04.01
An investigation of interphase FISH methods to provide a rapid
pre-natal diagnosis of chromosomal abnormalities in
uncultured amniocytes.
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2TG
The major aneuploidies diagnosed pre-natally involve the autosomes 13, 18 and
21, and the sex chromosomes X and Y. Fluorescence in situ hybridisation
(FISH) allows rapid analysis of chromosome copy number in interphase
cells. This study evaluated the use of three commercially available cen-
tromeric DNA probes (VYSIS CEP 18, X and Y) and two site specific DNA
probes (VYSIS LSI 13 and 21) for direct analysis of uncultured amniocytes.
Seventy-one clinical amniocentesis specimens were analysed by interphase
FISH and full karyotype, using the LSI 13 and 21 probes, and sixteen speci-
mens were analysed using the CEP 18, X and Y probes. This evaluation
demonstrated that FISH with the commercially available X, Y, 13, 18 and 21
probes could accurately and rapidly detect the copy numbers of these chro-
mosomes.

04.03
A rare complex chorionic villus structural chromosome
mosaicism involving isochromosome 18q.
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L(1);Health, M(1);Wilkinson, A(1);Laister, C(1);Hamilton, R(3)
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Road, Nottingham NG5 1PB, (2)Pregnancy Assessment Centre, Queens Medical
Centre, Nottingham, (3)Department of Obstetrics & Gynaecology, Rotherham District
General Hospital, Rotherham.
A chorionic villus sample was referred for pre-natal cytogenetic diagnosis
from a 26 year old woman because of an abnormal fetal ultrasound scan -
cleft lip and palate at 28 weeks gestation. The chorionic villus direct prepa-
rations showed a complex karyotype involving chromosones 3, 18 and 22
(45,XX,der(3)(3;18)(q29;p11.1),der(18;22)(q10;q10)). In contrast the chor-
ionic villus long term cultures showed a complex karyotype in which only
chromosomes 3 and 18 were involved, the der(18;22) absent and an isodi-
centric chromosome for the long arm of chromosome 18 present (isochromo-
some 18q syndrome) (46,XX,der(3)(3;18)(q29;p11.1),idi(18)(p11.1)).
The long-term culture result was confirmed in a fetal blood sample, although a
third mosaic cell line was also found (46,XX,idi(18)(p11.1)) in this tissue.
The pregnancy was terminated, the post-mortem report indicating a bilateral cleft
lip, an enlarged left kidney and a Meckel's diverticulum as the only significant
abnormalities in this fetus. This case clearly demonstrates the problems of
inter-tissue mosaicism in relation to isochromosome 18q diagnosis in chor-
ionic villus samples (false negative for this abnormality in the cvs direct
preparations). In addition, it provides further (circumstantial) evidence for
the involvement of the gene HPE4 (on chromosome 18p) in holoprosencephaly.
This fetus had neither holoprosencephaly or simple 18p- normally associated
with isochromosome 18q syndrome.

04.04
Prenatal diagnosis of a duplication of chromosome 11p13
14 in the absence of major phenotypic abnormalities.
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(1);Wilkinson, A(1);Whitehouse, M(1);Gill, K(1);Heath, M(1);Filter,
A(1);Bruce, J(4)
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Health Care NHS Trust, Salisbury District Hospital, Salisbury, Wilts., SP2 8BJ,
(3)Pathology Department, Queens Medical Centre, University Hospital, Nottingham,
NG2 2UH, (4)Department of Obstetrics & Gynaecology, City Hospital, Hucknall Road,
Nottingham NG5 1PB.
An amniotic fluid was referred for prenatal cytogenetic diagnosis because of
raised maternal age. Chromosome analysis revealed a female karyotype
with a de novo duplication of part of the short arm of one chromosome 11
(46,XX.dup(11)(p13p14)). Fluorescent in situ hybridisation studies using
probes specific for this region (EO6158,F1238,CO8166,FO2121,B2.1D11,
and cl-11G58) confirmed that the duplication included the WAGR region and
in addition suggested it to be a cryptic inversion. The pregnancy was termi-
nated. Intestinal duplications of this region are extremely rare, and have
been associated with multiple congenital abnormalities. This is in contrast to
our case in which only minor abdominal malformedations were noted. This
case illustrates the problems associated with the prediction of clinical out-
come in cases involving the WAGR locus - no specific malformations associ-
ated with this locus were present in this case.

04.05
A molecular genetic protocol to identify maternal contami-
nation in prenatal samples.
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It is imperative that prenatal diagnosis results are correct, as women will
base reproductive decisions on these results. One source of error is maternal
contamination. We recently counselled a family in whom a prenatal sample
showed gross maternal contamination, leading to a potentially incorrect
result. We therefore have devised a protocol to exclude maternal contamina-
tion. By using polymorphic minisatellites and Amelogenin in which there are
different sized alleles on the X and Y chromosomes, maternal contamination
of greater than 10% can be identified. This protocol is used in prenatal sam-
iples in which the genotype of the fetus may be the same as the mother, as in
cystic fibrosis in which the mother is a carrier of a known mutation and a
result showing the fetus to be a carrier of the same mutation could be either
a true result or maternal contamination and in X-linked conditions in which
normal female karyotype could be the result of maternal contamination. For
prenatal tests that involve biochemical testing, maternal contamination of
less than 10% may influence the results, and therefore in these situations,
our protocol may have limitations. We review the results of 10 prenatal sam-
iples in which this protocol was used.
04.06  
Prenatal detection of trisomy 21 by QF-PCR: Preliminary results, a novel alternative to DNA extraction and a cautionary tale on marker selection  
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Quantitative fluorescent polymerase chain reaction (QF-PCR) assays of highly polymorphic small tandem repeats (STRs) have been described as an effective alternative approach for the rapid detection of chromosome aneuploidies in uncultured prenatal specimens. We have employed a simple alkali lysis procedure to obtain target DNA from the amniocytes. This proved a robust and inexpensive alternative to DNA extraction. In a prospective study of 190 amniotic fluid samples we correctly identified 7 cases of trisomy 21 and these results were used to expedite the conventional analysis of the samples. Heavily bloodstained specimens were not excluded from this study and one such sample gave a false positive result. We used two published primer sets (loci D21S11 and D21S1414) reported to amplify across polymorphic tetranucleotide repeats on chromosome 21. Unexpectedly, although the markers gave different sized products, they revealed identical allele distributions in any given sample. Our preliminary results suggest that QF-PCR can consistently provide clear and reliable diagnoses, provided that visibly blood stained specimens are excluded.

04.07  
Early onset fetal hydrops and muscle degeneration in siblings due to a novel variant of type IV glycogenosis  
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We report 3 consecutively affected sibling fetuses, presenting at 18 weeks, 11 weeks and 13 weeks gestation with fetal hydrops, congenital limb contractures and akinesia. Post mortem examination of the 18 week fetus (sibling 1) showed subcutaneous fluid collections and severe degeneration of skeletal muscle. Histology demonstrated massive accumulation of diastase resistant PAS-positive material in the skeletal muscle cells and epidermal keratinocytes of all three fetuses. Enzyme studies of fibroblasts from the third fetus showed deficient activity of branching enzyme, indicating that this is a new, severe form of glycogenosis type IV with onset by early in the second trimester.

04.08  
Pre- and post-natal karyotypic discordance for a de novo unbalanced structural chromosome abnormality - an unusual case resulting from apparent instability of the 21q telomeric region.  
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De novo non-mosaic structural chromosome abnormalities (excluding supernumerary markers) detected in cultured CVS appear to be reliable indicators of the fetal karyotype. We present a case with pre- and post-natal discordance for an unbalanced karyotype. A 25 year old mother was referred for CVS with oligohydramnios at 28 weeks gestation. All 50 cultured CV cells examined had a de novo unbalanced female karyotype with additional satellites on the telomeric end of one 21q. The infant was born at 37 weeks. Examination showed features of monosomy 21 including growth retardation, microcephaly, flexion contractures, and facial dysmorphism with a broad nose. Cultured lymphocytes (150 cells) revealed a different unbalanced female karyotype with a normal chromosome X and 21, and a translocated dicentric chromosome involving exchange between Xq13 and the telomeric end of 21q: the abnormal X was late replicating. Involvement of the same chromosome 21 exchange site in both rearrangements suggests a linked origin, possibly some form of jumping translocation/telomeric association. No other tissues have yet been karyotyped. This case is particularly unusual in demonstrating several rare phenomena: 1) extra satellites, 2) such pre/post-natal karyotypic discordance, 3) apparent telomeric instability, 4) an X;autosomal translocation, 5) a stable dicentric chromosome, and 6) clinical features of true monosomy 21.

04.09  
Development of an antenatal screening programme for congenital abnormalities in a South Wales District  
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Two surveys on current practice in antenatal screening for congenital abnormalities, one of health professionals and the other of expectant mothers, were submitted to the Health Authority in 1996. The variations in practice described, as well as the sub-standard features detected were a cause for anxiety to mothers and health professionals. A multidisciplinary Professional Advisory Group was set up as a result, to review the existing patterns of care; to recommend a model of care and quality outcome measures which should form the standards for future audit and evaluation. The Group submitted its report in 1997, specifying the quality features to be implemented in all stages of the antenatal screening programme. It drew heavily on the scientific literature and the recommendations of professional bodies. Most importantly, the opinions and aspirations of the expectant mothers was 'the keystone' that informed this programme and was used to influence change. The Programme covered communication and patient information, amniocentesis, ultrasound scanning, termination of pregnancies for congenital anomalies, staffing and equipment, training and clinical audit. Implementation of the Programme is in progress and include the appointment of a Screening Midwife in each Obstetric Unit, a new leaflet, a computerised audit of outcome and a midwife training course.
04.10
Prenatal detection of an unstable de novo dicentric chromosome, dic(14;15)(p11.1;q13), with duplication of the Prader-Willi/Angelman syndromes critical regions (PWASCR).
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A 36 year old woman underwent amniocentesis for advanced maternal age. Cytogenetic analysis showed a dicentric chromosome 14 replacing the normal chromosome 14 in 86% of cells. The parental karyotypes were normal. The presence of the abnormality was confirmed in prenatal fetal blood. FISH studies using (-)satellite probes D14Z1/D22Z1 and D15Z1, chromosome 14 and 15 wcp, and the PWASCR probes, SNRPN and D15S10, indicated that the dicentric chromosome was derived from a translocation involving chromosomes 14 and 15, with the breakpoints at 14p11.1 and 15q13. Most cells thus showed a duplication of the segment 15pter(15q13), resulting in the presence of three copies of the PWASCR. However, 14% of cells showed mosaicism with monosomy 14 in 9% of cells and breakage of the dicentric chromosome in the inter-centromeric region resulting in two additional copies of the PWASCR occurring in some cells and loss of the additional copy of the PWASCR in others. Maternally derived duplication of the PWASCR has been associated with developmental delay. Chromosome 15 short arm polymorphism suggested a maternal origin for the dicentric chromosome in this case. The parents were counselled that the imbalance was likely to cause phenotypic abnormality and elected to terminate the pregnancy.

04.11
Prenatal diagnosis and characterisation of a de novo supernumerary marker chromosome originating by presumptive 3:1 segregation and resulting in pure trisomy 10p
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Amniocentesis was undertaken at 16 weeks gestation on a 26 year old woman referred with a raised serum screening risk of 1:287. Cytogenetic analysis showed a chromosome count of 47 with an additional acrocentric satellite marker chromosome in all cells examined. Conventional studies and FISH using a whole chromosome 10 paint, and chromosomes 10 and 14/22 specific centromeric probes (D10Z1 and D14Z1/D22Z21), showed that the marker chromosome was derived from the whole of the short arm of chromosome 10, including chromosome 10 centromeric elements, and the short arm and centromeric material of chromosome 14 or 22. The parental karyotypes were normal. The karyotype of the fetus was designated, 47,XY, +mar.ish der(10;14)(14q10:14q10) t(10;14)(14q10:14q10) de novo (wcp10+, D10Z1+, D14Z1/D22Z1+). A detailed ultrasound scan of the fetus showed multiple anomalies consistent with trisomy 10p. Following genetic counselling, the couple elected to terminate the pregnancy. Post-mortem examination of the fetus confirmed the ultrasound findings. Over 60 reports of complete and partial trisomy 10p exist, the majority being cases of inherited unbalanced translocations or inversion recombinants. However, trisomy resulting from a supernumerary 10p appears to be very rare. We report the first case of de novo trisomy 10p originating by presumptive 3:1 segregation.
34.15
Rapid prenatal diagnosis in 3 cases of fluid non-amniotic origin.

The Department of Fetal Medicine at Birmingham Women’s Hospital is a tertiary referral centre for normal scan cases from the West Midlands and further afield. We report three cases of prenatal diagnosis where cytogenetics has been performed on fluid aspirated from within the fetus. In case 1, fetal urine was aspirated at 14 weeks gestation from a distended bladder with hydrophrosis, a nuchal thickening of 4mm and a diagnosis of prune belly syndrome. Case 2 is that of hydrops fetalis in a 21 week fetus with fluid being taken from the hydrosac. In case 3, a large intra-abdominal cyst was observed at 36 weeks gestation. Cystic fluid was aspirated for rapid karyotyping prior to delivery. Results were obtained following short term suspension culture in the presence of PHA. These cases show that rapid prenatal karyotyping can be performed on effusions of fluid of origins which can be utilised as an alternative to fetal blood sampling or late placental biopsy for fetal karyotyping.

04.16
Mosaic trisomy 8 syndrome: A discordant amniocentesis result in a twin pregnancy.

Two amniotic fluid samples were received from a twin pregnancy in which twin 2 showed abnormalities on ultrasound scan; twin 1 appeared clinically normal. Mosaicism for trisomy 8 was detected in twin 2 with no evidence of a trisomy 8 cell line in twin 1. An inversion of chromosome 3 was also detected in both twins which had been inherited from the father. The pregnancy proceeded to term and blood samples taken at birth showed the presence of a trisomy 8 cell line in both twins. This result has been confirmed in two subsequent blood samples. Clinical examinations have shown features consistent with mosaic trisomy 8 syndrome in only one twin. The possible mechanisms for the origin of the trisomy 8 cell line are discussed, together with the clinical features of mosaic trisomy 8 syndrome.

04.17
Predictive value of common cytogenetic abnormalities detected following CVS: results from the Association of Clinical Cytogeneticists UK Collaborative Study

A Collaborative ACC Study has collected data from an unselected series of 20,865 CVS processed in 27 UK laboratories between 1987-93. This has been used to ascertain the reliability of common cytogenetic abnormalities, detected following CVS, in predicting the fetal karyotype. A total of 611 non-mosaic cases of trisomies 13, 18 and 21, monosomy X, XXX, XY and XYY were detected. All cases with follow-up data confirmed the prenatal findings; four involving numerical sex chromosome abnormalities were in mosaic form in the fetus. A further 92 cases of the above karyotypes were in a mosaic form (with a normal cell line) at diagnosis. Follow-up of this latter group showed 40% to be present in the fetus either in a mosaic or non-mosaic form; genuine cases of all the above mosaic abnormalities except XYY were detected. Interpretation of monosomy X is particularly problematic; 1 in 3 cases were mosaic, with 50% of the mosaic cases being associated with an abnormal fetal karyotype. Mosaic trisomies 13 and 18 were also disproportionately common. In contrast, only 5% of trisomy 21 findings were in the mosaic form. One false negative case, a trisomy 18 fetus with a normal extra-embryonic karyotype, was encountered.
04.19
Prenatal genetic diagnosis of coagulation factor VII deficiency in a consanguineous family
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Factor VII (FVII) deficiency, a rare autosomal recessive disorder, may in homzygotes result in a severe bleeding diathesis. The FVII gene (F7) comprises 8 exons spanning 12.8kb of genomic DNA located to chromosome 13q34-qter. Two F7 gene polymorphisms were utilised for prenatal diagnosis of FVII deficiency in a consanguineous family with an affected child who had died from intracranial haemorrhage. A 37bp tandem repeat in intron G of the gene was amplified by the PCR method. A promoter decanucleotide insertion in the 5'-flanking region of the F7 gene was PCR amplified and product digested with Sau3A1. PCR and digestion products were resolved by agarose gel electrophoresis. DNA analysis revealed the affected child was homozygous for repeat polymorphism allele A and presence (+) of the decanucleotide. The parents were informative for alternative polymorphisms, mother for repeat (A/B), father for insert (+/-). Analysis of DNA extracted from cord blood obtained at 20 weeks gestation showed the fetus had inherited the normal paternal (A-) and affected maternal (A+) alleles. Heterozygosity was confirmed by coagulation studies which showed fetal FVII activity of 8.6% (normal mid gestation range 14-24%). PCR analysis of F7 gene polymorphisms provides a rapid approach to genetic diagnosis of FVII deficiency.

04.20
Lack of evidence for translocations between the FHSD associated locus at 4q35 and the telomere of 10q26
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To look for evidence of exchange between 4q and 10q telomeres harbouring the D4Z4 tandem repeat, deletions of which are associated with facioscapulohumeral dystrophy, we performed Southern blot analysis on 78 control samples, from cystic fibrosis and myotonic dystrophy unaffected spouses. Previous studies have suggested that exchange rates could be as high as 20%. Since 10% of 10q26 fragments fall into the affected range of FSHD (<35 kb) we could expect 2% of control samples to produce false positive results when using standard Southern blot analysis. Results: 137 single digest fragments were derived from 152 control chromosomes, 21% of which were <35kb. On double digest 69 fragments were detected, only one of which was <35 kb. This fragment of 33kb may represent an extension of the normal range. Other evidence to suggest the presence of translocation was sought by looking for trisomy or monosomy in the double digest sample. No cases of trisomy were detected and no consistent loss of 3 or more alleles could be demonstrated. Conclusion: These results do not exclude the possibility of translocation, but produce no evidence to support the hypothesis. Since one allele was 33kb the cutoff of 35kb may still be too high.

04.21
Prenatal diagnosis of chromosome abnormalities in the West Midlands 1988-1998 and the application of FISH technology.
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The database of the Regional Genetics Laboratories in Birmingham comprises a series of 3000 amniocenteses processed from 1988 to May 1998. 942 (3.1%) abnormal karyotypes were observed, of which 502 were common trisomies (21,18 and 13), 77 were sex chromosome aneuploidies and 13 tripeids, all of which would have been detected with FISH probe combinations for 21,18,13, X and Y.12 unbalanced Robertsonian translocations would also have been detected using current FISH probes specific for 13 and 21. Therefore 604 of 942 (64%) abnormalities were detectable by FISH techniques. Of the 338 (36%) abnormalities which would not have been detected by the FISH approach, 34 (3.5%) were other trisomies or mosaic trisomies, 21, 18 or 13 or triploidy; 88 (9.3%) were de novo structural chromosomal abnormalities, comprising 54 unbalanced and 34 balanced rearrangements; 169 (18%) were familial rearrangements, of which 147 were balanced and 22 unbalanced; 32 (3.5%) were other (mosaic or non-mosaic) sex chromosome abnormalities; and 15 (1.6%) rearrangements were of unknown origin. These figures constitute a minimum estimate of 11.6% (109 cases) of abnormalities, or 36% of total referrals, of clinically significant unbalanced chromosome rearrangements which would not have been detected using FISH technology. The incidence of each abnormality is compared with published data.

04.31
A retrospective study of follow-up data on cases of prenatally diagnosed mosaicism.
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We present our data on the confirmation and follow-up of cases of prenatal diagnosis of level 2 and level 3 mosaicism in amniotic fluid cultures referred to this laboratory over a 10 year period. Initial data analysis suggests that 18036 amniotic fluid samples 5.2% had level 1 mosaicism, 0.96% had level 2 mosaicism and 0.3% had level 3 mosaicism. This compares with figures for 2.5-7.0%, 0.7-1.1% and 0.2% respectively, quoted by Gardner & Sutherland,
Prenatal diagnosis for Facioscapulohumeral muscular dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal muscular disorder characterised by weakness and atrophy of the muscles of the face, upper arm and shoulder girdle. Approximately, 10% of FSHD individuals represent new mutations. The FSHD gene has been mapped to 4q35 and the DNA probe p13E-11 (D4F10451) detects rearrangements on EcoR1/Bln1. This has been mapped in the majority of FSHD patients and in most cases the EcoR1/Bln1 fragment size is smaller than 35 kb. Over the last 5 years, we have been able to carry out prenatal diagnosis (PND) for FSHD in 9 pregnancies. These families were referred from genetic centres in Britain and Europe. In the first three families (1993-1995), PND was conducted using only one restriction enzyme (EcoR1) and closely linked 4q35 markers. In the remaining 5 families a differential double digest was used. Four pregnancies were assessed as being of high risk of FSHD. While the presence of genetic heterogeneity observed at FSHD locus, the high level of homology of FSHD candidate region to other parts of genome and the presence of sub-telomeric exchanges between the repeat loci on chromosomes 4q35 and 10q26 complicates the molecular testing for FSHD including prenatal testing, these important issues are counterbalanced by the known high sensitivity and specificity of the differential double digest.

Detection of aneuploidy on uncultured amniocytes using FISH

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FISH on uncultured amniocytes has been performed on 240 amniotic fluids between October 1997 and May 1998 at UCLH Cytogenetics Department. Using the Vysis aneuscreen probe set, uncultured amniocytes were screened for aneuploidy of the sex chromosome and chromosomes 13, 18 and 21. Successful hybridisation occurred in 100% samples. Six amniotic fluids (0.025% of cases) were unsuitable for FISH because of blood contamination or too few cells (1 case). Of the successful cases, abnormalities were detected in 6.2% (15 cases). The majority of abnormalities were trisomy 21 or trisomy 18, but trisomy 13, 15X and 47 XXX were also detected. Two cases gave mosaic results for trisomy 21 and both were non-mosaic trisomy 21 on cultured amniocytes. Two chromosome abnormalities were found on cultured amniocytes not detected using aneuscreen; these involved a familial structural rearrangement and a fetus with two markers and scan anomalies. Maternal cell contamination was evident in 6% of male fetal samples, but this was less than 1% in all but one case. FISH of uncultured amniocytes enables the patient to have a rapid result for specific chromosomal differences with 48 hours. Cost implications and application of FISH for specific categories of referral reasons will be discussed.

Uniparental disomy studies in two cases of trisomy 16 mosaicism detected at amniocentesis

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Two cases of trisomy 16 mosaicism were found during routine cytogenetic analysis of amniotic fluid cultures. Both patients were referred following detection of raised maternal serum alpha fetoprotein (MSAFP) and very high human chorionic gonadotrophin (HCG) levels, giving Down Syndrome risks of 1:10 (case 1) and 1:59 (case 2). In case 1 ultrasound showed early intrauterine growth retardation (IUGR) and a 'cystic placenta'. Trisomy 16 was found in 2 independent cultures in 2 out of 23 cells. Molecular analysis, using 4 chromosome 16 markers, detected uniparental heterodisomy in the amniocyte DNA. The pregnancy was terminated at 23 weeks gestation. Autopsy showed only minor congenital anomalies but the fetus was growth retarded and the placenta was haemorrhagic and infarcted. Cytogenetic analysis confirmed trisomy 16 mosaicism in the placental membranes, but a normal karyotype was found in skin, intestine, lung and kidney. In case 2 ultrasound showed a normal fetus and placenta. Trisomy 16 was found in 2 independent cultures in 3 out of 29 cells. The pregnancy was terminated at 21 weeks. Autopsy showed an absent right adrenal gland and a horseshoe kidney. Uniparental disomy studies and fluorescence in-situ hybridisation studies on various tissues from both fetuses will be presented.

Uniparental disomy: a clinical dilemma

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A forty two year old multigravid lady requested chorionic villus biopsy (CVB) for maternal age. The direct preparation showed a karyotype of 47, XX, +14, but the long term culture showed 47, XX, +14 in 3 cells and 46, XX in 47 cells. The discrepancy between the direct and the long term culture raised the possibility of uniparental disomy (UPD) for chromosome 14 in the euploid cells, and therefore an aneuploidy was performed. This showed a normal female karyotype in all 200 cells and UPD studies revealed biparental inheritance of chromosome 14. Confined placental mosaicism complicates 1% of all CVB results. The presence of mosaicism requires further investigation by either aneuploidy or fetal blood sampling. The finding of a normal karyotype in this situation still raises the possibility of UPD. UPD for chromosome 15, for example, has less implications for the child, but for chromosome 14, these implications are less clear. In this case if UPD for chromosome 14 had been proven, this would present a clinical dilemma in parental counselling. This scenario is just one instance where technology may have outstripped clinical practice.
04.36
False positive results in the diagnosis of trisomy 18 on chorionic villus biopsy
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The objective of this study was to establish the incidence of false positive trisomy 18 results in chorionic villus biopsies (CVB), when analysing both the direct and the long term cultures. This was a retrospective study in the Yorkshire Regional Cytophotogenetics Unit, reviewing all the CVB showing trisomy 18 from 1987 to 1997. There were a total of 3,387 CVB performed; 38 showed trisomy 18. In 33 cases the direct and long term cultures confirmed trisomy 18; in 2 cases the direct result showed a normal karyotype, but the long term culture showed trisomy 18, and in 2 cases there was confined placental mosaicism on the direct, with a normal karyotype on the long term culture. In the final case, the direct analysis showed a balanced 13:14 translocation with trisomy 18, but the long term culture showed no evidence of trisomy 18. There was a false positive rate of 9%, and a false negative rate of 0.06%. Confined placental mosaicism complicates a significant proportion of karyotypes in the diagnosis of trisomy 18. This represents a dilemma in the use and interpretation of the direct analysis of CVB.

04.37
Transcervical retrieval of fetal cells in the first trimester of pregnancy and the results of isolation of fetal cells by density gradient centrifugation and MACS.
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Transcervical retrieval of cells was performed by a method described before (Daryani et al. Prenat. Diagn. 1997; 17: 243-248). Fetal sex was correctly predicted in 85% of cases (40/47) of which 19 were male pregnancies (specificity=85% and sensitivity=78.3%). These cells are mixed with a great number of maternal cells. Therefore in order to isolate the fetal cells an enrichment method may require. To choose an enrichment method of choice the cell contents and viability of 40 CV suspensions, 19 endocervical mucus, and 7 placental biopsies were studied and compared. On average, 90% of the cells in CV suspensions were RBCs compared to 50% in CW mucus and 40% in CVS samples. This shows that RBCs are the major cell component of samples obtained from transcervical washings. There were far more squamous cells in the CW suspension (5.4 times) than in the endocervical mucus. No association was found between the number of nucleated cells and RBCs in all three different samples. If the higher number of RBCs in the samples was an indicator of more invasiveness, this indicates that more invasiveness will not necessarily increase the number of nucleated cells. For enrichment, a single density gradient cell centrifuge above 1.063 was used to isolate trophoblastic cells. Fetal sex was correctly predicted in all female pregnancies but only 2 of the 9 male fetuses. A magnetic cell sorter (MACS) was also used to deplete the unwanted cells. HLA w6/32 and CD45 was used for this purpose. Only depletion by w6/32 showed 100% correct fetal sex prediction (3 male and 6 female pregnancies). However many cells were lost during the process. The findings show that cells of fetal origin are indeed present in the delivery canal in the first trimester of pregnancy. However the right method for enrichment is yet to be identified since the cells of interest may also be lost during enrichment.