Appendix 1. Mother-Fetus or Infant Dyads Included in this Systematic Review for Ultrasound and Congenital Zika Virus Exposure

Reference	Country of Zika exposure	USPSTF internal validity rating;* NOS†	Number of dyads included for ultrasound
Brasil, 2016 (1)	Brazil	II-2;	55
Hoen, 2018 (2)	France (Martinique and Guadeloupe)	II-2; 5	37
Pomar, 2017 (3)	France (French Guiana)	II-2; 6	5
Rodo, 2019 (4)	Central or South America, or Dominican Republic	II-2; 4	8
Sanz Cortes, 2018 (5)	Colombia	II-2; 4	12
Sarno, 2016 (6)	Brazil	II-2; 4	31
Walker, 2018 (7)	"countries with local transmission"	II-2; 7	42
Acosta-Reyes, 2017 (8)	Colombia	III	1
Aragao, 2016 (9)	Brazil	III	5
Arauz, 2016 (10)	Panama	III	1
Azevedo, 2018 (11)	Brazil	III	2
Beaufrère, 2019 (78)	Martinique, Guadaloupe, Venezuela	III	3
Benjamin, 2017 (13)	Venezuela	III	1
Besnard, 2016 (14)	France (French Polynesia)	III	8
Brito, 2018 (15)	Brazil	III	1
Calvet, 2016 (16)	Brazil	III	2
Melo, 2016 (17)	*Brazil*	*III*	*(2) ‡*
Carvalho, 2016 (18)	Brazil	III	19
Castro, 2017 (19)	Brazil	III	8
Chen, 2017 (20)	Unites States (Florida)	III	1
Contreras-Capetillo, 2017 (21)	Mexico	III	2
Culjat, 2016 (22)	Brazil	III	1
Davila-Castrodad 2018 (23)	US (Puerto Rico)	III	1
De Oliveira Dias, 2017 (24)	Brazil	III	1
De Oliveira Melo, 2016 (25)	Brazil	III	1
Diaz-Menendez, 2016 (26)	Colombia, Venezuela, or Honduras	III	1
Driggers, 2016 (27)	Mexico, Guatemala, and Belize	III	1

Giovanetti 2018 (28)	Brazil	III	1
Ho 2018 (29)	Colombia	III	1
James-Powell, 2017 (30)	Jamaica	III	1
Juca, 2016 (31)	Brazil	III	1
Laavanya, 2017 (32)	Brazil	III	1
Lemos de Carvalho 2018 (33)	Brazil	III	1
Lovagnini-Frutos, 2017 (34)	Bolivia	III	1
Martines, 2016 (35)	Brazil	III	3
Linden, 2017 (36)	Brazil	III	2
Linden, 2016 (37)	Brazil	III	3
Martines, 2016 (38)	*Brazil*	*III*	*(3) ‡*
Schwartz, 2017 (39)	*Brazil*	*III*	*(3) ‡*
Lockrow 2019 (40)	Mexico	III	1
Mattar, 2017 (41)	Colombia	III	1
Meaney-Delman, 2016 (42)	an area with risk of Zika	III	3
Mendonca, 2018 (43)	Brazil	III	2
Mlakar, 2016 (44)	Brazil	III	1
Strafela, 2017 (45)	*Brazil*	*III*	*(1) ‡*
Moron, 2016 (46)	Brazil	III	1
Mulkey, 2018 (47)	Dominican Republic	III	1
Nogueira, 2017 (48)	Brazil	III	50
Oliveira, 2016 (49)	Brazil	III	1
Parra-Saavedra, 2017 (50)	Colombia	III	12
Perez, 2016 (51)	Venezuela	III	1
Pereira, 2019 (52)	Brazil	III	8
Rabelo, 2017(53)	Brazil	III	1
Rajapakse, 2018 (54)	Guatemala, Brazil	III	4
Rodo, 2017 (55)	Colombia	III	1
Santos, 2017 (56)	Brazil	III	2
Siqueira Mello, 2019 (57)	Brazil	III	1
Sarno, 2016 (58)	Brazil	III	1
Saulino, 2017 (59)	El Salvador	III	1
Zacharias, 2017 (60)	*El Salvador*	*III*	*(1) ‡*
Schaub, 2017A (61)	France (Martinique)	III	8
Schaub, 2017B (62)	*France (Martinique)*	*III*	*(8) ‡*
Soares de Souza, 2016 (63)	Brazil	III	2
Sotelo, 2017 (64)	Brazil	III	1
Souza, 2016 (65)	Brazil	III	1
Spiliopoulos, 2019 (66)	Jamaica	III	1
Sulleiro, 2019 (67)	Colombia	III	1

Suy, 2016 (68)	Colombia	III	1
Turley, 2018 (69)	"endemic area"	III	4
Valdespino-Vazquez, 2019 (70)	Mexico	III	1
Ventura, 2016 (71)	Venezuela	III	1
Vesnaver, 2017 (72)	Brazil	III	1
Villamil-Gomez, 2019 (73)	Colombia	III	1
Werner, 2016 (74)	Brazil	III	1
Werner, 2016 (75)	Brazil	III	1
Yarrington, 2019 (76)	Haiti	III	1
76 total papers	15 unique countries	X	385 total cases

*United States Preventive Services Task Force (USPSTF) Assessment of Internal Validity: I= Randomized controlled trial (RCT); systematic review or meta-analysis of homogeneous RCTs; II-1=Well-designed controlled trial without randomization; II-2= Well-designed cohort or case-control study; II-3= Multiple time-series, with or without the intervention; III= Opinions of respected authorities, based on clinical experience; descriptive studies or case reports; reports of expert committees.(91)

†Newcastle-Ottawa Quality Assessment Scale: A maximum score of 8 points awarded in three categories, selection (3), comparability (2), and outcomes (3) (92)

‡Italics denote cases that were reported in more than one paper, where both papers provide unique information. Each case appears once in total case count; all papers that provide unique information are included in total reference count.

Appendix 2. Mother–Fetus or Infant Dyads Included in this Systematic Review for Amniocentesis and Congenital Zika Virus Exposure

Reference	Country of Zika exposure	USPSTF internal validity rating;* NOS rating for cohort studies†	Number of dyads included for amniocentesis
Pomar, 2017 (3)	France (French Guiana)	II-2; 6	2
Rodo, 2019 (4)	Central or South America or Dominican Republic	II-2; 4	2
Acosta-Reyes, 2017 (8)	Colombia	III	1
Adhikari, 2017 (77)	Honduras	III	1
Barzon, 2018 (78)	El Salvador	III	2
Beaufrère, 2019 (12)	Martinique, Guadaloupe, Venezuela	III	3
Chimelli, 2017 (79)	Brazil	III	3
De Oliveira Melo, 2016 (25)	Brazil	III	4
Driggers, 2016 (27)	Mexico, Guatemala, and Belize	III	1
Eijk, 2016 (80)	Suriname	III	1
Herrera, 2016 (81)	Honduras	III	1
Ho, 2018 (29)	Colombia	III	1
Lovagnini-Frutos, 2017 (34)	Bolivia	III	1
Meaney-Delman, 2016 (42)	an area with risk of Zika	III	1
Pereira, J. P., Jr. 2019 (52)	Brazil	III	8
Perez, 2016 (51)	Venezuela	III	1
Rajapakse, 2018 (54)	Guatemala, Brazil	III	1
Rodo, 2017 (55)	Colombia	III	1
Sarno, 2016 (58)	Brazil	III	1
Schaub, 2017A (61)	France (Martinique)	III	10
Schaub, 2017B (62)	*France (Martinique)*	*III*	*(8)* ‡
Sotelo, 2017 (64)	Brazil	III	1
Souza, 2016 (65)	Brazil	III	1
Sulleiro, 2019 (67)	Colombia	III	1
Suy, 2016 (68)	Colombia	III	1
Turley, 2018 (69)	"endemic area"	III	3
Valdespino-Vazquez, 2019 (70)	Mexico	III	1
Vorona, 2016 (82)	Barbados	III	1
Wongsurawat, 2018 (83)	Thailand	III	1
27 total papers	14 unique countries	x	56 total cases

*United States Preventive Services Task Force (USPSTF) Assessment of Internal Validity: I= Randomized controlled trial (RCT); systematic review or meta-analysis of homogeneous RCTs; II-1=Well-designed controlled trial without randomization; II-2= Well-designed cohort or case-control study; II-3= Multiple time-series, with or without the intervention; III= Opinions of respected authorities, based on clinical experience; descriptive studies or case reports; reports of expert committees.(91)
†Newcastle-Ottawa Quality Assessment Scale: A maximum score of 8 points awarded in three categories, selection (3), comparability (2), and outcomes (3)(9, 92)

‡Italics denote cases that were reported in more than one paper, where both papers provide unique information. Each case appears once in total case count; all papers that provide unique information are included in total reference count.

Appendix 3. Fetuses Without Prenatal Congenital Zika Syndrome Findings on Prenatal Ultrasound but With Congenital Zika Syndrome Abnormalities After Pregnancy Completion

SR_ID (reference)	Latest prenatal ultrasound, weeks' gestation (weeks elapsed from maternal infection or symptoms)	Abnormalities described after pregnancy completion
19 Benjamin, 2017 (13)	32w (11)	abnormal neurologic exam with clonus, hyperreflexia, posturing; abnormal electroencephalogram; abnormal funduscopic exam with pale optic nerve
25 Brasil, 2016 (1)	36w (14)	abnormal neurologic exam with hyperreflexia, clonus of upper and lower extremities, posturing, head lag; and abnormal funduscopic exam
32 Brasil, 2016 (1)	33w (19)	hypoplasia of the inferior portion of the vermis, large for gestational age, congenital heart disease, hypoactive
110 James-Powell, 2017 (30)	16w (UNK)	overlapping sutures limiting visualization of brain parenchyma, dilated lateral ventricles but normal third and fourth ventricles, and calcifications; abnormal clinical exam with severe microcephaly, prominent occiput, bitemporal narrowing, collapsed skull bones, small anterior fontanelle, low posteriorly-rotated ears, horizontal nystagmus, redundant skin folds at the nape, wide spaced nipples and large hands with clinodactyly, respiratory distress, generalized cracking and peeling of the skin, hypertonia in the upper limbs with brisk reflexes, severe arthrogryposis with hand and feet contractures, flexion deformities of both hips, hyperextension of the knees with inability to dorsiflex, severe restriction in abduction, hypertonia and hypereflexia of the upper limbs, "rocker-bottom" feet with prominent calcaneii, sandals gap and abnormal creases on the soles, displaced anus with lax anal tone, neonatal seizures; abnormal neurologic exam at six weeks with confirmation of these dysmorphisms, and irritability, bi-frontal facial narrowing, wide nasal bridge, mild retrognathia, high arched palate, diastasis of recti abdominis, small umbilical hernia, external rotation of the hips, hyper-extension at the knees, extra crease beneath patella of both knees, wasting of left lower limbs distally more than proximally, shortening of the left lower limbs, fixed contractures to knees, distal tapering of fingers; abnormal eye exam with bilateral optic atrophy

119 Martines, 2016 (35)	20w (16)	neonatal demise; pathology exam with normal division of hemispheres of the brain, lissencephaly, ventriculomegaly with lateral ventricle hydrocephalus, cerebellar hypoplasia, contractures
176 Nogueira, 2017 (48)	First trimester (maternal symptoms at 28 weeks)	chorioretinitis, right eye
178 Oliveira, 2016 (49)	39w (13)	intracranial calcifications, reduced brain parenchyma, notably in the frontal and parietal lobes, ventriculomegaly; microcephaly; abnormal neurologic exam at six months with neuropsychomotor developmental delay, global hypertonia and spastic hemiplegia, with the right dominant side more severely affected
200 Santos, 2017 (56)	33w (UNK)	subependymal hemorrhage, multiple subcortical punctiform calcifications, reduced corpus callosum area and cerebral parenchyma, reduced cortical thickness, ventriculomegaly; microcephaly; exotropia
249 Ventura, 2016 (71)	UNK (UNK)	intracranial calcifications and mild neuronal migration anomaly; circular hypopigmented lesion superior to the optic nerve left eye
271 Hoen, 2018 (2)	18w (13)	moderate microcephaly, disproportionate
272 Hoen, 2018 (2)	32w (26)	bilateral club feet
273 Hoen, 2018 (2)	36w (29)	moderate microcephaly, small for gestational age
274 Hoen, 2018 (2)	30w (22)	moderate microcephaly, small for gestational age
275 Hoen, 2018 (2)	35w (26)	moderate microcephaly, small for gestational age
286 Hoen, 2018 (2)	36w (23)	moderate microcephaly, small for gestational age
289 Hoen, 2018 (2)	36w (22)	moderate microcephaly, small for gestational age
290 Hoen, 2018 (2)	35w (21)	moderate microcephaly, small for gestational age
291 Hoen, 2018 (2)	36w (21)	moderate microcephaly, small for gestational age
292 Hoen, 2018 (2)	37w (18)	moderate microcephaly, small for gestational age
294 Hoen, 2018 (2)	35w (16)	moderate microcephaly, small for gestational age
296 Hoen, 2018 (2)	18w (-4)	moderate microcephaly
298 Hoen, 2018 (2)	35w (13)	moderate microcephaly, small for gestational age
301 Hoen, 2018 (2)	36w (11)	moderate microcephaly, small for gestational age
302 Hoen, 2018 (2)	32w (5)	moderate microcephaly, small for gestational age
303 Hoen, 2018 (2)	36w (7)	moderate microcephaly
304 Hoen, 2018 (2)	35w (5)	moderate microcephaly, small for gestational age

305 Hoen, 2018 (2)	37w (5)	moderate microcephaly, small for gestational age
306 Hoen, 2018 (2)	34w (0)	moderate microcephaly
319 Mendonca, 2018 (43)	UNK (UNK)	microcephaly, ventriculomegaly, calcifications, cerebral atrophy, hydrocephaly; seizures at 5 months
320 Mendonca, 2018 (43)	UNK (UNK)	microcephaly, ventriculomegaly, calcifications, corpus callosum abnormalities, cerebral atrophy; neuropsychomotor development delay at 7 months
354 Siquieira Mello, 2019 (57)	UNK (UNK)	Microcephaly
376 Walker, 2018 (7)	UNK (UNK)	Microcephaly
419 Yarrington, 2019 (76)	33 (UNK)	Microcephaly

SR_ID Systematic review identification number, UNK unknown, w weeks

Appendix 4. Fetuses With Negative Zika Nucleic Acid Test (NAT) Results on Amniotic Fluid Specimens, but With Congenital Zika Syndrome Abnormalities After Pregnancy Completion

SR_ID	Gestational age at amniocentesis (time elapsed since maternal infection or symptoms)	Abnormalities described after pregnancy completion
2 Adhikari, 2017 (77)	UNK (UNK)	Hydranencephaly
103 De Oliveira Melo, 2016 (25)	17w (10)	cerebral volume reduction, lissencephaly, ventriculomegaly, callosal hypoplasia, abnormalities of the basal ganglia and or thalami, cerebellar hypoplasia, brainstem hypoplasia, subcortical calcifications, basal ganglia, brainstem or thalamic calcifications, neonatal death
104 De Oliveira Melo, 2016 (25)	35w (25)	Microcephaly
105 De Oliveira Melo, 2016 (25)	25w (17)	microcephaly, neonatal death
255 Turley, 2018 (69)	30w (10)	severe bilateral ventriculomegaly, absent cavum septum pellucidum, white matter and corpus callosal volume loss, multiple subependymal nodules; seizures, developmental delay
256 Turley, 2018 (69)	23w (UNK)	colpocephalic configuration of the lateral ventricles and callosal dysgenesis; developmental delay
258 Turley, 2018 (69)	23w (UNK)	severe supratentorial volume loss; severe microcephaly; neonatal death
321 Pereira, 2019 (52)	34w (27)	microcephaly, SGA, cortical thinning, hydrocephalus, calcifications, cerebellar hypoplasia
326 Pereira, 2019 (52)	30w (18)	microcephaly, calcifications, ventriculomegaly, pachygyria, arthrogryposis, optic nerve hypoplasia, macular atrophy, SGA
357 Valdespino-Vasquez, 2019 (70)	28w (14)	microcephaly, micrognathia and retrognathia, low-set ears, and a depressed nasal bridge, as well as arthrogryposis; hypoplastic cerebral lobes and brain stem; lissencephaly, ventriculomegaly, calcifications.

NAT nucleic acid test, SR_ID Systematic review identification number, UNK unknown, w weeks

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