Investigation of Perinatal Loss, Molecular Genetic and Pathological Features

Adrian Charles[*,1,3], Ashleigh Murch[2,3], Catherine Douglass[4] and Loryn Sellner[5]

[1]*Department of Paediatric Pathology, Pathwest Laboratory Medicine, King Edward Memorial Hospital, Australia*

[2]*Cytogenetics Department, Pathwest Laboratory Medicine, King Edward Memorial Hospital, Australia*

[3]*School of Pathology and Laboratory Medicine, University of Western Australia, Australia*

[4]*Department of Obstetrics, King Edward Memorial Hospital, Australia*

[5]*Faculty of Medicine, University of Western Australia, Australia*

Abstract: This review is a perinatal pathologist's approach to the stillbirth with emphasis on the use of molecular techniques. Stillbirths are a heterogeneous group, with diverse aetiologies. The stillbirth investigation needs to be based on a good review of the history, antenatal scans, the autopsy, placental examination and ancillary investigations. Often a multidisciplinary team is required to identify and evaluate the factors. However an increasing understanding of the pathophysiology of stillbirths and the development of molecular techniques in both the diagnostic and research laboratories are introducing additional ways of identifying causal factors in stillbirths.

The main areas where molecular techniques are currently useful in assessing a stillbirth are to identify or confirm:

- Syndromes/genetic diseases from amniocentesis and foetal samples.
- Infective aetiologies from amniocentesis and foetal tissues.
- Maternal and foetal thrombophilia mutations which lead to an increased risk of stillbirth.

However current research and probable future routine techniques includes the following areas;

- The use of array technology instead of classical cytogenetics to identify chromosomal imbalances.
- The identification of markers or polymorphisms related to intramniotic infection.
- The use of foetal DNA in maternal blood for diagnosis.
- Proteomic techniques to identify protein profiles in maternal serum or amniotic fluid to identify pregnancies at risk.
- The identification of molecular markers of the interaction between the foetal and maternal immune systems at the placenta - decidua/maternal blood interface.
- Molecular techniques to understand the mechanisms of the foetal/developmental origins of adult disease.
- The identification of the role of micro RNA in stillbirths and placental function.
- Molecular techniques to refine poorly understood areas of placental maldevelopment.

Keywords: Perinatal loss, mutations, stillbirth.

INTRODUCTION

Stillbirth is a heterogeneous condition with many different causes and risk factors [1]. Stillbirths are not common, with a rate of around 7 per 1000 births in the developed world, so few obstetricians will see more than a couple a year whereas a perinatal pathologist will see many more and is in a privileged position to offer insights in this area. The stillbirth has received rather scant attention in mainstream medicine, but it has potentially major implications for the mother, her family, as well as the healthcare system. Stillbirths are around 10 times more common than sudden infant death syndrome (SIDS). If stillbirths are the fatal end of the spectrum of fetal disease and as foetal wellbeing and optimal growth are the focii of the science of the developmental origins of adult disease, stillbirth should be the subject of more scrutiny [2].

Stillbirths are important markers of the delivery of healthcare. Although most other measures of life expectancy are improving, the stillbirth rate which had fallen has now been fairly stable for the last 15-20 years and may be starting to rise as shown by a variety of state and national reports from the US, UK and Australia. The reasons for the failure of improvement are probably multifactorial; mothers are older, more likely to have a raised BMI (body mass index) and more pregnancies are achieved through assisted fertility techniques. Each of these increases the stillbirth risk and has compensated any underlying improvement in the healthcare.

*Address correspondence to this author at the Department of Paediatric Pathology, Pathwest Laboratory Medicine, King Edward Memorial Hospital, Australia; Tel: +61 08 9340 8687; Fax: +08 9340 8552;
E-mail: Adrian.Charles@health.wa.gov.au

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The definition of stillbirth varies from country to country and state to state, based on gestational age and/or weight [3]. Which predominates depends on the country, with those using population wide first trimester screening programmes favouring gestational age. But the criteria of age and weight also vary in different places, and there are other inclusion/ exclusion criteria such as when a fetus is known to have deceased prior to the age of registration, or is delivered after interruption of the pregnancy for a fatal foetal condition may or may not be included.

The risk of foetal death varies according to the gestational age. There is an initial fall in risk during the first half of pregnancy, plateauing from the mid trimester, rising in the late third trimester with a particular rise very late in the third trimester and post term [4]. The risk is increased for multiple pregnancies. The various causes of stillbirth vary in their incidence according to the gestational age, with chorioamnionitis being more common in the mid second trimester, pre-eclampsia later and unexplained stillbirths later in the third trimester [3]. Congenital abnormalities are seen in around 20% of foetal deaths. Many of the otherwise unexplained third trimester stillbirths show evidence of growth restriction, particularly if customised birth weights are used [5]. The stillbirth rate also shows a U shaped curve with maternal age and there is also an increased risk with diabetes and obesity both of which are a growing problem in current obstetric patients.

The cause of a stillbirth can sometimes be certain and a complete explanation of the foetal demise, such as overwhelming infection, or a heart anomaly with hydrops. However often the examination shows one or several factors that are less clearly lethal such as growth restriction, minor anomalies such as unilateral renal agenesis, or placental changes such as villitis. Many of these factors have an increased risk of stillbirth but the pathogenic pathway to foetal mortality is not clear. Some of these factors are recurrent, may benefit from further investigations, and may affect the management of subsequent pregnancies. A good systematic review with a multidisciplinary team will improve the understanding of causality [6]. In a minority of cases (15-20%) the cause of death is unexplained even after a thorough assessment [7, 8]. This probably reflects the complex physiology of pregnancy and the pathophysiology of abnormal conditions such as growth restriction which are not well understood. There are, for example, more congenital abnormalities in stillbirths than in live born babies, which does not appear to be due to ascertainment or termination of pregnancy. The mechanism of the increased loss of foetuses with minor anomalies is not clear.

There are usually a number of noteworthy factors identified in any stillbirth. There may be maternal, placental and foetal factors to be considered, with medical, social and cultural aspects. The importance of each factor is related to its role in that death, the ease with which that factor may be managed to reduce future risks, that it highlights a system failure or that it is a common though relatively minor factor in a number of deaths. Though it is simpler to have a single factor as the cause of death, stillbirths, rather like deaths in the elderly, are often due to multiple factors. The use of more refined classification systems may help record this [9-11].

The investigation of stillbirths has been hampered by many factors including lack of evidence based investigation protocols and compliance to these protocols. Resources are also required for the multidisciplinary team investigation. There are recent good guidelines for investigating a stillbirth such as the CESDI/CEMACH publications (http://www.cmace.org.uk/Publications/CEMACH-Publications/CESDI-Publications.aspx), PSANZ (http://www.psanz.org.au) and the ACOG [1].

Media coverage of the autopsy and particularly organ and tissue retention in the UK and other countries have affected the practice of paediatric pathology and particularly the consent processes for retaining tissues [12]. This is likely to impact on research as additional consent is often needed to use tissues to research disease, although when approached parents are often are very keen for this to proceed [13].

The basic molecular paradigm is that DNA is transcribed to mRNA in the nucleus and mRNA is transported into the cytoplasm where it is translated into proteins. However the *in vivo* situation is infinitely more complicated and subtle with many levels of control. The DNA has sites to allow transcription, with start sites, promoters and repressors and there are also higher level controls that are very important. The chromosomes have an organised structure within the nucleus, and the genes may be organised on the chromosome so that there may be sequential order of genes, replicating the expression order, as in the homeobox genes. The structure of the chromosomes is controlled by the histones and nuclear architecture which allow the transcription activators to attach to the DNA. Certain specific areas of the genome are controlled by methylation of the backbone of the DNA in cytosine-guanine (CG) rich areas. The most well known methylated regions are the inactivated X chromosome and the 11p15 area associated with Beckwith Wiedemann syndrome and some paediatric tumours. In addition to the genomic DNA there is also mitochondrial DNA which is derived wholly from the mother, with an associated group of metabolic disorders, though many mitochondrial genes are in fact encoded in the nuclear genome. The mRNA is also modified before translation, with introns removed, and there may be variable splicing to give functionally different proteins. The mRNA is also controlled by stabilising factors and controlled destruction. Not all RNA is translated; much is non coding, such as the H19 gene at 11p15. More recently it has been realised that there are a large number of small non coding RNAs that appear to be controllers of genes, through interaction with the mRNA in development and cancer [14]. The importance of this area is only just being addressed, and this is likely to be a very significant factor in a large number of control pathways and groups of genes with related functions [15]. The mRNA is translated into proteins which may be controlled by localisation, phosphorylation, cleavage or glycosylation to affect their function, and there are also controls such as the ubiquitin pathway for their turnover. This complexity undoubtedly allows subtle control of the cellular processes, and demonstrates that there is not a simple relationship between DNA, mRNA, the protein and function. Molecular tests need to be interpreted with this complexity in mind.

MOLECULAR TECHNIQUES

There are many molecular techniques. One could argue that immunohistochemistry specifically detecting a protein is such a technique. The commonest molecular technique is the polymerase chain reaction (PCR) or one of its variants, including RT-PCR for RNA, PCR *in situ*, leaving a product that can be visualised in the section (Primed *In Situ* labelling - PRINS). The PCR product can be run electrophoretically on a slab gel, but now fluorescent primers are often used and the PCR products detected by capillary gel electrophoresis.

PCR reactions can be used for many purposes including spanning a region of variable size that has clinical significance, such as the number of repeats in Fragile X syndrome, a viral or bacterial DNA sequence can be specifically amplified and detected or specific bacterial ribosomal RNA can be isolated to confirm bacteria are present, in suspected infection. Pathologic DNA or RNA polymorphisms or mutations can be found by sequencing the products, or by using mutation specific probes or primers. Assessing the highly polymorphic microsatellite regions present on each chromosome, for one, two or three alleles can be used as a simple, cheap assessment, of aneuploidy [16]. It will detect a duplication of one allele, or loss of heterozygosity such as occurs in uniparental isodisomy with loss of one chromosome and duplication of the partner. One can use this technique when investigating a suspected partial mole by using decidua as maternal tissue to determine whether the extra chromosomal material in a triploid fetus does not come from the mother, thereby identifying the dipaternal triploid fetus with the partial mole phenotype without getting into paternity issues. The technique known as Quantitative Fluorescence PCR-QF-PCR is semi-quantative, detecting allele copy number change even where alleles are identical.

Another technique that can be used to detect aneuploidy and partial chromosomal loss (and other conditions) is Multiplex Ligation-dependent Probe Amplification (MLPA) which is now available as commercial kits, can be used for amniocentesis samples [17], and also foetal material including paraffin fixed tissues (unpublished observations). This technique uses fluorescent labelled probes in a modified PCR-based multiplex method to co-amplify up to 30-40 DNA targets. The relative amounts of products generated are used to identify deletions or duplications of genes, part genes, or entire chromosomes (monosomies or trisomies). The distinction between triploidy and diploidy can be difficult as the entire genetic complement is uniformly increased, hence no relative changes are identified [18].

The detection of imprinting anomalies is becoming increasingly important. One important genetic mechanism underlying imprinting is methylation of the DNA in the chromosomes at specific sites. The use of PCR techniques with and without bisulfite treatment can detect methylation changes [19]. Methylation is important in X-inactivation, and explains why digynic and diandric triploidy cases have a different phenotype.

Cytomolecular techniques can be used to mark the location, or presence or absence of of a specific chromosomal region *in situ* in metaphase chromosomes or in interphase nuclei. A labelled nucleic acid probe for the region of interest is hybridised to the target nucleic acid, such as a specific chromosomal centromere, or location, viral DNA or RNA. The technique was originally developed using fluorescently labelled probes (FISH - fluorescence *in situ* hybridisation). As well as being used on cell preparations this can be applied to histological sections (Fig. **1**). As there are a number of fluorochromes which are excited by different wave lengths, and fluoresce at different wavelengths, multiple targets can be assayed at once. The detection system can be automated [20]. Modifications of this technique include using chromagens that can be used in normal light microscopes rather than fluorescent microscopes using secondary antibodies tagged with peroxidise. This is called CISH. The use of silver techniques is called SISH. The common sites used in this field are centromeric specific probes to detect chromosome number, but detection of specific areas of the chromosome such as the Di George locus and other regions of microdeletion or microduplication, or subtelomeric specific probes are also used. A technique called PRINS is a combination of FISH and *in situ* polymerisation, where a probe is elongated *in situ* with labelled nucleotides that allow identification of the site [21]. This technique has also been used on fetal cells circulating in the maternal blood [22].

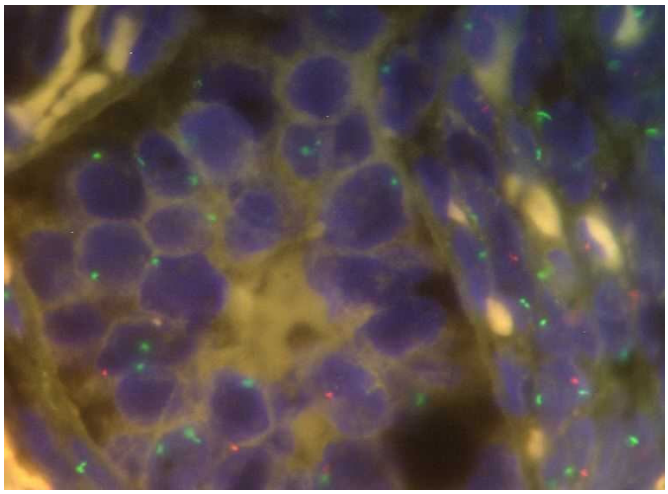

Fig. (1). Patient with Turners phenotype and a 45 XO/46 XY karyotype and a dysgenetic gonad with sex cords and ovarian stroma. The mixed XY and X0 signals are clearly seen in both the sex cords and ovarian stroma like areas (Green X chromosome Red Y chromosome, sex cord left, ovarian stroma on right. DAPI - blue nuclear counterstain).

More recently the hybridisation technique is used the other way around. A test sample of DNA or RNA is added to a "chip" containing an array of many different targets, which can be DNA widely sampled at high resolution across the genome to assess whether the sample has gain or loss of chromosomal material, or by having targets to transcribed RNA so one can assess mRNA (messenger RNA) profiles. The degree of hybridisation is measured in a semi-quantitative manner, and the vast amount of information created is analysed using complex bioinformatic software. Chips are now available that cover the whole genome and the range of messenger RNAs, and also micro RNA. These chips are moving from the research to the diagnostic domain [23, 24].

The DNA array chip has replaced an older technique where DNA extracted from a diagnostic sample is cohybridised with a control sample to a normal metaphase spread to allow one to assess chromosomal gains or losses in the test sample relative to the normal control (called comparative genomic hybridisation-CGH) [25]. The chips replacing this (array CGH) allow very much greater genomic resolution. The advantage is that the DNA can be used from frozen tissue or even from paraffin embedded material, unlike classic karyotype analysis that requires live cells. The technique only detects deletions or duplications of DNA (often referred to now as copy number variation - CNV) [26]. A balanced translocation will not result in net gain or loss of material, and so will not be identified by CGH, even though the translocation may alter a protein and affect the cell function. DNA chips may also target some of the polymorphisms in the DNA - the single nucleotide polymorphism - and this can be used to assay changes such as uniparental isodisomy, where a chromosome is duplicated from one parent as well as being used for a high resolution screen of the chromosome [26]. Array technology usually needs high quality nucleic acid (DNA or RNA) in reasonable quantities, but recently amplification of the DNA sample by PCR techniques has overcome the use of small samples and DNA from paraffin sections can also be used [27].

Recently the area of proteomics has taken a larger role. Tandem mass spectrometry and chromatography techniques produce a spectral plot, which can then be analysed using subtraction analyses to identify differences in protein/ protein fragments present in various conditions. These can then be used as biomarkers. The function of these biomarkers can be used as diagnostic cues, and the biological function of the biomarkers ascertained.

The different techniques allow a large number of questions to be answered and some of the uses will be addressed below.

GENETIC AND MOLECULAR INVESTIGATIONS OF A STILLBIRTH

It is a moot point whether every stillbirth should have cytogenetic analysis. The test is labour intensive and thus expensive. Moreover, the success rate can be poor, particularly in macerated foetuses, resulting in increased workload for no return. A recent review [28] showed around 5% of stillbirths have cytogenetic anomalies, but most of these were non recurrent. Another review found a low yield of cytogenetic abnormalities of foetuses where autopsy showed no dysmorphic features of aneuploidy [29]. This suggests a targeted analysis might be a more cost effective approach. The local practice should be negotiated with the cytogenetics laboratory. Amniocentesis performed after confirmation of fetal death is recommended because of a higher yield of cytogenetic and microbiological culture compared with foetal samples taken following birth [1]. If there are obvious syndromic features such as those of a chromosomal or other anomaly, then clearly testing is indicated. In the presence of a major anomaly, significant growth restriction, or a mildly unusual appearance, cytogenetics should be performed especially in a macerated fetus. The cultures for cytogenetics can be obtained from foetuses a few days after delivery but are best taken as soon as is practical. In macerated foetuses where the yield is low the placenta should be sampled as the yield is improved as the placenta is maintained by maternal circulation. The placenta may occasionally differ cytogenetically from the fetus as in confined placental mosaicism (see below). In these cases newer techniques, discussed below, can be helpful in identifying genetic changes using non viable foetal tissues. Many chromosomal anomalies are not recurrent, so do not directly affect management, but identifying a chromosomal anomaly and thereby a likely aetiological factor for the stillbirth may prevent other tests, investigations, as well as answers for the parents and the clinicians. Parental karyotype is indicated in recurrent losses, to exclude balanced translocations in the parents giving rise to unbalanced chromosomes in the gametes as a cause of recurrent loss. In the future, molecular techniques, especially microarray, are likely to replace routine cytogenetic analysis in stillbirth studies as the technique is much more sensitive and does not require viable tissue for culture. Ideally some tissue (or foetal blood on a Guthrie card) should be kept for potential DNA analysis at autopsy on all foetuses. Whilst this may not be feasible for all stillbirths, samples should be retained on dysmorphic foetuses unless the diagnosis is clear and further examination is unnecessary.

The newer techniques, FISH, QF-PCR or MLPA described above can be applied with minimal modifications to foetal tissues, when cytogenetic culture is not possible either due to maceration, or because the specimen has been fixed in formalin. Tissues used for FISH or PCR are best where there is still nuclear preservation on the H&E stain, so the placenta, lung or brain are usually best whereas liver and other tissues showing marked autolysis show poor nuclear staining and also tend to yield little useful DNA. These techniques will identify the common trisomies. Both FISH and MLPA can also be used to target specific abnormalities such as the Di George region microdeletion, and are worthwhile performing if the autopsy shows anomalies of the cardiac outlet especially if a cleft palate or other features are present. For fetuses with a range of anomalies where the chromosomal analysis is normal, subtelomeric FISH probes may be useful [30]. FISH techniques work well with adaptation on paraffin sections but sections of 6 or 7 microns mean that many nuclei will be sectioned giving rise to a nuclear truncation artefact. In our laboratory we tend to use a nuclear disaggregation technique using a small biopsy of a paraffin block to produce whole nuclei when we are assessing for aneuploidy (Fig. **2**). Clearly there are times when the histological structure is needed and this technique is not feasible then (Fig. **1**). FISH techniques can be used where there is mosaicism such as in Pallister Killian syndrome with isochromosome 12p and often in Turner syndrome.

An interesting cause of significant foetal growth restriction which may be severe is confined placental mosaicism, where the fetus has a normal karyotype but the placenta shows areas or specific tissues showing aneuploidy [31]. This is a labour intensive investigation, and not normally performed routinely in most laboratories. There are usually only slight if any histological clues of this genetic change [32].

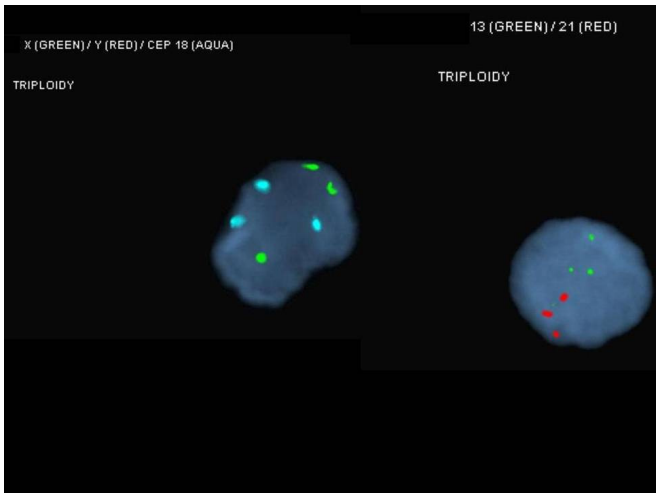

Fig. (2). Separated nuclei FISH. Composite picture of two hybridisations. For suspected Triploidy. DAPI nuclear counterstain, deeper blue, 3 Green X probes, 3 light blue chromosome 18 probes, 3 Green chromosome 13, and three Red chromosme 21 probes. consistent with Triploidy.

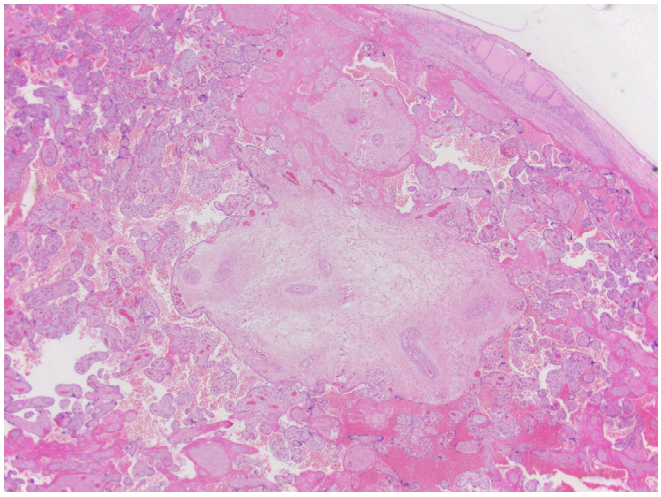

Fig. (3). Large term stillbirth (4.4 kg) showing mesenchymal dysplasia of the placenta which also showed absent p57 staining (a maternal expressed gene at 11p15). Beckwith-Wiedemann syndrome was confirmed with dipaternal methylation pattern at 11p15.

Methylation studies using the specific PCR techniques as above are important in Beckwith-Wiedemann Syndrome, which is genetically heterogeneous and there are some genotype-pheonotype correlations [33]. This syndrome appears to be increasing in incidence due to IVF technology [34].

Viral infections can be a cause of stillbirth and are sometimes overlooked. PCR is now frequently used as the main diagnostic test, either on liver or on a placental sample taken especially at autopsy, but now frequently used on paraffin scroll taken from paraffin embedded tissue blocks. PCR is a very sensitive technique and mere detection of the presence of a virus may not mean it is of pathological significance. It may be acquired during delivery, or may be being carried harmlessly without pathological sequelae or even a contaminant. Most viral infections that cause fetal demise and stillbirth cause significant pathology, with villitis, inclusions, or areas of necrosis in the fetus, but there are also increased stillbirths in mothers with viral infection without fetal or placental infection, possibly mediated by cytokines, and more subtle viral infections which may mediate stillbirth through other mechanisms [35]. The identification of the prokaryotic specific ribosomal 16s RNA is generally used more in research to identify if bacteria are present.

There are molecular tests for thrombophilia which are indicated in some cases. These are usually performed on the mother often 6 weeks or so after the loss. Pregnancy is physiologically a hypercoagulable state, but mothers with acquired or inherited thrombophilia have an increased fetal loss [36], and the mother is also at risk of venous thromboembolism both during and after pregnancy [37]. Investigating the mother with thrombophilia tests should be considered if some placental lesions are found, such as massive perivillous fibrin and foetal thrombotic vasculopathy [38]. These investigations will normally be instituted by the obstetrician rather than the pathologist, though may be suggested in the autopsy and/or placental report.

Over the last ten years there has been growing interest in identifying circulating fetal DNA in the maternal circulation and using this for making a diagnosis [39] and thereby avoiding the 0.5-1.0% of risk from amniocentesis or chorionic villus sampling. This is now being used clinically for sex determination and some aneuploidies [40]. This technology may affect stillbirths by earlier detection of aneuploidy.

An unusual condition is where a fetal genetic disorder gives rise to a maternal disease, and this is the lethal fatty acid disorder associated with a deficiency of fetal long chain 3-hydroxy-acyl-coenzyme A (CoA), and maternal HELLP syndrome (Haemolysis, ELevated liver enzymes, Low Platelets) [41].

FIRST TRIMESTER SCREENING

Although this is not strictly considered a molecular technique, it is of interest that a low PAPPA (Pregnancy Associated Plasma Protein A) at the first trimester dating scan (11-12 weeks pregnancy), is associated with a poor pregnancy outcome including stillbirth and growth restriction. PAPPA is a protein produced by the placenta. This test by itself is not sensitive enough to use clinically but the fact that it may be associated with a poor outcome many weeks before the problem manifests is useful. This raises the possibility of using this as a marker for further screening and intervention or at least closer monitoring. The first trimester screen has been combined with later tests such as AFP (Alpha FetoProtein) to try to increase the sensitivity of testing [42]. Poor placentation appears to play a significant factor in a large number of stillbirths. Inadequate reconstruction of maternal vessels is seen in some early second trimester losses [43] and it may well be an effect of this that the screening is detecting. This area may give some further clues as to the aetiology of stillbirths as growth restriction is often due to poor placentation.

CURRENT RESEARCH OR FUTURE ROUTINE TECHNIQUES

There are several areas where one considers there are likely to be changes sooner or later. Pathologists must stay attuned to these developments to incorporate them sensibly and efficiently into practice.

Routine karyotyping is a technician intensive technique, and it takes time for the cells to culture. Arrays offer a faster way of assessing the genome, at a higher resolution and in quicker time. The downside is that balanced translocations may be missed. It is likely within a few years this will be the first choice of screening as the price of the chips decreases. There are already reports in the literature of the use of the technique in investigating first trimester miscarriages, however it has as yet not greatly increased the pick up of abnormalities in this group [44]. The subtle copy number changes detectable by array techniques may suggest the technique is likely to play an important role in investigation of this group.

There is a large amount of research examining the way the maternal and foetal immune systems interact, and how the foetus avoids or adapts the maternal immune surveillance. This involves non classical HLA epitopes such as HLAG, the specialised Natural Killer cells found in the decidua and the receptors associated with the immune system [45]. It is likely that as the immune system becomes better understood ways of assessing this may be helpful in understanding cases of recurrent villitis or poor placental implantation.

One of the most frequent causes of midtrimester losses is infection, and polymorphisms of innate immunity systems such as the MBL and the cytokine systems are being explored to examine the immune basis for this tendency [46].

An exciting development is the screening of maternal blood to identify protein signatures that may be altered earlier in pregnancies that end suboptimally. This may give rise to novel biomarkers, or the nature of the protein(s) identified may give an insight into the pathogenetic pathway. As stated earlier, it is clear that many pregnancies that end poorly have abnormal first trimester screens or are due to poor implantation, something that happens early in pregnancy. The benefit of this is that if the pregnancy is at risk, then there is the potential of monitoring closely and delivering early. This needs careful assessment of the risks of preterm delivery, with the difficulties of accurate and sensitive enough monitoring techniques. Studies have used amniotic fluid [47] or maternal blood [48] and showed good correlation of the proteomic findings with fetal sepsis and funisitis.

Since Barker described the risk of adult disease in babies who were born small [2] a whole field of medical investigation has developed in this area. So far little has been undertaken in the examination of stillbirths, many of which are growth restricted and some of which appear to be associated with mothers who have metabolic syndrome. Since there is now evidence that epigenetic changes occur in laboratory animals that are growth restricted and this fits with observations from the Dutch famine mothers, there is the likelihood that the fetuses may also show similar epigenetic changes [49]. As the stillbirth can be seen as the fatal end of the suboptimal intrauterine environment this may aid our understanding of the epigenetic changes related to the field of DoHAD (developmental origins of adult disease), and how intrauterine growth restriction causes the metabolic changes found decades later.

Non coding RNAs are now recognised to have a crucial role in the control of gene expression, both in development and cancer [14, 15]. There are now array techniques available for assessing these, and it is likely that these will aid our understanding of maldevelopment and disease. RNA tends not to be stable in cells, but interestingly micro RNAs (miRNA) appear more stable in paraffin sections than messenger RNA. One suspects that this field of epigenetic testing will also explore more closely the interaction of chromatin, histones, RNA, and chromosomal structure in abnormal development.

THE FUTURE

Stillbirths have been rather neglected in medical research, but the field is interesting, challenging and rewarding, requiring input from different professionals to reduce what currently appears a rather intractable statistic. Stillbirths are not uncommon, and not clearly decreasing in incidence, despite our medical progress in other areas.

However a methodical, systematic approach to investigate stillbirths, record and analyse the data and determine ways to improve the factors that appear to predominate in that population should lead to an improvement in the stillbirth rate. In view of the role of growth restriction in stillbirths, better ways of monitoring and assessing foetal wellbeing and foetal growth are needed. The number, range and sophistication of molecular tests available are likely to grow, and as they are not cheap need to be introduced in a systematic way. The establishment of biobanks with well categorised specimens including placenta and maternal blood with bioinformatic resources may be very helpful. It is also likely that the better understanding of the causes of stillbirth may aid our understanding of the effects of a suboptimal intrauterine growth and development that appears to be the basis for a myriad of Western adult diseases.

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