Zika virus detected in amniotic fluid and umbilical cord blood in an in vitro fertilization-conceived pregnancy in Venezuela

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Objective: To describe the consequences of Zika virus infection at 10 weeks of gestation in an IVF-conceived pregnancy in Venezuela.
Design: A case report.
Setting: Private assisted reproduction unit.
Patient(s): A 36-year-old patient who conceived her first pregnancy through IVF and became infected with Zika virus at 10 weeks' gestation in Venezuela.
Intervention(s): In vitro fertilization with fresh ET. Clinical, laboratory, and imaging Zika diagnostic methods.
Main Outcome Measure(s): Zika virus detection by real-time polymerase chain reaction (PCR) in maternal plasma, PCR in amniotic fluid and umbilical cord blood. Ultrasonography findings of anatomic abnormalities.
Result(s): Zika infection was confirmed at 10 weeks' gestation by real-time PCR; ultrasound results appeared normal. At 19 weeks' gestation, an ultrasound revealed biometry on three SDs below the means for all parameters but with no apparent anatomic abnormality. Zika virus was positive in maternal urine and amniotic fluid by PCR at 19 weeks' gestation. Ultrasound at 21 weeks + 4 days of gestation showed fetal cerebellar hypoplasia with ventricular dysmorphia, particularly marked on the left, consistent with microcephaly and ventriculomegaly. Because of the poor prognosis, pregnancy was interrupted at 24 weeks' gestation, in France. The PCR in umbilical cord blood taken in this procedure was positive for Zika virus.
Conclusion(s): Initial ultrasound findings in pregnancy may not be informative. Only at 21 weeks + 4 days of gestation did an ultrasound reveal fetal microcephaly and ventriculomegaly. Combined clinical, laboratory, and imaging findings provided a complete picture of the severe damage caused by Zika infection. (Fertil Steril® 2017;107:1319–22. ©2017 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Key Words: Zika virus, microcephaly, congenital malformations, ventriculomegaly, amniotic fluid

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Recently, an epidemic of Zika virus (ZIKV) occurred in South and Central America. ZIKV, an arbovirus (arthropod-borne virus), is transmitted by domestic mosquitos such as *Aedes aegypti* and, to a lesser extent, by *A. albopictus*. It was isolated for the first time in 1947, from a febrile *Rhesus* monkey, in the Zika Forest (Uganda) and recognized in men in Nigeria in 1953 (1).

The last epidemiological update of the World Health Organization, dated September 8, 2016 (2), reports that, since 2015, 46 American countries and territories confirmed native cases by vector transmission, and 5 countries reported sexually transmitted Zika cases. At present, vertical transmission (mother to fetus) is also recognized.

The virus, as opposed to other arboviruses, such as dengue and chikungunya, presents a strong neurotropism (3). On April 13, 2016, the Centers for Disease Control and Prevention (CDC) of the United States recognized the causal relationship between ZIKV infection during pregnancy and fetal microcephaly and other serious cerebral anomalies (4).

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However, at present, the incidence of fetal anomalies related to ZIKV infection during pregnancy is unknown.

Providing counseling is challenging because currently available data are limited. To determine the gestational consequences of ZIKV infection could be useful for preconception and postconception counseling.

CASE REPORT

A French couple, married for 2 years, with no known medical or family history, attended a consultation in November 2015, because of a 2-year primary infertility. The woman was 36 years old, nulliparous, and her husband was 34 years old at the time. The couple had lived in Caracas, Venezuela, for 4 years.

A fertility study showed the infertility to be caused by endocrine factors, positive antithyroglobulin antibodies, and insulin resistance. Hence, we suggested an IUI; but given the lengthy period of infertility, they decided on IVF.

In February 2016, the couple returned to undergo the procedure. Controlled ovarian hyperstimulation (COH) was performed with 225 IU of recombinant FSH (Gonal-F, Merck Serono) for 12 days and fixed antagonist protocol: 0.25 mg cetrotide (Merck Serono), 81 mg baby aspirin, 5 mg folic acid. Comorbid pathologies were treated with 50 μg/d levothyroxine, 1,500 mg/d metformin, and prophylactic antibiotic therapy with 1 g of azithromycin at the beginning of the stimulation cycle. The trigger injection with 250 μg of recombinant hCG (Ovidrel, Merck Serono) was performed when the follicular measurements of at least one or two follicles had ranged $\geq$18 mm in diameter. Thirty-five hours after hCG, follicular aspiration was performed under IV sedation. Eight oocytes were obtained and, afterward, four blastocysts. Only one blastocyst was transferred to the uterus. Support of the luteal phase was started 2 days after follicular aspiration, with micronized P soft gelatin capsules (Utrogestan, Laboratorios Seid), 200 mg every 8 hours administered intravaginally until the ninth week of gestation.

Real-time polymerase chain reaction (PCR) for ZIKV in plasma was performed at 10 weeks gestation at Instituto de Higiene Rafael Rangel, Universidad Central de Venezuela, Caracas, Venezuela. The PCR of amniotic fluid and umbilical cord blood were performed at 19 and 24 weeks of gestation, respectively, at the Hôpital Necker in France. Institutional Review Board approval was obtained.

RESULTS

Pregnancy was confirmed by quantitative β-hCG in plasma (549.8 mU/mL) 10 days after blastocyst transfer. Cardiac activity was confirmed at 6 weeks with the first transvaginal ultrasound. Obstetric examinations were conducted on appropriate dates, and prenatal tests were performed, including the TORCH profile (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex), all of which were negative.

At 10 weeks of gestation, the patient presented with fever and erythematous and pruriginous cutaneous exanthema. The IgG and IgM antibodies for dengue and chikungunya were tested, and real-time PCR for ZIKV in plasma was performed. Only ZIKV returned a positive result. Ultrasound findings after the clinical expression of infection, performed at 12 weeks gestation, showed a cranial-caudal longitude of 51.8 mm and nuchal translucency of 0.4 mm. The couple was informed of the probability that the fetus would be affected given that infection manifested in the period of morphogenesis. Echography at a gestational age of 14 weeks + 3 days revealed a biparietal diameter of 27.1 mm and femur length of 11.5 mm, without detection of any anatomic abnormality.

Given the patient's age and the ZIKV infection during the early gestational period, we suggested genetic amniocentesis and virus detection in amniotic fluid at 15 weeks (according to CDC Guidelines: Amniocentesis performed at $\geq$15 weeks of gestation is associated with lower rates of complications than when performed at earlier gestational ages). Nonetheless, the couple refused to undergo such procedures.

Toward the end of June 2016, the patient traveled to France, where she underwent ultrasonic examination at 19 weeks of gestation; biometrically, less than three SDs below the average was evidenced for all parameters, with no anatomic abnormality. Given these findings, the couple accepted amniocentesis for ZIKV detection in the amniotic

TABLE 1

Evolution of laboratory and ultrasound findings.

Gestational age (wk+d)	10	12	14+3	19	21+4	24
Weeks after infection	1			9		14
Symptoms	Fever exanthema					
Ultrasound		Normal	Normal	<3 SD below average for all parameters; No anatomic abnormalities	Cerebellar hypoplasia and ventricular dysmorphia	
ZIKV RT-PCR maternal blood	Positive					
TORCH	Negative					
CMV PCR toxoplasma PCR				Negative		
ZIKV PCR urine and amniotic fluid				Positive		
ZIKV PCR cord blood						Positive
Pregnancy interruption						X

Note: CMV = Cytomegalovirus; PCR = polymerase chain reaction; RT = real-time; ZIKV = Zika virus.

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fluid and maternal urine; at 19 weeks, both tests confirmed the presence of ZIKV.

In the next echography, performed at 21 weeks + 4 days of gestation, fetal cerebellar hypoplasia with ventricular dysmorphia, particularly marked on the left, was evidenced, leading to a diagnosis of microcephaly and ventriculomegaly. The couple decided to undergo echography follow-up in 15 days. The multidisciplinary committee of prenatal diagnosis of Hôpital Necker explained to them that there was a strong probability that the fetus had microcephaly and ventriculomegaly, recognized as incurable.

The couple decided to interrupt pregnancy at 24 weeks of gestation. Induced delivery produced a male fetus weighing 395 g. The PCR was performed for ZIKV in umbilical cord blood, which came out positive. Information regarding pathological anatomy was not available to us at the time of writing. Table 1 shows the evolution of laboratory and ultrasound findings.

DISCUSSION

The World Health Organization declared the ZIKV epidemic a world health issue (5), and beyond doubt, its progressive growth represents an important problem, not only for obstetric patients but also for those with the intention to reproduce. It is challenging to provide counseling because of the limitations of currently available data. Although there are guidelines regarding the time to wait after the onset of illness before attempting pregnancy (6), infection may still occur during the course of fetal development. In areas where climate favors reproduction of the vectors, this entails a challenge for medical personnel.

Early ultrasound findings in pregnancy may offer no lasting reassurance to couples; in this case, only at 21 weeks + 4 days of gestation did an ultrasound reveal microcephaly and ventriculomegaly in the fetus. Combined findings from clinical examination, laboratory tests, and imaging provide a complete picture of the severe damage caused by ZIKV infection.

Information about the presence of the ZIKV in the amniotic fluid of pregnant women with microcephalic fetuses is scarce in the literature (7–10). ZIKV was detected in the amniotic fluid of two women having symptoms of ZIKV infection in their first trimester of pregnancy in Brazil. Ultrasounds at 21–22 weeks' gestation confirmed microcephaly. Amniotic fluid tested at 28 weeks confirmed the presence of ZIKV genomes as well as Zika antibodies. Tests were negative for other infectious diseases, and samples of blood and urine were negative for ZIKV (7). There is a technical report in Venezuela of a microcephaly case associated with a recent ZIKV infection, confirmed by PCR in amniotic fluid and cord blood 4 weeks + 3 days after infection (9). In Colombia, ZIKV was documented by real-time PCR in amniotic fluid at 10 weeks and, in cord blood, at 28 weeks after infection (10). ZIKV load in the amniotic fluid was three times higher than that in the maternal serum.

The fact that the first-trimester placenta is susceptible to ZIKV infection, along with data showing the presence of the virus in the amniotic fluid (7), prompted El Costa et al. (11) to investigate whether the virus targets the umbilical cord. They found that, in the mouse, umbilical cords are permissive to ZIKV infection.

In our study, ZIKV was detected by PCR in amniotic fluid even at 9 weeks after infection and in umbilical cord blood at 14 weeks (Table 1). These results support the view that ZIKV can cross the placental barrier and strengthen the causal association between ZIKV and cases of microcephaly and ventriculomegaly. Intrauterine viral load results suggest persistent replication, partly explained by the reduced immune system response of the fetus, as has been noted in the pathogenesis of congenital cytomegalovirus (7).

Patients undergoing IVF need counseling about Zika to prevent infection during pregnancy. Together with the preventive measures (environmental, contraceptive), we must be emphatic in transmitting awareness regarding the risk entailed in contracting ZIKV infection during pregnancy. On the other hand, if a couple wants to assume the risk of getting pregnant in endemic zones or in times of pandemic, exercising their right to reproduce, they deserve access to assisted reproduction technology (ART), with prior informed consent. They should be offered alternatives to immediate gestation, such as oocyte and embryo vitrification or cryopreservation of semen. By learning about all options and being aware of their needs and goals, patients undergoing IVF should be prepared to make the best decisions.

The following resources can be helpful in offering before or after conception counseling: *Guidance for Providers Caring for Women and Men of Reproductive Age with Possible Zika Virus Exposure*, published by the American Society for Reproductive Medicine (www.asrm.org/Guidance_for_providers_caring_for_patients_exposed_to_zika); CDC Zika Clinical Guidance (www.cdc.gov/zika/hc-providers/clinical-guidance.html); the CDC's *Interim Guidelines for Pregnant Women During a Zika Virus Outbreak* (www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm); as well as various World Health Organization and Latin American Network of Assisted Reproduction (REDLARA) resources.

Because there is no vaccine, no treatment, no good serologic test, vector control is difficult, and because of the severe consequences, abortion access should be offered. The Zika crisis exposes the social injustice of lack of access to abortion in Latin American countries and promotes reproductive tourism. In Venezuela, there is no access to legal therapeutic abortion services. Abortion in Venezuela is illegal except in cases of threat to the life of the pregnant woman. Governments now have the opportunity—and the responsibility—to close such gaps in reproductive rights (12).

REFERENCES

1. Minassian ML. Virus Zika: Crónica de una pandemia ¿impensada? Rev Argent Microbiol 2016;48:97–9.
2. Pan American Health Organization/World Health Organization (PAHO/WHO). Zika epidemiological update. Available at: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=36079&lang=en. Accessed September 8, 2016.
3. Ticconi C, Pietropolli A, Rezza G. Zika virus infection and pregnancy: what we do and do not know. Pathog Glob Health 2016;110:7–8.

4. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. N Engl J Med 2016;374:1981–7.
5. World Health Organization (WHO). WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations. Available at: http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-Zika/en/. Accessed February 1, 2016.
6. Ball DG, Bracero N, Bustillo M, Davis O, Hershlag A, Kawwass J, et al. Guidance for providers caring for women and men of reproductive age with possible Zika virus exposure ASRM. Available at: https://www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/News_and_Research/Press_Releases/2016-04/Zika%20Guidance%2004-06-16%20(2).pdf. Accessed October 1, 2016.
7. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis 2016;16:653–60.
8. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol 2016;47:6–7.
9. Suárez R, Martínez Urbina N, Torres J, Moros Z. Zika—Venezuela: infección temprana en embarazada, microcefalia, mortinato, confirmación. Technical report. Promed mail. Archive Number: 20160303.4067220. Available at: http://www.promedmail.org/post/4067220. Accessed March 24, 2017.
10. Suy A, Sulleiro E, Rodó C, Vásquez E, Bocanegra C, Molina I, Esperalba J, et al. Prolonged Zika virus viremia during pregnancy. N Engl J Med 2016;375:2611–3.
11. El Costa H, Gouilly J, Mansuy JM, Chen Q, Levy C, Cartron G, et al. ZIKA virus reveals broad tissue and cell tropism during the first trimester of pregnancy. Sci Rep 2016;6:35296.
12. Roa M. Zika virus outbreak: reproductive health and rights in Latin America. Lancet 2016;387:843.