Effect of acute Zika virus infection on sperm and virus clearance in body fluids: a prospective observational study

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Summary

Background Evidence of human sexual transmission during Zika virus emergence is a matter of concern, particularly in procreation, but to date, kinetics of seminal shedding and the effects of infection on human reproductive function have not been described. To investigate the effects of Zika virus infection on semen and clearance of Zika virus from semen and body fluids, we aimed to study a cohort of Zika virus-infected men.

Methods This prospective observational study recruited men presenting with acute Zika virus infection at Pointe-à-Pitre University Hospital in Guadeloupe, French Caribbean, where a Zika virus outbreak occurred between April and November, 2016. Blood, urine, and semen were collected at days 7, 11, 20, 30, 60, 90, and 120 after symptom onset, and semen characteristics, such as total sperm count, sperm motility, vitality, and morphology, and reproductive hormone concentrations, such as testosterone, inhibin, follicle-stimulating hormone, and luteinising hormone, were assessed. At days 7, 11, and 20, semen was processed to isolate motile spermatozoa. Zika virus RNA was detected by RT-PCR using whole blood, serum, urine, seminal plasma, semen cells, and motile spermatozoa fractions. Zika virus was isolated from different sperm fractions on Vero E6 cultures.

Findings 15 male volunteers (mean age 35 years [SD 5; range 25–44) with acute Zika virus infection and positive Zika virus RNA detection in blood or urine were enrolled. Total sperm count was decreased from median 119×10^6 spermatozoa (IQR 22–234) at day 7 to $45\cdot2\times10^6$ ($16\cdot5$–$89\cdot6$) at day 30 and 70×10^6 ($28\cdot5$–$81\cdot4$) at day 60, respectively, after Zika virus infection. Inhibin values increased from $93\cdot5$ pg/mL (IQR 55–162) at day 7 to 150 pg/mL (78–209) at day 120 when total sperm count recovered. In motile spermatozoa obtained after density gradient separation, Zika virus RNA was found in three of 14 patients at day 7, four of 15 at day 11, and four of 15 at day 20, and replication-competent virus was found in the tested patient. Seminal shedding kinetics seemed heterogeneous among patients. Whole blood was the fluid most frequently positive for Zika virus RNA (62 of 92 samples) and three patients remained positive at day 120.

Interpretation Semen alterations early after acute Zika virus infection might affect fertility and could be explained by virus effects on the testis and epididymis. Frequency of shedding and high viral load in semen, together with the presence of replicative virus in a motile spermatozoa fraction, can lead to Zika virus transmission during sexual contact and assisted reproduction procedures. Whole blood seems to be the best specimen for Zika virus RNA detection, diagnosis, and follow-up.

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Introduction

Zika virus is an *Aedes* spp mosquito-borne flavivirus, first isolated in the rhesus monkey in Uganda in 1947 and in man in 1952.[1] The first large outbreaks occurred in the Yap islands in Micronesia (2007)[2] and then in French Polynesia (2013–14).[3] A recent outbreak in Brazil extended to several countries of the Americas. Acute Zika virus infection is symptomatic in 20–50% of cases. Microcephaly and CNS abnormalities in fetuses and newborn babies have been described after infection during pregnancy. In adults, neurological diseases such as Guillain-Barré syndrome and myelitis have been reported. In February, 2016, WHO designated the Zika virus epidemic "a public health emergency of international concern". This emergency situation ended mid-November, 2016. Nevertheless, WHO underlined the urgent need for greater understanding of the pathophysiology of Zika virus disease and transmission.

Zika virus has been isolated from numerous human fluids[4] and identified in human semen[5] and vaginal secretions.[6] In semen, Zika virus RNA can persist up to 188 days after symptom onset.[7] The localisation of Zika virus in the human genital tract and its consequences are not yet known. However, studies in monkeys[8] and mice[9–11] have evidenced Zika virus in different male genital organs and have shown the consequences of genital infection. A Zika virus antigen was detected inside the spermatozoa of an infected man.[12] Sexual transmission has been described.[13] In Brazil, higher Zika virus incidence in sexually active women than in men suggests that sexual transmission is involved in viral spread.[14]

Research in context

Evidence before this study

In February, 2016, when this study was proposed, very few data were available on Zika virus and human semen and no prospective study was available to determine the kinetics of Zika virus in semen and its association with presence of the virus in other body fluids. During the major 2016 Zika virus epidemic, sexual transmission of the virus, in particular from male to female, and adverse fetal outcomes linked to in utero transmission, such as microcephaly, were confirmed. To date, with an exception of a prospective study, only several case reports or very short series have been published showing that Zika virus persisted in semen after clinical remission, with a prolonged risk of sexual transmission. We searched PubMed with the terms "Zika" and "semen" or "sexual transmission" for articles published before June, 2017, in any language and identified 27 articles. Animal studies show Zika virus localisation in the testes and also in several other parts of the male genital tract. In an immune-modified male mouse model, severe damage due to Zika virus infection was observed in the testes and epididymis. However, no data were available on semen characteristics in men during and after infection. The precise localisation of Zika virus in semen (seminal plasma, semen cells, and spermatozoa) was not known, despite a case report of Zika virus antigen within spermatozoa. In February, 2017, the preliminary results of a prospective study reported clearance of the virus in different body fluids, except whole blood samples, and including semen but semen characteristics and Zika virus localisation in semen were not analysed.

Added value of this study

This prospective study provides longitudinal data showing that replicative Zika virus can persist in semen after clinical remission despite its clearance in other fluids such as blood and urine. This study investigates semen characteristics during 120 days follow-up and Zika virus localisation in different fractions of semen. Depending on semen viral load, different shedding patterns were observed. Moreover, we show quantitative and qualitative harmful effects on spermatozoa production. Finally, replication-competent virus was found in seminal plasma and also in spermatozoa after spermatozoa isolation by semen preparation methods.

Implications of all the available evidence

Data for frequency and duration of replicative Zika virus shedding in semen are of vital importance in planning strategies for prevention of sexual transmission. Moreover, although they were transient in this study, quantitative and qualitative sperm production alterations challenge human reproductive potential. Detection of replication-competent virus in isolated spermatozoa from an infected patient is also a matter of concern for sexual transmission, reproduction, and particularly for assisted medical procreation guidelines. Further studies are necessary to identify the factors responsible for Zika virus shedding in semen and for sperm alterations. Last, whole blood is shown as the most sensitive sample for molecular diagnosis of Zika virus infection. The results of this study increase our knowledge of Zika virus infection and its effect on human male reproduction and will help physicians to counsel infected patients and public health specialists to make policy recommendations.

Understanding the localisation of Zika virus within the male genital tract, its dynamics, and shedding in semen, is of paramount importance to prevent sexual transmission. The consequences of Zika virus infection on the testis, genital tract, and fertility in men are unknown. Mice studies using immune-deficient animals, modified Zika virus strains, or both have shown a highly deleterious effect of Zika virus infection on the testis and epididymis.

In human beings, the association of a mosquito-borne virus with sexual and transplacental transmissions and with birth defects had not previously been described. This association raises increased concerns about the consequences of the Zika virus outbreaks.

We aimed to identify Zika virus in semen and within the different semen compartments, and to determine semen characteristics and reproductive hormone concentrations immediately following infection in men.

Methods

Study design and participants

This was a prospective observational study that enrolled men aged between 18 years and 45 years who were diagnosed with Zika virus infection. Exclusion criteria included men with other acute illnesses, inability to provide a semen sample, ejaculation disorders, semen volume less than 1·5 mL, or negative Zika virus RNA in serum or urine. Patients attended follow-up visits 7, 11, 20, 30, 60, 90, and 120 days after symptom onset (appendix p 4). Whole blood, serum, urine, and semen samples were collected at each visit.

See Online for appendix

19 male patients presenting with clinical symptoms of acute Zika virus virus infection were recruited through physicians after a press information campaign in Guadeloupe Island and underwent a Zika virus RNA test in urine and serum. Of these 19 men, three men with a negative Zika virus RNA test and one with very low semen volume were excluded. 15 men diagnosed with acute symptomatic Zika virus infection and positive for Zika virus RNA in serum or urine were enrolled at Pointe-à-Pitre University Hospital, Guadeloupe. This area was officially designated a Zika virus outbreak area from April to November, 2016. One patient had been taking levothyroxine for hypothyroidism for several years and one patient had chronic migraine treated by oxetorone. Seven patients were married or cohabiting and eight were single. 11 men were of African or mixed descent and

four were white. All volunteers gave written informed consent and received compensation (€400) for their participation. At each visit, a questionnaire was completed about any adverse events since the last visit to the laboratory.

This study is registered with ClinicalTrials.gov, number NCT02874456, and was approved by the institutional ethics review board (CPP Sud-Ouest et Outre-Mer II).

Procedures

100 semen samples were obtained by masturbation after a recommended 3–6 days abstinence period and processed within 1 h of ejaculation for analysis. Seminal plasma and whole semen cells were isolated from a semen aliquot by centrifugation at 600 g and frozen at –80°C. Semen analysis was done according to WHO guidelines on an aliquot (200 µL) of semen (appendix p 3). At days 7, 11, and 20, an aliquot of semen was processed to isolate spermatozoa cell populations (90% fraction and swim-up fraction) according to previously published methods used for HIV-infected men (appendix p 3).[15] Seminal plasma and spermatozoa underwent Zika virus RNA analyses and viral isolation.

Urine, whole blood, and sera were collected in the morning and frozen until Zika virus RT-PCR assays. Serum follicle-stimulating hormone, luteinising hormone, and testosterone concentrations were assessed by automated immunoassay (Cobas 8000e602, Roche Diagnostics, Meylan, France). Serum inhibin B concentrations were quantified in duplicate using a manual ELISA assay (AnshLabs, Webster, TX, USA) with a quantification limit of 4·6 pg/mL.

RNA was extracted from whole blood, serum, urine, and semen fractions with the MagNA Pure 96 instrument (Roche Diagnostics, Meylan, France) using the DNA and Viral NA Small Volume Kit (Roche Diagnostics; input and output volumes 200–100 µL). For semen cell fractions, input volumes were adjusted to 2×10^6 cells. Zika virus RNA was quantified using the RealStar Zika RNA RT-PCR kit 1·0 (Altona Diagnostics GmbH, Hamburg, Germany; limit of detection 2·48 log copies per mL). The manufacturer's internal control was systematically used to check for PCR inhibitors.

Samples from one patient (patient 13) positive for Zika virus RNA in seminal plasma and cell fractions, including motile spermatozoa, were tested for infectivity in Vero E6 cell cultures (appendix p 3).

Anti-Zika virus IgG and IgM antibodies were detected using Diapro Zika virus IgG or IgM ELISA immunoassay (Launch Diagnostics, Longfield, England, UK) according to the manufacturer's protocols. Results are expressed as signal-to-cut-off ratios (S/CO). Anti-dengue virus IgG and anti-chikungunya virus IgG antibodies were detected using dengue virus IgG and chikungunya IgG ELISA immunoassays (Diapro, Diagnostic Bioprobes Srl, Milano, Italy). HIV and human T-lymphotropic virus type 1 (HTLV-1) screening tests were done on Abbott Diagnostics Architect i2000SR (Abbott, Rungis, France).

All frozen samples were transferred to the GERMETHEQUE national biobank (BB-0033-00081; coordination: Toulouse, France) and data from case report forms were centralised at Toulouse University Hospital (Toulouse, France).

Statistical analysis

Data are presented as median (IQR) due to the number of patients and as boxplots for graphic representation. Data were compared between positive and negative Zika virus RNA samples (whole blood, serum and, seminal plasma) using the non-parametric Mann-Whitney test for quantitative variables (semen parameters, sperm pellet). To study association between quantitative variables, Spearman's rank correlation coefficient was used.

Sperm characteristics were compared between day 7 and the following days (days 11, 20, 30, 60, 90, and 120) using the Wilcoxon signed rank sum test. Hormone values were compared between day 7 and days 30, 60, and 120. Because there were multiple comparisons, a Bonferroni correction was used and p=0·0083 was considered significant for sperm characteristics and of p=0·016 for hormone values.

Data were analysed with SAS software (version; 9.3, SAS Institute). p=0·05 was considered significant in the case of no Bonferroni correction.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

15 Zika virus-positive men (mean age 35 years [SD 5, range 25–44) were enrolled in the study. All patients were symptomatic (appendix p 7). They were followed up prospectively for 120 days, except one patient who withdrew after day 30 visit (no reason was given). All were negative for HIV and HTLV-1 antibodies. Four participants provided further samples at day 150, and two at day 180.

All patients developed an immune response against Zika virus. Anti-Zika virus IgM antibodies were detected as early as day 7 in 12 patients (80%) and in all patients at day 20. After day 20, anti-Zika virus IgM seemed to decrease but with an average still above unity (appendix p 5).

Two patients had negative anti-dengue virus IgG in sera: one (patient number 7) had no Zika virus in semen and the other (patient number 10) had only semen Zika virus excretion at day 7. 13 patients were anti-dengue virus IgG positive: three of these patients had no semen excretion of

Zika virus (numbers 1, 12, and 14). Only patients 4, 6, 13, and 15 had their day 7 serum reactive for anti-chikungunya virus IgG.

Zika virus RNA detection in the different body fluid samples during follow-up is shown in figure 1, and viral load for each body fluid is shown in the appendix (p 11).

Zika virus RNA was detected in at least one serum sample in all patients. 36 (39%) of 92 sera were Zika virus RNA positive, maximum value 4·38 log copies per mL. Four patients still had positive serum samples at day 30. All volunteers but one had at least one Zika virus RNA-positive whole blood sample. 62 (67%) of 92 samples were Zika virus RNA positive (highest viral load: 4·70 log copies per mL). Three volunteers (23%) remained Zika virus RNA-positive at day 120. All volunteers had at least one Zika virus RNA-positive urine specimen. 36 (40%) of 91 urine specimens were Zika virus RNA positive (highest viral load: 5·38 log copies per mL). Two urine samples tested positive at day 30.

11 men (73%) had at least one Zika virus RNA-positive seminal plasma or semen sample at day 7. In four patients (27%), Zika virus RNA was never detected in semen or its fractions.

Only one sample exhibited PCR inhibitors. 35 samples (35%) were Zika virus RNA-positive (highest seminal viral load: 10·20 log copies per mL). Of the four patients who provided seminal plasma after 120 days, one remained Zika virus RNA positive at day 160 (3·40 log copies per mL).

Zika virus RNA was detected in 27 (27%) of 100 native semen cell pellets. The highest viral load in native semen cell pellets was 9·12 log copies per 2×10^6 cells.

The results of Zika virus RNA detection in seminal plasma showed three different patterns of viral seminal shedding (figure 2): non-shedding patients, with consistently negative Zika virus RNA detection in seminal plasma during follow-up (figure 2A; n=4, patients 1, 7, 12, and 14); seminal shedders with concomitant sera, urine shedding, or both (figure 2B; n=6, patients 2, 3, 4, 8, 9, and 10); and persistent seminal shedders after virus clearance in sera and urine—ie, discordant shedding patients (figure 2C; n=5, patients 5, 6, 11, 13, 15). Intermittence of seminal excretion was observed for three patients (patients 5, 11, and 15) from the discordant shedding patient group.

We isolated spermatozoa fractions at days 7, 11, and 20 in all semen samples except one (appendix p 10). 11 (25%) of the 90% fractions containing only spermatozoa were Zika virus RNA-positive. All were from semen with high Zika virus RNA load in seminal plasma (>5 log copies per mL). All these positive 90% fractions were submitted to a swim-up method to isolate motile spermatozoa. Seven (64%) swim-up fractions later tested positive for Zika virus RNA (maximum 7·20 log copies per 2×10^6 cells).

No sample was positive in cell fractions obtained after sperm preparation if native semen was negative. Seminal plasma was positive and semen cells of native semen were negative in seven (16%) of 45 samples, and seminal plasma was negative and native semen cells were positive in two (2%) of 45 samples.

Zika virus was isolated from seminal plasma, 90% fractions (spermatozoa), and swim-up fractions (motile spermatozoa) in the one volunteer for whom the isolation of virus was done (patient 13). Viral replication was observed in all these specimens, as assessed by cytopathic effect observation and measurement of increased Zika virus RNA levels on Vero E6 cell culture (appendix p 6).

Semen characteristics are reported in table 1 and figure 3. Total sperm count and total motile sperm count were significantly decreased (about 50% lower) at day 60 compared with at day 7. The multiple anomalies index was already higher at days 30 and 90 than at day 7. At day 120, median values of total sperm count, sperm motility, vitality morphology, and multiple anomalies index did not differ from day 7.

Compared with day 7, follicle-stimulating hormone concentration was higher at day 30 and decreased after this time with lower values at day 60 and day 120 than at day 7. Lowest inhibin β concentration was observed at day 7 with a progressive increase over time (table 2). Although luteinising hormone concentrations decreased after day 7, testosterone values were not significantly different at the different timepoints studied, while a trend toward lower values was observed at day 7.

When Zika virus RNA was positive in native semen (seminal plasma or semen cell pelets), semen volume, total sperm count, and total motile sperm count were significantly lower compared with Zika virus RNA-negative semen (p<0·05; appendix p 8).

Discussion

This prospective study is the first, to our knowledge, to: undertake a longitudinal assessment of different biological samples (whole blood, serum, urine, and semen) jointly

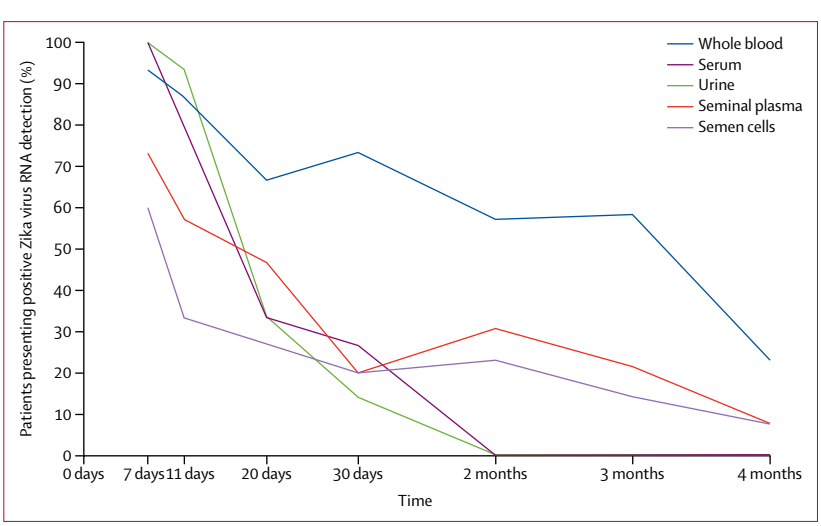

Figure 1: **Frequency of Zika virus RNA detection in the different fluids according to timepoints after Zika virus infection symptoms onset in 15 patients**

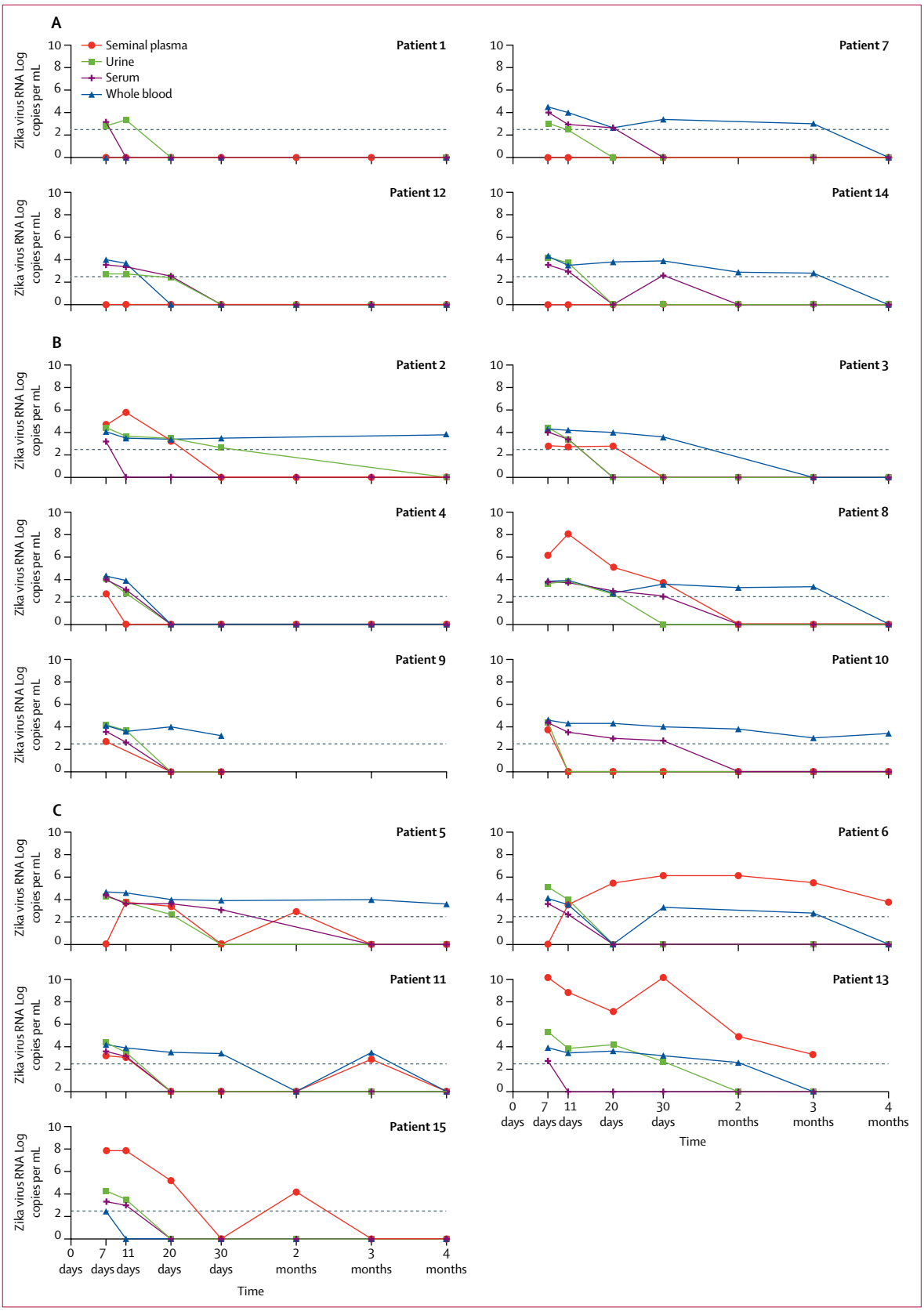

Figure 2: **Zika virus viral load (Zika virus RNA Log copies per mL) in the fluids studied for all 15 patients**
According to the seminal viral load, three patterns were identified: (A) non-shedding patients (patients 1, 7, 12, 14); (B) seminal shedders with concomitant blood, urines shedding, or both (patients 2, 3, 4, 8, 9, 10); (C) long-term seminal shedding patients discordant with urine or blood shedding (patients 5, 6, 11, 13, 15). The dotted line represents the detection limit of the kit (2·48 log copies per mL).

	Day 7 (n=15)	Day 11 (n=15)	Day 20 (n=15)	Day 30 (n=15)	Day 60 (2 months; n=13)	Day 90 (3 months; n=14)	Day 120 (4 months; n=13)
Volume (mL)	2·75 (1·9–4)	2·9 (2–3·6)	2·6 (1·7–4·2)	2·8 (2–4·2)	2·5 (1·9–4)	2·7 (1·7–4)	3·35 (2–4)
Sperm count (millions per mL)	44 (11·5–83)	26·6 (14–63)	14·9 (11·5–47)	13 (5–37)	20·2 (11·5–37)*	21 (7·7–61·5)	31·5 (14·5–67·5)
Total sperm count (millions per ejaculate)	119·0 (22–234)	52·0 (33·6–229·5)	80·46 (16·1–130·2)	45·2 (16·5–89·6)	70·0 (28·5–81·4)*	44·92 (17·6–171·5)	86·9 (26·32–168·75)
Total motile sperm count (millions per ejaculate)	49·01 (5·5–108·61)	19·89 (13·2–103·28)	36·21 (5·04–50·72)	10·12 (3·3–44·8)	24·42 (11·16–35)*	13·81 (3·52–47·88)	34·76 (9·135–75·9375)
Motility (%)	35% (35–45)	40% (35–45)	40% (30–45)	35% (20–45)	40% (30–50)	40% (20–42)	40% (35–45)
Vitality (%)	60% (56–73)	61% (55–66)	62% (51–66)	54% (49–63)	64% (61–69)	55% (47–62)	67% (53–70)
Normal sperm (%)	21% (15–33)	20% (17–31)	16% (13–31)	13% (7–27)	16% (10–22)	13% (11–19)*	20% (12–26)
Multiple anomalies index	1·85 (1·72–2·17)	1·995 (1·84–2·12)	2·06 (1·82–2·26)	2·08 (1·74–2·34)*	2·17 (1·92–2·31)	2·22 (2·02–2·36)*	2·09 (1·96–2·13)

Data are median (IQR). *p<0·0083 between day 7 and days 11, 20, 30, 60, 90, or 120.

Table 1: **Semen characteristics of the 15 Zika virus-infected patients according to timepoints after infection symptom onset**

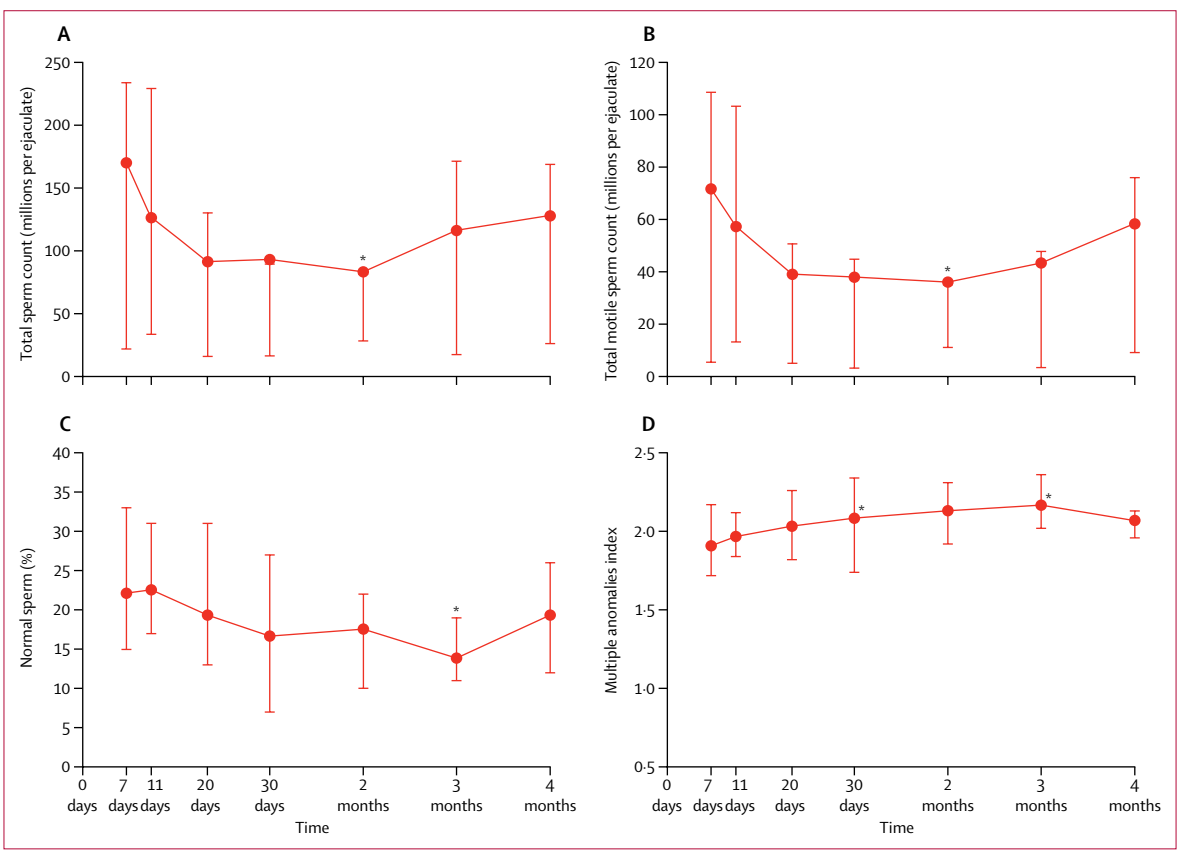

Figure 3: **Semen characteristics of the 15 Zika virus-infected patients according to timepoints after infection symptom onset**
(A) Total sperm count (millions per ejaculate). (B) Total motile sperm count (millions per ejaculate). (C) Normal sperm (%). (D) Multiple anomalies index. Data are median (IQR). *p<0·083 between day 7 and days 11, 20, 30, 60, 90, or 120.

with the investigation of semen characteristics and reproductive hormones following acute Zika virus infection, and determine the detection and clearance of Zika virus RNA in different semen fractions and the presence of replicative virus (including motile spermatozoa fractions, generally used in assisted reproductive procedures).

15 symptomatic patients provided the first samples at day 7 after clinical onset. All patients showed an immune response to Zika virus because IgM antibodies were systematically detected 7–20 days after clinical onset. All patients but two were immunised against dengue virus.

	Day 7 (n=14)	Day 30 (n=8)	Day 60 (2 months; n=7)	Day 120 (4 months; n=13)
Follicle-stimulating hormone (IU/L)	4·45 (3·6–7·8)	5·85 (2·75–8·75)*	3·3 (2·1–4)*	3·7 (3–5·4)*
Luteinising hormone (IU/L)	6·95 (4·9–7·5)	4·9 (3·55–5·55)*	5 (3·2–6·1)	4·4 (3·2–5·5)*
Inhibin (pg/mL)	93·5 (55–162)	125·5 (53–196·5)*	144 (70–220)*	150 (78–209)*
Testosterone (ng/dL)	344·5 (246–477)	517 (338–543)	515 (365–591)	415 (300–460)

Data are median (IQR). *p<0·0166 between day 7 and days 30, 60 or 120.

Table 2: **Hormone concentrations in sera of Zika virus-infected patients according to timepoints after infection symptom onset**

Serum and urine samples were Zika virus RNA positive for all patients at day 7 after clinical onset. In agreement with Paz-Bailey and colleagues,[16] Zika virus RNA detection was more frequent in serum than in urine during follow-up. Two urine samples and four sera were Zika virus RNA positive at day 30 but none after. Notably, Zika virus RNA was detected in whole blood in all patients except one. This whole blood-negative patient showed only brief and low viraemia or viruria without viral excretion in semen. In our study, whole blood was more frequently Zika virus RNA positive than were urine or sera, and remained so for longer periods (up to 120 days). Similarly, Murray and colleagues[17] detected Zika virus RNA in erythrocytes after infection. We confirm the higher sensitivity of Zika virus RNA detection in whole blood than in urine or serum compartments.[18] This finding could have an effect on the recommendations of the samples to be used for molecular detection of Zika virus RNA detection, whether for diagnosis of an acute infection or for detection of the virus in a biological product of human origin.

Several case reports have described Zika virus shedding in semen from symptomatic and asymptomatic men, and sexual transmission of the virus has also been reported. Sexual transmission is a major concern, particularly for pregnant women and couples wishing to conceive, because of the adverse effects of the virus during pregnancy. However, very few cohort studies have analysed the persistence and clearance of Zika virus in semen.[16,19] Most (73%) of our Zika virus-infected patients excreted the virus in semen 7 days after symptom onset. Our prevalence of patients with Zika virus-positive semen seems to be higher than that reported in other studies.[16,19] These differences could be explained by the fact that in our study, unlike other studies, Zika virus RNA was detected both in seminal plasma and in semen cells. This could have led to increased detection in semen samples, and consequently is a better reflection of the presence of Zika virus in semen. According to Zika virus RNA seminal shedding, we defined three patient patterns: non-shedding patients, with consistently negative Zika virus RNA detection in seminal plasma during follow-up (27%), seminal shedders with concomitant blood, urine shedding, or both (40%), and discordant shedder patients with persistent seminal shedding after virus clearance in sera and urines (33%).

The causes of these different patterns need further investigation because several hypotheses could be suggested: differences in viral seeding and local replication within genital organs and cells, differences in local host innate and adaptive immune defences, or differences in virus strain tropism (although all patients came from the same localised area and were recruited over a short period of time). Thus specific male organs or cells could act as mid-term or long-term reservoirs for Zika virus once infected, as has been reported for a number of systemic viruses.[20,21] Our results confirm that seminal viral loads can be much higher than blood and urine viral loads.[5]

The precise replication site of Zika virus in semen is currently unknown in man. The fact that vasectomised men[22,23] can shed Zika virus in semen suggests that male genital organs distal from the testis could contribute to viral production. The persistence of Zika virus-infected cells in the testis, seminal vesicles, and prostate of non-human primates with cleared viraemia has also recently been reported.[8] The haematospermia reported during acute Zika virus infection[24,25] could suggest local genital tract infection.

Density gradient centrifugation and swim-up methods are effective in obtaining HIV-free or hepatitis C virus-free spermatozoa populations.[15] In some of our patients, high Zika virus RNA loads were still detected in the swim-up fraction, which contains only motile spermatozoa. This finding could reflect Zika virus adherence to spermatozoa or their infection, although technical contamination of the preparation due to the high viral load in semen cannot totally be ruled out, as reported for HIV virus.[26] In support of Zika virus interaction with sperm cells, we previously detected a Zika virus antigen in spermatozoa from an infected patient.[12] The fact that we were able to rescue replication-competent virus from all fractions, including the swim-up fraction of motile spermatozoa, argues against contamination with genomic RNA or defective virus particles.

To date, the effect of acute Zika virus infection on semen and hormonal characteristics in men has not been studied. Studies in mouse models described major effects such as orchitis and epididymitis, following systemic infection of immune-compromised animals.[10,11] In non-human primates, foci of Zika virus-infected cells were localised in the testes, prostate, and seminal vesicles.[8] We studied sperm characteristics over time following Zika virus infection. As early as 30 days post symptom onset, the multiple anomalies index increased, the median percentage of normal forms was significantly decreased at day 90, and recovery of both parameters was observed at day 120. The lowest median values of total sperm count and total motile sperm count were found at day 30, with significance at day 60 after clinical onset. Recovery was seen at days 90–120. Soon after Zika virus symptom onset,

follicle-stimulating hormone concentrations were higher and inhibin β concentrations were lower than at day 120, which could be related to an initial direct or indirect effect of infection on Sertoli cell function. This could be associated with subtle early Leydig cell dysfunction because there was a trend for low testosterone concentrations and significantly higher luteinising hormone at day 7. This finding is concordant with the decreased inhibin and testosterone concentrations observed in testis homogenates from Zika virus-infected immune-deficient mice at 14 days and 21 days of Zika virus inoculation.[10,27] Early sperm alterations could result from viral infection of the epididymis with a direct or indirect effect on spermatozoa during epididymal transit (mean duration 12 days) or testis infection, or both, affecting late spermiogenesis (spermiogenesis duration 23 days). The sperm modifications might also be related to fever effect. This has previously been reported, but after high fever.[28,29] However, although most of our patients reported subjective fever, pyrexia during Zika virus infection is usually moderate. Moreover, we found that semen characteristics were more altered in seminal plasma samples with positive viral loads than in those with undetectable loads. This finding suggests that Zika virus infection by itself could probably be directly responsible for sperm alterations, although further studies with a large number of men are needed to confirm our results.

One limitation of this study was the absence of a control group—ie, non-infected men, for the study of semen and hormonal characteristics. Studies, particularly on Zika virus-infected and asymptomatic men (ie—without fever) including also a control group, as well as in relevant animal or ex vivo human models, are needed to decipher the origins of sperm alterations and to investigate the infectivity and functions of genital glands following Zika virus infection.[5,30]

In conclusion, this study provides a longitudinal assessment of the detection and clearance of Zika virus in different body compartments. We show that Zika virus RNA is detected in whole blood longer than in serum and urine. We describe three different patterns of Zika virus excretion in semen according to their viral excretion in semen, blood, and urine. Importantly, we show that Zika virus infection modifies semen characteristics in men and that replication-competent virus can be isolated from motile spermatozoa. Although our knowledge of Zika virus infection and the reproductive tract is incomplete, these findings have implications for public health policy, contributing to increased diagnostic efficiency and limiting of sexual transmission of Zika virus, as well as guiding counselling of Zika virus-infected patients and couples who wish for a child.

Contributors

LB, CP, and GJ did the literature search and designed the study. LP, GJ, and PL recruited the volunteers. LP, NP, and GJ did the clinical and biological follow-up of the volunteers. SG did the first biological Zika virus diagnosis tests for volunteer inclusion. J-MM and CP did all virological and immune analyses. GM and NDR did the virus isolation. SH did the hormonal investigations. MW carried out all data treatment, statistical analyses, and designed the article figures. GJ, J-MM, GM, MW, LP, NP, ND-R, CP, and LB participated in interpretation of the data and writing the discussion. All the authors have approved the manuscript. The study protocol and manuscript writing was coordinated by LB.

Declaration of interests

We declare no competing interests.

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