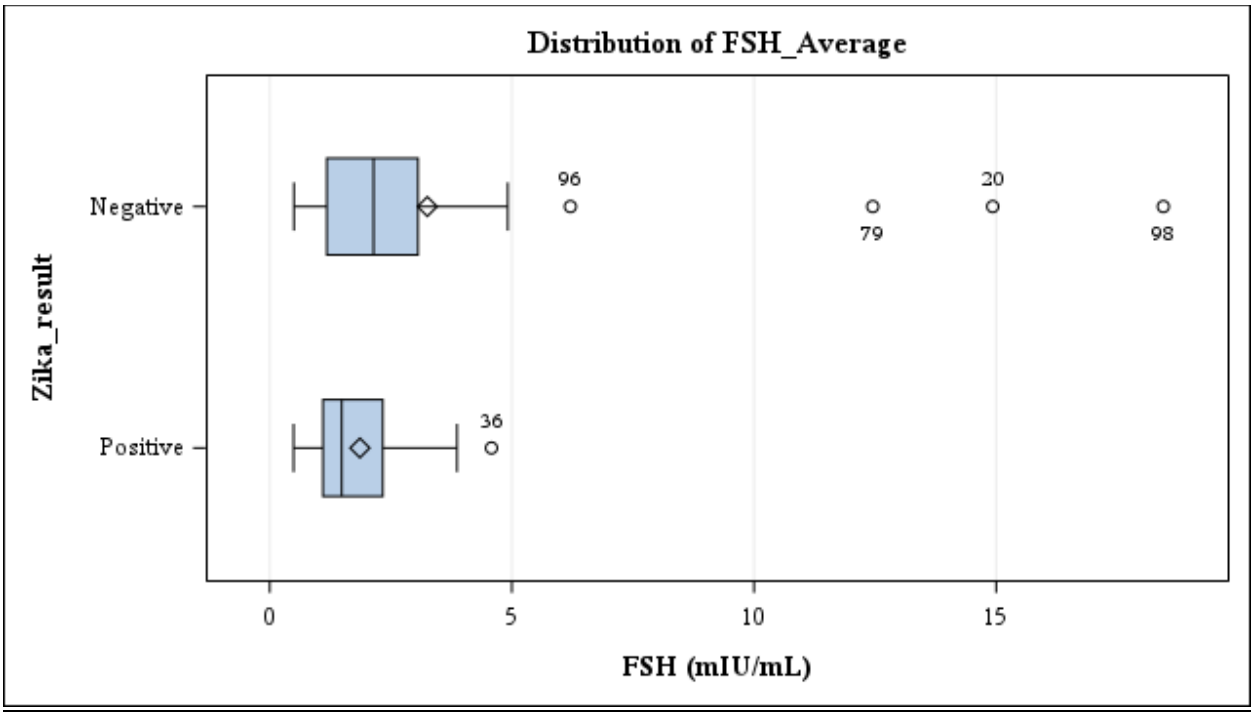

Figure 1: Boxplot depicting differences of FSH (mIU/mL) between ZIKV seropositive and seronegative groups after adjustment for patient febrility before sample collection, prior chikungunya infection, and novel STI infection.

Discussion

The study explored how prior Zika infection, signified by antibody presence in blood tests, may affect several markers of male fertility. Based on previous studies, and considering what is known about the behavior of ZIKV in infecting germline cells, we expected to observe decreases in measures such as sperm motility and count in ZIKV seropositive individuals overall. Ultimately, we did not see any significant differences between our ZIKV seropositive and seronegative groups in our chosen measures of fertility, with one notable exception. Average FSH levels were significantly higher in the seronegative group. When confounders such as fevers preceding visits, novel STI diagnoses, and prior chikungunya infection were adjusted for, however, the p-value was observed to be above the 0.05 alpha level for significance. Thus, these data show that there is not ample evidence to suggest a having a prior ZIKV infection alone has a significant effect on male fertility indicators. Crucially, these results do not refute the possibility that ZIKV may negatively affect those factors immediately following infection.

Limitations

One limiting factor of the conclusions of this paper is the lack of time sensitivity of the ZIKV infections. Some participants may have been infected at the start of the 2015-2016 epidemic, while others could have been infected in the weeks preceding the study. This gap in experimentation prevented further insight into the relevance of recency of infection to any potential effects on male fertility indicators. Secondly, the extensive loss to follow up and experimental miscoordination between the two study sites ultimately presented some unavoidable skew into our data due to our relatively small sample sizes. In future studies, more study sites with greater participation in regions where Zika is now endemic in the Americas will allow for greater power and more conclusive data.

References

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