Appendix 1. Characteristics of 17 Pregnant Women With Confirmed Prenatal Zika Virus Infection and Ultrasound Findings Consistent With Congenital Zika Syndrome in the Fetus, Barranquilla and Ibagué, Colombia, 2015–2016

Patient Number	Age category (years)	Obese, >30 kg/m^2	Gravida/ Para	Gestational age at time of Zika symptoms	Maternal Zika symptoms	First ultrasound with head circumference, weeks since Zika symptoms	Zika virus RNA positive samples (weeks of gestation)	Zika virus RNA negative samples
1	25-29	Yes	2/1	7 weeks	Rash, fever	9 weeks after Zika	Infant serum	N/A
2	<20	No	1/0	8 weeks	Rash, conjunctivitis, arthralgia	20 weeks after Zika	Maternal serum (12)	N/A
3	25-29	No	1/0	8 weeks	Rash	13 weeks after Zika	Maternal serum (10)	Amniotic fluid (26 weeks)
4	<20	No	1/0	9 weeks	Rash, fever	2 weeks after Zika	Amniotic fluid (37)	N/A
5	≥30	Yes	1/0	10 weeks	Rash, fever	1 week after Zika	Amniotic fluid (32)	Maternal serum
6	20-24	No	2/0	10 weeks	Rash, fever	4 days after Zika	Placenta, umbilical cord	N/A
7	25-29	No	1/0	10 weeks	Rash, pruritus	5 weeks after Zika	Maternal serum	N/A
8	≥30	No	4/3	11 weeks	Rash, conjunctivitis	5 weeks after Zika	Umbilical cord	Placenta
9	20-24	No	1/0	12 weeks	Rash, fever	1 week after Zika	Placenta, urine, cord blood	Umbilical cord
10	25-29	No	1/0	12 weeks*	Rash, pruritus	8 weeks after Zika	Umbilical cord, placenta	Urine
11	20-24	No	1/0	13 weeks*		3 weeks after Zika	Amniotic fluid (34)	N/A

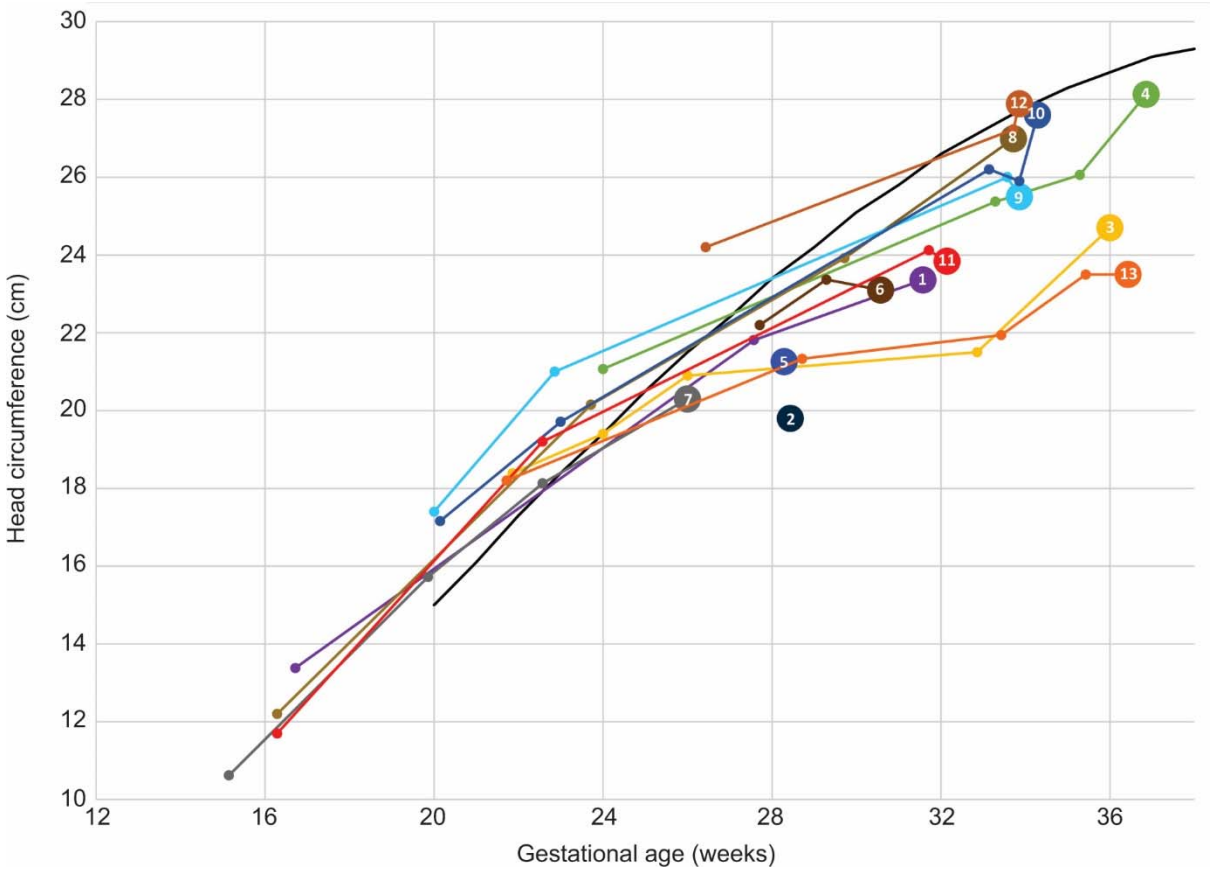
Parra-Saavedra M, Reefhuis J, Piraquive JP, Gilboa SM, Badell ML, Moore CA, et al. Serial head and brain imaging of 17 fetuses with confirmed zika virus infection in Colombia, South America. Obstet Gynecol 2017; 130.
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12	20-24	No	1/0	13 weeks	Rash, fever	6 weeks after Zika	Placenta	Maternal serum, umbilical cord
13	<20	No	1/0	13 weeks	Rash, fever, arthralgia, pruritus	8 weeks after Zika	Amniotic fluid (22)	Maternal serum
14	≥30	Yes	1/0	14 weeks	Rash, fever, conjunctivitis, arthralgia, pruritus, edema	5 weeks after Zika	Amniotic fluid (34)	N/A
15	<20	No	1/0	Asymptomatic		26 weeks gestation[†]	Amniotic fluid (28)	N/A
16	25-29	No	3/1	Asymptomatic[*]		14 weeks gestation[†],	Amniotic fluid (20)	N/A
17	20-24	No	1/0	Asymptomatic		29 weeks gestation[†],	Amniotic fluid (33)	N/A

[*] Partner had Zika virus symptoms during pregnancy, [†] asymptomatic patients, reasons for follow up: patient 15--threatened abortion at gestational week 7; patient 16--vanishing twin at week 8; and patient 17--small for gestational age at 29 weeks.

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Appendix 2. Sequential ultrasound head circumference measurement relative to the –3 standard deviations based on Intergrowth 21st (*black line*) for 13 pregnancies with first-trimester Zika virus symptoms and postnatal evidence of congenital Zika syndrome in the infant, Barranquilla and Ibagué, Colombia, 2015–2016. Head circumference measures below the black line indicate microcephaly; for that gestational age the measure is more than 3 standard deviations below the mean based on Intergrowth 21st fetal growth standards. The linear connectors between the ultrasound measurements are intended to guide the reader, and do not imply linear changes between the observed data points.



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Appendix 3. Summary of Published Case Series Reports on Timing of Prenatal Microcephaly Diagnosis in Pregnancies Affected by Zika Virus Infection

Author (reference) and case number of informative cases	Gestational age at Zika virus exposure	Zika virus diagnostics	Ultrasound without microcephaly (weeks)	Any abnormality detected (weeks)	Microcephaly diagnosed (weeks)
Mlakar (24), one case	13	Symptoms only	14, 20	29	32
Brasil (22)		82% of women were positive by polymerase chain reaction in blood, urine or both			
Fetus 19	8				35
Fetus 24	12				29
Calvet (3)					
Case 1	18	Amniotic fluid 28 wks	16		21
Case 2	10	Amniotic fluid 28 wks	22		25
Carvalho (9)					
Fetus 1	8	Symptoms			28
Fetus 2	8	Symptoms			31
Fetus 3	9	RT-PCR positive			31
Fetus 4	11	Symptoms			36
Fetus 5	8	Symptoms			37
Fetus 6	10	Symptoms			38
Fetus 7	16	Fever			38
Fetus 8	8	Fever			28
Fetus 13	11	Symptoms			27

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Fetus 14	16	Symptoms			29
Fetus 15	10	Symptoms			28
Fetus 18	9	Symptoms			22
Driggers (23), one case	12	IgM+/IgG+	13, 16, 17	19	*
Werner (25), one case	12		12, 21		32
Soares (11)					
Confirmed1	6	Amniotic fluid	17-20 (1)		25-30
Confirmed2	8	Amniotic fluid	14-30 (4)		31-35
Confirmed4	10	Amniotic fluid	21-35 (2)		≥36
Confirmed7	12	Amniotic fluid	17-20 (1)		25-30
Confirmed8	18	Amniotic fluid, cord blood, brain	17-30 (2)		31-35
Confirmed11	12	Brain	21-35 (3)		≥36
Confirmed12	10	Cord blood	14-24 (3)		≥36
Confirmed13	12	Cord blood	17-20 (1)		≥36
Presumed2	6	Symptoms (rash)	14-30 (3)		31-35
Presumed3	6	Symptoms (rash)			31-35
Presumed5	8	Symptoms (rash)	17-24 (2)		25-30
Presumed6	8	Symptoms (rash)			≥36
Presumed7	8	Symptoms (rash)	17-24 (2)		31-35
Presumed9	10	Symptoms (rash)	21-24 (1)		31-35
Presumed11	11	Symptoms (rash)			31-35

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Presumed14 Presumed15	12 12	Symptoms (rash) Symptoms (rash)			≥36 25-30
Vorona (26), one case	7	Serum, Amniotic fluid		16	21
TOTAL: 37 fetuses	Median: 10 Range 6-18				Median: 32 Range 21-38

* A reduction from 47th to 24th percentile head circumference was observed from 16 -20 weeks gestation, pregnancy was terminated at 21 weeks

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