Unlike most the flaviviruses, there is growing evidence to suggest that Zika virus (ZIKV) is transmitted sexually and recent studies have verified ZIKV presence in semen, but not blood, of a previously Zika-infected patient for 14 up to 88-days after Zika onset. In women, ZIKV has been detected for up to 44-days post-infection. Strong circumstantial evidence also suggests that ZIKV can be transmitted between sexual partners. Even given these findings, there is limited information on the association of the virus with human sperm. Since human reproductive organs are immunologically privileged sites, ZIKV antibody produced by the host’s adaptive immunity may not reach the male reproductive organs (testis) efficiently. Hence, transmission of ZIKV may still occur in vaccinated or ZIKV infected men, potentially keeping the sexual transmission route of infection viable. In our studies, we sought to assess the cellular targets of ZIKV in the semen. For this purpose we artificially infected human sperm with purified Zika virions at the multiplicity of infection (MOI) of 1.0 and 10.0 and evaluated the primary targets of ZIKV in the semen. We carried out real time RT-PCR (RTPCR, in situ RT-PCR (ISRTPCR) Results: ZIKV was present in the spermatozoa, epithelial and mononuclear cells of the semen in all three semen specimens analyzed. Both the sperm and mononuclear cells of the semen were found to be infected with ZIKV. In order to determine whether Tyro3 receptors, which are known to be expressed on human spermatozoa, play a role in ZIKV entry into spermatozoa, we co-localized of the ZIKV by ISRTPCR and Tyro3 by immunoflorescent (IFA) antibody to Tyro3 and found that, in the majority of cases, Tyro3 and ZIKA were co-stained. The most striking finding was that ZIKV only bound to the mid-piece of the spermatozoa and not the head, ruling out the potential of germ-line infection by ZIKV. Conclusions: Our data strongly suggest a potential sexual/horizontal route of transmission for ZIKV via infected sperm and other seminal cells. ZIKV most likely enters the sperm via the Tyro3 receptor found at the mid-piece of the mature spermatozoa. Our results thereby support rejection of germ-line infection by ZIKV.