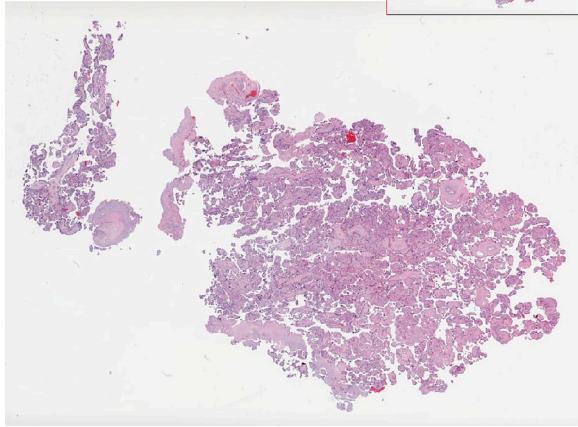
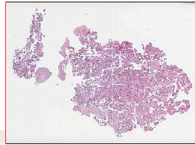


CASE 2



Richard Lieberman, MD

Description

31 year old G1 with an intrauterine fetal demise at 20 weeks estimated gestational age. Induction and delivery of a stillborn followed. IUGR (intrauterine growth retardation) and oligohydramnios (decreased amniotic fluid) were noted previously at ultrasonography at 15 weeks gestation for evaluation of elevated serum γ -fetoprotein. The patient is a health-care provider who noted that, while not getting sick herself, she was in direct contact with many patients suffering with “the flu” (upper respiratory, not gastrointestinal) during her first trimester. She asks her clinician, who in turn asks us, if there is anything in the placental pathology to explain her loss.

CASE 2: Fetal Demise Associated With Influenza A Infection

DIAGNOSES: Intrauterine fetal demise (second trimester). Diffuse villous fibrosis, perivillous and intravillous fibrin deposition associated with chronic villitis and intervillitis (placentitis), histiocytic type. Influenza A virus infection (type not specified) with ultrastructural, immunohistochemical, and PCR confirmation.

CLINICAL HISTORY: 30 year old G1 with an intrauterine fetal demise (IUFD) followed by induction and delivery at approximately 20 weeks EGA. The patient was Rubella immune, O positive. Antepartum course complicated by an abnormal Quad Screen; elevated maternal serum alpha-fetoprotein MSAFP at 288, 7 multiples of the median (MOM); and inhibin A at 590, 3.4 MOM; with estradiol and beta-hCG around 1 MOM. Ultrasound at 15 weeks demonstrated symmetric intrauterine growth retardation (IUGR) and oligohydramnios.

The patient is a healthcare worker who noted that, while not remembering getting sick herself (other than the “usual” symptoms of early pregnancy), she spent a month during her first trimester in direct contact with many patients suffering with upper respiratory, “flu” symptoms. The institutional influenza vaccination program was not available until the last week of her assignment.

LABORATORY EVALUATION: TORCH screen negative for antibodies to CMV and Toxoplasmosis. Factor V Leiden negative, Protein S & Protein C normal. PT/PTT normal. Chromosome analysis, normal, 46XY.

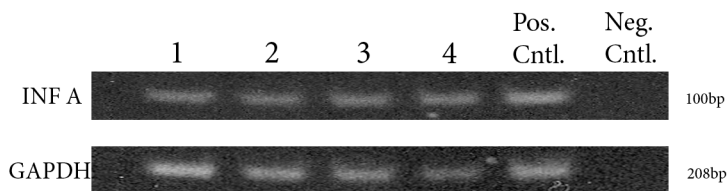
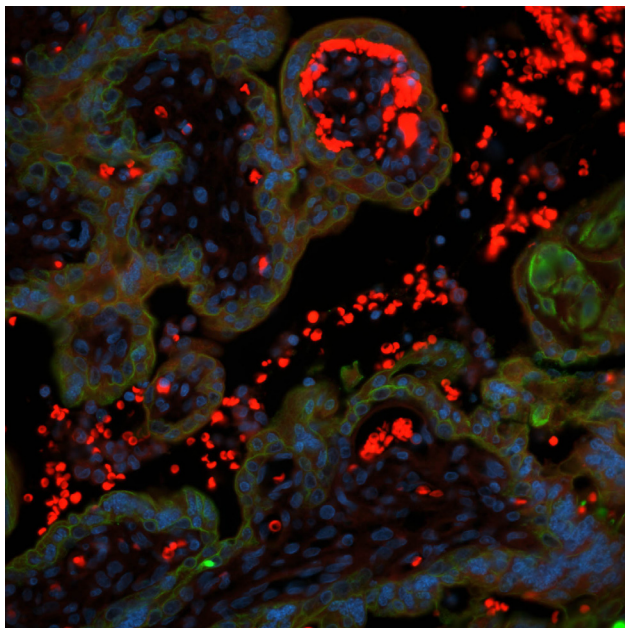
MICROSCOPIC DESCRIPTION:

The placenta demonstrated non-specific changes often associated with those seen following fetal demise. They included diffuse villous fibrosis, vascular occlusion and organized thrombosis. In addition, this case demonstrated an abundance of histiocytes, both in the maternal (intervillous) space,

and within the chorionic villi. Of note, the histiocytes had a prominent foamy appearance, some of which also demonstrated brown-pigment changes. Fetopsy revealed non-specific findings also. Normal organogenesis was observed in the heart, lungs, liver and other sampled organs. Subtle infiltration of foamy histiocytes was also observed. No evidence of viral inclusions were noted in the H&E samples of placenta or fetal organs.

ELECTRON MICROSCOPY DESCRIPTION: Formalin fixed placental tissue was processed and scanned with transmission electron microscopy (EM). Histiocytes from the intervillous and intravillous spaces were identified and noted to have several characteristics of viral production. Along the nuclear envelope were numerous electron hypodensities. The cytoplasm demonstrated organelles which appear at first, ballooned or degenerating, but also are associated with numerous electron densities of relatively uniform size (100-150 nm). A few larger, well-formed viral capsid-like structures were also discovered in the cytoplasm, suspicious for influenza virus.

IMMUNOFLUORESCENCE AND PCR: Dual staining of cytotrophoblast (cytokeratin) and Influenza A M1 Capsid protein demonstrated intracellular viral antigen both within the maternal histiocytes (intervillous space) and the fetal Hofbauer cells (intravillous histiocytes). Polymerase chain reaction (PCR) with positive controls for Influenza A confirm the presence of Influenza A virus in the maternal and fetal spaces.



DISCUSSION:

Influenza virus, an airborne infection, has demonstrated a significant risk of morbidity and mortality to pregnant women during both seasonal and epidemic outbreaks. As a result, the American College of Obstetrics and Gynecology has long recommended influenza virus vaccination to all pregnant women. Seasonally, influenza virus infection may be noted to occur an nearly ¼ of all pregnant woman. While

infections in the second and third trimester have not been shown to significantly impact pregnancy outcome or demonstrate evidence of transplacental transmission of influenza virus, the impact on first trimester exposure is less well studied. In fact, during recorded influenza outbreaks in the last century, there have been notable *associations* of maternal infection with higher rates of pregnancy loss in the first trimester, as well as congenital malformation. However, confirmation of diagnosis of a viral infection during the first trimester of pregnancy, much less causation of adverse outcome, is not straightforward.

While accurate numbers are elusive due to incomplete work-up of most IUFD cases, stillbirths and fetal deaths have been associated with an infectious agent in approximately 20% or more of cases. The earlier the stillbirth occurs in gestation, the more likely the association with an infectious etiology. Placental histopathological findings that have been reported include acute chorioamnionitis and funisitis, chronic villitis, massive chronic intervillitis, and massive perivillous fibrin deposition (aka maternal floor infarction). The entity of “chronic villitis” is considered the classic lesion in TORCH (toxoplasmosis, other [usually parvovirus], rubella, cytomegalovirus, and histoplasmosis) infections occurring during pregnancy. In actual practice, a causative infectious agent is only identified in 1-10% of all cases of chronic villitis, with most attributed to maternal immune-regulated rejection of the fetal allograft.

In our case, the patient’s history of early exposure to potential infectious agents and the unusually prominent histiocytic infiltration of the maternal and fetal spaces compelled us to look further for etiologies of this mid-trimester demise. Very little exists in the literature regarding ultrastructural analysis of placental parenchyma in infectious (or any other pathological) condition. Our EM of this placenta was remarkable in the findings of intranuclear and intracytoplasmic evidence of viral production. Visual cues of the viral particles (resembling influenza morphology) in conjunction with the patient’s history pointed us in the direction of the specific pathogen. Confirmation of the presence of Influenza A Capsid M Protein (sensitive across all HxNx serotypes), we believe represents the first documentation of first trimester viral infection associated with subsequent intrauterine demise.

Several case reports and studies document transplacental passage of Influenza A using maternal and fetal serologic testing, with one case documenting viral antigen via amniocentesis. Ours documents the histopathological location of the virus in both the maternal and fetal compartments. Does it document a cause-effect relationship of the patient exposure to the adverse outcome? While a compelling association, additional information, such as acute and convalescent antibody titers in the mother along with specifying the Influenza A subtype would also be beneficial.

SUMMARY: This case illustrates a novel use of EM in directing our efforts at identifying the etiology of a mid-trimester pregnancy loss. Confirmatory immunofluorescence and PCR was made possible by knowing that virus was present. New molecular diagnostic techniques have real potential to make more rapid assessment of infectious antigens possible.

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