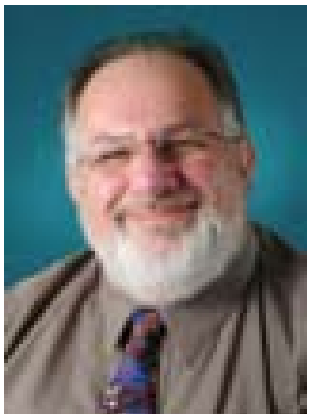
What happens when a baby dies: stillbirth investigations for infection and other aetiologies



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Infections in stillbirths are common, often clinically silent and need to be screened. The microbiology laboratory needs to have the appropriate culture techniques and expertise. The results of the clinical features, the pathology findings of the fetus and the placenta and the microbiological and serological features need to be interpreted together; individual results should not be considered in isolation.

Stillbirth is defined in Australia as the loss of a fetus who shows no signs of life at birth and is at least 400 grams in birth weight or at least 20 weeks' gestation. In Australia in 2005, there were 1,979 reported fetal deaths, at a rate of 7.3 per 1,000 births, about 19% of which are at term, making stillbirth a far more common condition than SIDS [1].

Stillbirth has been a hidden medical issue but has an immense effect on the woman as well as family members, physicians and nurses. It may be a potential marker of maternal or inherited disease but, despite this, it is an area that is under investigated. This paper sets out the investigations that may explain the cause of death and which may be valuable in counselling parents about recurrence risk in subsequent pregnancies, with an emphasis on information regarding the infectious aetiology of stillbirth.

Currently, the principal risk factors and causes of stillbirth in developed countries are congenital anomalies, preeclampsia-related complications, intrauterine growth restriction and intrauterine infections. There is an association with older women, obesity and infertility treatment that are factors likely to be increasing the rate of stillbirths. Other common factors associated with stillbirth include placental disorders (e.g. placental abruption and vascular under-perfusion), complications of multiple gestation, and umbilical cord abnormalities or accidents. Several factors may coexist in individual cases. In approximately 15-25% of stillbirths no cause is identified.

Infection, which may involve mother, fetus or placenta, is associated with 10-25% of stillbirths in developed countries; it is a more frequent cause of stillbirth in developing countries [2]. Infection may cause stillbirth by a number of mechanisms including direct infection, placental damage, and indirect mechanisms without identifying infection of the fetus or placenta or severe maternal illness. Assigning a specific infection as a cause of death may not be straightforward. Firstly, stillbirths may have multiple causes and infection may be one of them. Secondly, in an already compromised fetus due to maternal or fetal cause, infection may accentuate or precipitate the demise. Thirdly, despite finding a specific organism on culture or serological evidence of recent infection, these agents may not be the actual cause of death. The earlier (in gestation) the stillbirth, the more likely it will be associated with infection. It is not clear why ascending infection is so common in midtrimester.

The important clinical point is that fetal and placental infection is often clinically silent, and therefore each stillbirth needs to be

investigated with infection in mind, especially the non-macerated cases. Around 70% of all acute chorioamnionitis is clinically silent. Chorioamnionitis can also present with bleeding and abdominal pain like abruption or be associated with premature prelabour rupture of membranes and so, even if symptomatic, may not be recognised. Most of the patients with histological chorioamnionitis have no maternal symptoms such as fever, uterine tenderness or maternal leukocytic response.

There are two major microorganism-related mechanisms associated with significant perinatal mortality and morbidity. First, ascending genital tract infection, almost always bacterial, which ranges from localised choriodecidual inflammation to frank chorioamnionitis with fetal sepsis; this is a major cause of mid trimester miscarriage and severe preterm delivery (Figure 1). Second, haematogenous spread of maternal systemic infection, be it bacterial, viral or parasitic [3].

Organisms mostly associated with ascending infection are the genital mycoplasma species *Ureaplasma urealyticum* and *Mycoplasma hominis*, but a large variety of bacteria can colonise the female genital tract without causing any symptoms in pregnant woman. Listeria infects by the haematogenous route and is readily identified culturally and by special stains; it leads to abscess formation in the placenta and is one of the definitive causes of stillbirth [4]. In case of parasitic infection, as in malaria, it is not uncommon to find parasitaemia in intervillous spaces of the placenta of infected mothers with uninfected fetal blood. Toxoplasmosis is a worldwide zoonosis. Fetal infections result from parasitic disease of placenta that can destroy the fetus or lead to varying degrees of fetal/congenital infection. The organism can be detected by placental tissue or body fluid culture and specific polymerase chain reaction (PCR) testing of these samples. However, the overall contribution of toxoplasma to fetal death is relatively small. Stillbirths or neonatal deaths occur in 5% of pregnancies with first trimester toxoplasmosis, in 2% with second trimester infection and in 0% in third trimester infection [5].

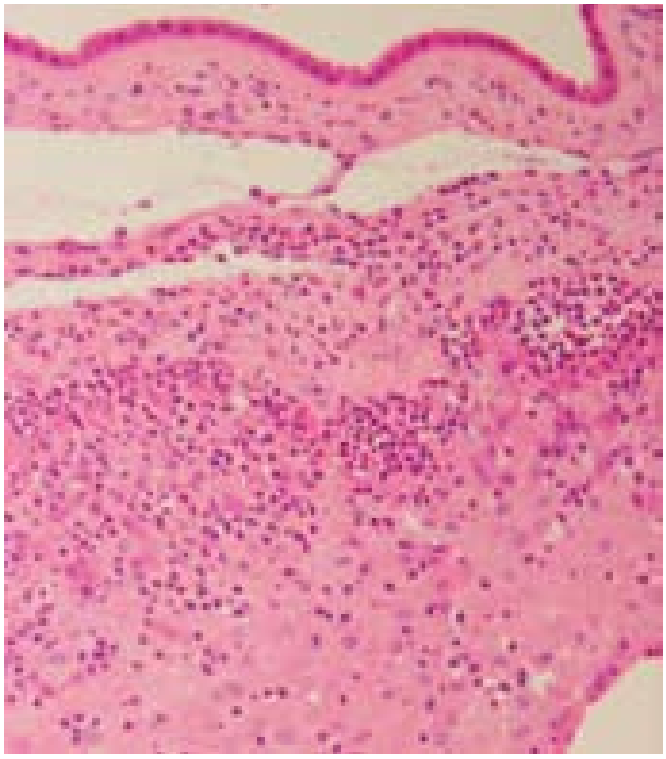

Figure 1a. Acute chorioamnionitis.

Although it is clear that viruses can cause stillbirth, the overall nature of this relationship is unclear. Serological or PCR evidence of an infection does not prove causation. Parvovirus B19 (Figure 2) appears to have strongest association with stillbirth as it can either infect fetal erythropoietic tissue – leading to severe fetal anaemia and/or hydrops, both potential mechanisms for fetal death – or infect myocytes – leading to myocarditis and *in utero* heart failure. The risk of stillbirth is greater for parvovirus infection occurring prior to 20 weeks' gestation [6].

Enteroviruses, including Coxsackie A and B, echoviruses and other enteroviruses are also associated with stillbirth. Coxsackie viruses can cross the placenta and cause villous necrosis, inflammatory cell infiltration, calcific pancarditis and hydrops. Cytomegalovirus (CMV) is one of the common congenital infections and placental involvement is well documented. The virus can be detected through serology and culture/PCR but the exact mechanism of how CMV causes stillbirth is not clear. Rubella virus can cause endothelial damage and thrombosis in placental and fetal vessels, leading to stillbirth. The risk to the fetus is greater at early gestation and decreases with increased gestational age. However, with the development of rubella vaccine and its widespread adoption, this virus has little contribution in stillbirths in developed countries.

Syphilis is one of the major causes of adverse pregnancy outcomes in developing countries and high rates are reported in parts of rural Australia. With primary and secondary syphilis, stillbirth, neonatal death or prematurity occurred in 50% of cases, whereas with early latent or late syphilis, stillbirths occurred in only 10% [5].

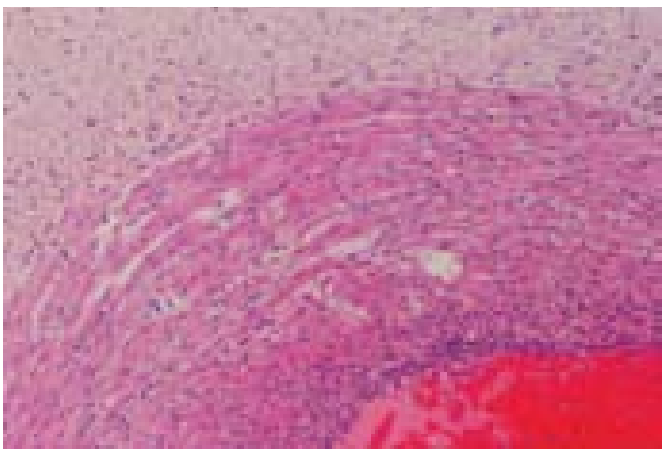

Figure 1b. Funisitis, with margination and infiltration of neutrophils through the venous wall of the umbilical cord into the stroma.

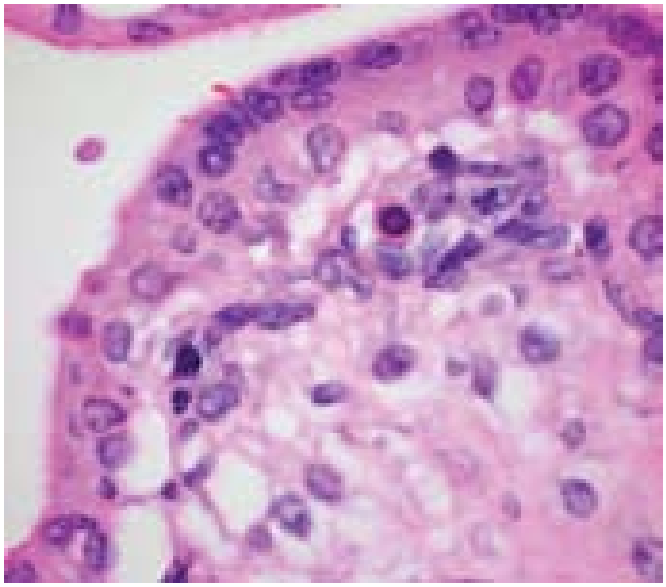

Figure 2. Parvovirus inclusion in an early pregnancy placental villus.

Protocols for investigation of stillbirth

It is important to have a set of rules or protocols to investigate stillbirths. Almost all protocols follow the same set of investigations. It is important that the microbiology laboratory is set up for the investigation of stillbirths and is able to culture both for the unusual and more fastidious organisms such as genital mycoplasma and Haemophilus species. The fetus is often delivered through the vagina and exposed to the vaginal flora, and it is easy for fluid to be moved as the fetal chest and abdomen are compressed, and for bacterial contamination to occur. Bacterial overgrowth is particularly common after a prolonged delay from delivery until post mortem. The interpretation of the microbiology results is ideally a communication between the clinician, the perinatal pathologist and the clinical microbiologist.

Full autopsy

The most important part of the workup of a fetal demise is the autopsy of the fetus. The decision to proceed with an autopsy must be made by the parents and informed consent is necessary. The healthcare providers should strongly emphasise that the result of the autopsy may be useful to the patient and her family in planning future pregnancies. However, despite the autopsy, cause of death may remain undetermined in 12-50% of cases [7].

If consent given

The standard fetal autopsy includes a comprehensive external examination, photography and radiography of the fetus and gross and histological and supplementary laboratory investigations such as microbiological, cytogenetic and metabolic studies [8]. Examination of the internal organs (weights, detailed macroscopic and histological examination) should also be carried out. There should also be examination of the placenta and umbilical cord (Figure 3) with cultures if unable to be taken from the fetus.

Outside the context of infectious aetiologies of stillbirth, the autopsy could reveal other causes of death. Careful external examination and measurements could reveal growth restriction as an associated finding of stillbirth. Other causes obvious on external examination are hydrops fetalis (which may have an infectious aetiology), congenital malformations, such as skeletal dysplasias, or karyotypic anomalies, such as trisomy 18 or triploidy. Internal examination could reveal numerous congenital malformations or corroborative evidence of growth restriction. Uteroplacental vascular insufficiency, often associated with maternal hypertensive disorders of pregnancy, and placental abruption could be confirmed by placental examination.

A microbiological examination must be taken – swabs from lung, stomach and liver for microbiological cultures. Group B Streptococcus (GBS), *Escherichia coli* and *Ureaplasma urealyticum* are the organisms commonly associated with chorioamnionitis and fetal infection. Stillborn infant blood (cord blood or, if this is not possible, infant cardiac blood) is collected for microbial cultures of GBS, Haemophilus species, Listeria and/or coliforms. Maternal serology testing of syphilis, parvovirus, toxoplasmosis, rubella, herpes simplex virus (HSV) and CMV, as well as specific PCR testing of fetal tissues can be done. Mycobacterium testing is of interest if TB is suspected or the cause of death is not obvious. In ambiguous situations, elevated fetal serum β2 microglobulins can be used as a reliable marker for intrauterine infection due to CMV or toxoplasmosis [9].

If consent not given

If consent is not given, a limited fetal evaluation should be discussed with parents who are resistant to a complete autopsy. However, there is no adequate substitute for a full fetal autopsy. Some less invasive alternatives that are acceptable by parents are MRI, needle biopsy of tissue and the non-invasive component of the standard autopsy such as external fetal examination and placental examination (see below).

Placental examination

Culture

Subamniotic swabs for at least aerobic and, ideally, anaerobic cultures including for bacterial vaginosis-associated organisms

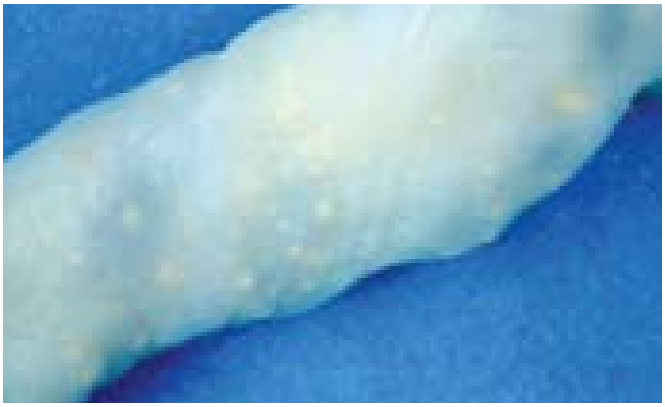

Figure 3. Candida colonies on surface of umbilical cord on careful inspection.

if resources are available, are recommended for all cases. Fresh placental tissue is collected for tissue culture if infection is suspected (GBS, Listeria, Haemophilus, coliforms and viruses). If TB is suspected, a separate sample is collected for mycobacteria.

Atay *et al.* showed that histopathological chorioamnionitis and placental culture positivity rates in control and study group were 64.7% vs 0%. Bacteria were recovered from 90.9% of placentas and 36.4% of fetal lungs of the cases with histopathological chorioamnionitis [10]. Pankuch *et al.* showed that bacteria were recovered from 72% of placentas with histological chorioamnionitis and from 82% of clinical chorioamnionitis, all of which had histological chorioamnionitis. Nearly 50% bacteria recovered from placentas were anaerobes [11]. Viral cultures and serology are often not available or are of relatively low sensitivity. The microorganisms most commonly recovered from the chorioamnion include *Ureaplasma urealyticum*, facultative and anaerobic Gram-positive cocci, *Gardnerella vaginalis* and Bacteroides species. *Haemophilus influenzae* (usually non-typable), *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are rarely recovered from placentas [12].

Histopathological examination

Histological acute chorioamnionitis (Figure 1) is defined as a maternal neutrophilic response to bacterial infection with or without an accompanying fetal neutrophilic response. Acute chorioamnionitis is usually the result of infection in the female genital tract. Other less common routes are haematogenous seeding of placenta and contiguous spread of organisms from adjacent pelvic viscera. Fetal inflammatory response does not necessarily mean fetal infection; it is an indication of activation of fetal immune system. Immune activation in turn leads to increased levels of cytokines in fetal blood that are associated with increased risk of brain injury and chronic lung disease.

Histopathological examination of placenta contributes to a better understanding of the cause of intrauterine fetal death. Rayburn *et al.* showed that significant histological aberrations were found in placentas of 98% cases. The most frequent abnormalities were those of vascular insufficiency, haemorrhagic endovasculitis, retroplacental haematoma, acute chorioamnionitis with fetal involvement, and erythroblastosis/hydrops [13]. Mayo *et al.* showed that histological chorioamnionitis occurred in 2.6 times more often in women with stillbirths than in women with live births [14].

Chorioamnionitis with acute villitis is seen with fetal bacterial sepsis especially by streptococci and Gram-negative bacilli. The presence of mixed lympho-histiocytic infiltrate in the terminal villous stroma is the feature of chronic villitis (Figure 4). Chronic villitis is usually associated with placental infection with CMV, syphilis or toxoplasma. Less common agents like HSV and coxsackie viruses are occasionally associated with chronic villitis. Less virulent organisms such as the genital mycoplasma species cause asymptomatic maternal infection; however, they are associated with histological chorioamnionitis and adverse pregnancy outcome.

Cytogenetics

Where relevant, if karyotyping has not been done, then placental tissue can be used for cytogenetics studies.

Maternal investigations

Clinical history

When stillbirth and neonatal death occurs, the obstetric history, including exposure (e.g. medications and viral infections), history of amniocentesis, intrauterine contraceptive device and family history with three generation pedigree, if possible, should be reviewed. Maternal history of fever, abdominal pain or other evidence of lower genital tract infection such as offensive vaginal discharge should also be recorded. Consideration of performing an antenatal ultrasound scan prior to stillborn delivery is recommended by most protocols (whenever possible, following confirmation of stillbirth) for the identification of unknown abnormalities. The ultrasound findings may be helpful when the family does not consent to a full autopsy [15].

Gram-staining and fibronectin test should be carried out on vaginal or cervical secretions. A positive test is strongly associated with chorioamnionitis and neonatal sepsis [16]. Also, at the time of stillbirth confirmation and prior to delivery, collection of amniotic fluid by amniocentesis is recommended. This results in good microbiological specimens and material for cytogenetic analysis. It may be screened for leukocyte count, Gram-stain, pH, glucose concentration, endotoxin, lactoferrin, cytokine levels (e.g. interleukin-6). The cytokines commonly quantified in either the amniotic fluid or the blood include interleukin-6, TNF alpha, interleukin-8.

Further, PCR testing can be used to identify agents such as human immunodeficiency virus, CMV, HSV, parvovirus B19, toxoplasmosis and bacterial DNA in amniotic fluid. Screening for GBS carriage should be done by combined vaginal/rectal swabs and a white blood cell (WBC) count done in maternal blood. Serology tests for parvovirus B19, toxoplasmosis, CMV, syphilis, rubella, HSV and HIV are recommended as core investigations. Maternal blood cultures should be taken if the

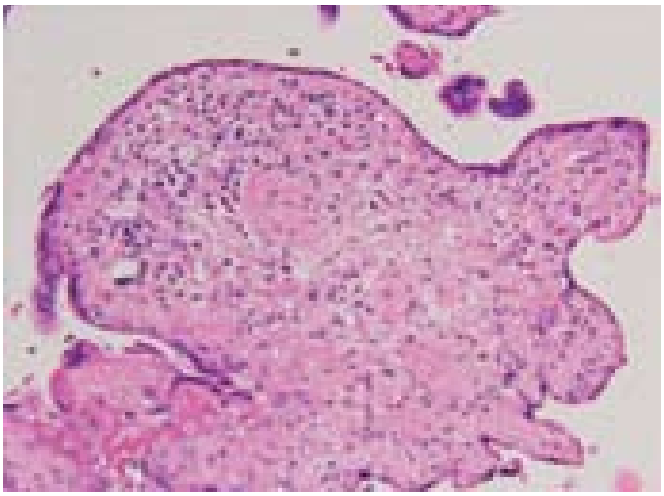

Figure 4. Chronic villitis: chronic inflammatory cells in the stroma of a terminal villus.

clinical findings suggest active chorioamnionitis, with additional cultures being performed when indicated (e.g. faeces for Listeria and campylobacter infection).

High vaginal/low vaginal swabs (HVS/LVS) should also be taken. There is an association between bacterial vaginosis and premature labour but antenatal management of bacterial vaginosis as a means of preventing premature labour remains controversial. Possible further research would include serum concentrations of interleukin-6, interleukin-8 and tumour necrosis factor, and non-cytokine markers of infection, including serum C-reactive protein and serum ferritin.

Interpretation

Acute chorioamnionitis is considered to have virtually always an infective aetiology, even though bacteria are only cultured in some of the cases. This discrepancy may be due to maternal antibiotic administration, failure to culture for implicated organisms such as the genital mycoplasma species or bacterial vaginosis-associated organisms, or the reporting of potentially pathogenic organisms as 'normal' flora with no further specification.

A fresh stillbirth with no evidence of chorioamnionitis does not exclude an infectious aetiology. Organisms such as GBS can cause fetal infection with intact membranes. The infection may be overwhelming, with insufficient time to initiate an inflammatory reaction. Furthermore, there may be no inflammatory response at autopsy as the cellular immune system in an extremely premature fetus may be immature. In a fresh stillbirth with evidence of chorioamnionitis, the possibility of isolation of organism from fetal tissue or placenta is always high (with the caveats mentioned earlier) and an infectious cause must be considered.

Caution is advised in the interpretation of organisms cultured in these scenarios as being either 'vaginal contaminants' or normal flora, since they may, in fact, be very significant. In cases of macerated stillbirths, the presence of chorioamnionitis does not necessarily mean infection unless a fetal reaction is identified. In a macerated stillbirth where there is no evidence of chorioamnionitis and no organism isolated, other causes of stillbirth should be considered. Most macerated stillbirths appear to have a low yield of identification of a causative bacterial agent, although syphilis and viral causes may be found.

In practice, every non-macerated, and not obviously dysmorphic fetus should have bacterial cultures taken and assessed in the light of histology. Viral infections should be considered in growth restriction and in stillbirths with rashes, localised areas of necrosis (e.g. liver) and hydrops in both macerated and non-macerated stillbirths.

Conclusion

Infections remain a frequent factor in stillbirths and are often clinically silent. Stillbirths require active screening for infections with close collaboration of all involved specialists in the interpretation of results.

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