

# Persistence of Zika Virus in Gradient Sperm Preparation

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Received date: June 14, 2017; Accepted date: June 16, 2017; Published date: July 20, 2017

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#### Abstract

In April 2016 ZIKA virus RNA was detected during control before performing in vitro fertilization in adult-man semen that contracted the infection in French Guyana and travel back to France. The infection had been detectable in the sperm by qRT-PCR and the virus persists in the semen even after routinely ART sperm migration performed in a bilayer gradient. Zika virus (ZIKV) is an arbovirus transmitted by *Aedes aegypti* mosquitos. The virus has spread rapidly in the *A. aegypti* endemic region (South America, Central America, the Caribbean, the Pacific islands, Singapore, and Thailand) but the ability of ZIKV to be transmitted sexually has enhanced the transmission of the disease in non-endemic countries. It has been reported that during pregnancy ZIKV infection can lead to developmental abnormalities (microcephaly, cerebral calcification, fetal loss) and that the virus is also associated with Guillain-Barré syndrome. Guidelines have been drawn up regarding patients who come from countries affected by ZIKV epidemic and who intend to use Assisted Reproductive Technology (ART). The international guidelines impose analysis for ZIKV detection in blood, urine and semen of the patient resident in or travelling back from ZIKV endemic countries in a 6-month-span. These screening analyses must be performed in semen before ART procedures.

Keywords: Zika virus; Blood; Semen

## **Case Study**

We report a case of a French man coming to Paris from Guyana, an endemic area, to perform ART for cryptozoospermia diagnostic [1]. The patient described an episode of symptoms compatible with ZIKV which occurred 1 month before his arrival in France: high fever (temperature around 40°C), cephalgia, myalgia and arthralgia.

His illness lasted 5 days and he had no symptoms of itchy rash and prostatitis or gross hematospermia. In our laboratory we collected and frozen his semen before the ART attempt [2-5]. The first standard serologic analysis (HCV, HBV, HIV, TPHA) were negative. But the ZIKV detection was positive by rRT-PCR in both: Total sperm and in the sperm fraction used in ART after bilayer gradient centrifugation. Second tests performed 10 days later detected IgG and IgM in the serum and ZIKV RNA was always present in sperm bilayer gradient preparation. Other control tests have been performed both in ejaculated sperm and in a sperm selected fraction resulting positive. These results have been confirmed by Arbovirus CNR (Marseille). The first negative result appeared on the 112<sup>th</sup> day after the onset of symptoms.

## Discussion

According to our knowledge this is the first report in which the ZIKV persists in the fraction. The characteristics of spermatozoa infection and the time window of risk of sexual transmission are still on debate and the detection of ZIKV on sperm head by immunohistochemistry has been showed [6].

The viral persistence in semen is one of the key issues and until a solution to this matter can't be found it would be better to follow specific measures of prevention, specifically in the ART procedures. Usually in ART the semen preparation by bilayer density gradient centrifugation coupled to the intra-cytoplasmic sperm injection (ICSI) largely decreases the transmission risk to viruses. We know that HCV isn't present in the last fraction used for ART [7] invers the ZIKV persist in this fraction like HIV and HBV [8].

### Conclusion

Before including a couple coming from endemic area in IVF program it's mandatory to perform a deeply virus research not only in the total sperm but in the last sperm fraction used in ART.

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