The examination of embryonic and fetal material in diagnostic histopathology laboratories

COLIN L BERRY
From the Department of Morbid Anatomy, The London Hospital Medical College, London E1 1BB, UK

Introduction
In many histopathology laboratories the amount of time and attention spent on assessing products of conception is derisory, often comprising no more than the examination of a single block to establish that pregnancy has occurred. This contrasts with the examination of putatively non-malignant lesions of the breast from which several blocks may be taken, sometimes with x-ray assistance, and where, if certain epithelial changes are present, step sections are frequently cut. The probability of finding a lesion of prognostic importance in the breast is extremely low. The probability of recurrence of a defect of the central nervous system or cardiovascular system or of a skeletal abnormality in an embryo or fetus exceeds 5% in non-assortative matings; for abnormalities associated with single genes of large effect the probability will be much higher. Much clinically valuable information is thus not gathered, and genetic counselling or antenatal investigation may not be carried out where it is necessary. If we assume that more than 10% of aborted embryos will be abnormal (see below), it is salutary to examine pathological records and to see how frequently such anomalies are recorded.

What may usefully be done in a routine laboratory? Serial sections of early embryos are clearly not practicable, nor are they likely to be helpful since they would present problems in interpretation for pathologists without an embryological interest. I believe that such material should be studied in detail, and that it forms a valuable potential link between departments of anatomy and morbid anatomy, but it is clearly not a task for a consultant with a general service to provide. Towards the other end of the gestational period, larger fetuses—say, greater than 200 mm crown-rump length—can be examined in the same way as stillbirths or neonatal deaths, and the present review will not deal with this aspect of the problem. It is the intermediate stages, fetuses of 30-200 mm crown-rump length (say, 8 weeks' to 24 weeks' gestation), that can provide much valuable information from relatively little extra work.

![Fig. 1 Causes of embryonic and fetal loss in early pregnancy.](image-url)
Background data

The majority of potential human fertilisations do not result in the production of a fetus. Figure 1, after Witschi, indicates where much of this reproductive loss occurs. From a number of studies it appears that about one-quarter of ova either fail to become fertilised or do not achieve the blastula stage. Failure of implantation occurs in a further 20% or so, and thus at 14 days after exposure of eggs to sperm almost half of the loss has occurred. Much of this reproductive failure is due to major chromosomal anomalies, including absence of any one autosome and some trisomic states.

In the next 28 days a further 10% or so of embryos are lost. Many of these are chromosomally abnormal, and some have major malformations. Subsequently there is little change in the number of conceptions going to term.

It can be seen that the 'normal' process of development is, in fact, exceptional. Errors are common and of varying type. The loss of an entire autosome obviously results in loss of too much genetic information to be tolerable. Massive failures of morphogenesis will also result in embryonic death; at its most obvious this may be expected when an amniote embryo fails to develop an effective circulation. Acardiac embryos develop only in the presence of a twin whose circulation perfuses the developing placenta effectively. Other reasons for spontaneous termination of pregnancy are less clear. Table 1

<table>
<thead>
<tr>
<th>Malformation</th>
<th>Prevalence per 1000</th>
<th>% Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural tube</td>
<td>13·1</td>
<td>1·0</td>
</tr>
<tr>
<td>Cleft lip and palate</td>
<td>24·4</td>
<td>2·7</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>9·0</td>
<td>0·9</td>
</tr>
<tr>
<td>Cyclopia and cebophage</td>
<td>6·2</td>
<td>0·1</td>
</tr>
</tbody>
</table>

shows the prevalence of certain defects in abortions and at birth. Although death in early intrauterine life might be expected in massive neuropinal dysraphism, it is not clear why so many embryos or fetuses with polydactyly or cleft lip and palate are lost unless other unsuspected abnormalities are present. In the same way, the Turner phenotype is obviously compatible with extrauterine life, but losses of embryos of this genotype are severe and probably exceed 90%.

Human malformations, defined as 'macroscopic abnormalities of structures attributable to faulty development and present at birth', occur at a frequency of around 25 per 1000 births in most large studies. These defects are clearly successful anomalies in terms of survival but represent a small percentage of abnormal conceptions. How small is uncertain, and better pathological studies are necessary to clarify this point.

Examination of aborted embryos

History

Historical details are as important in this field as they are with any other surgical specimen. Maternal age materially affects the probability of abnormality; menstrual dates are a valuable aid to dating. Drug ingestion (Table 2) or coincidental illness, for example, rubella, are clearly important in teratology and are discussed more extensively elsewhere. The previous reproductive history is also valuable, as is the state of health of other children or a family history of congenital or metabolic defects. If alpha-fetoprotein determinations have been made this should be stated; surprisingly, this information is not always given. Finally, the method used to induce abortion should also be stated.

Measurements

The purpose of measurements is to assist in checking gestational ages.

Table 2 Agents known to be teratogenic in man*†

<table>
<thead>
<tr>
<th>Agent</th>
<th>Reference</th>
<th>Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td></td>
<td>Microcephaly, mental retardation</td>
</tr>
<tr>
<td>Folic acid antagonists</td>
<td>Murphy*</td>
<td>Growth retardation, anencephaly, hydrocephaly, cleft lip and palate, skull defects</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Lenz and Knapp*</td>
<td>Limb reduction and cardiovascular abnormalities</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Becker et al.*</td>
<td>Nasal and facial abnormalities, stippled epiphyses</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Lemoine et al.*</td>
<td>Abnormal facies, microcephaly, oblique palpebral fissures, pre- and post-natal growth retardation</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>Collins and Turner*</td>
<td>Cleft lip and palate and congenital heart disease, minor skeletal abnormalities</td>
</tr>
<tr>
<td>Methymercury</td>
<td>Amin-Zaki et al.*</td>
<td>Blindness, deafness, hyperactive reflexes</td>
</tr>
</tbody>
</table>

*Initial reports or those which contain data useful to the pathologist are cited here. Facies in the fetal alcohol syndrome are well illustrated in the paper of Collins and Turner.

†Testosterone and synthetic agents with progestational effects may cause virilisation of female fetuses.
LINEAR DIMENSIONS

Measurements of the crown-rump or crown-heel length are not easy to make. Just as it has been realised that measurement of growth in children requires specialist techniques, so the work of Bagnall and his colleagues\textsuperscript{18} has illustrated the importance of techniques in fetal measurement. In practice, most pathologists are not able to use a carefully standardised procedure to measure fetuses, and the degree of flexure of the spine and limbs is difficult to control. Crown-heel length is also variably affected by fixation.

For these reasons, measurements of foot length are to be preferred (Fig. 2). Foot length correlated well with crown-rump length and is not significantly affected by fixation. In instances where the fetus is fragmented by abortion the feet generally remain intact. In certain circumstances where malformation affects the external form of the fetus, foot length is the only practicable measurement, and it is normal (in terms of length/gestational age) in anencephaly.\textsuperscript{18}

WEIGHT

The weight of a fetus is prone to alter, depending on the method of abortion used, the type of fixation, or the delays in delivery to the laboratory. We have not found it to be useful unless fresh specimens can be obtained. However, there is valuable information to be obtained from fresh material. Golbus\textsuperscript{16} has illustrated the growth deficiency occurring with trisomic states, in particular, with trisomy 13 and 18, which is evident in the second trimester. Trisomy 21 fetuses apparently grow normally, as Kučera and Doležalová\textsuperscript{17,18} have found. Nevertheless, the wide range of organ weights and other measurements found in the study of Golbus and Berry\textsuperscript{19}, who examined 133 fresh fetuses between 90 and 170 postmenses, emphasises the lack of value of this type of measurement for pathologists examining only a few cases a year.

EXTERNAL EXAMINATION

External examination may reveal many defects. Useful points to note in dating the fetus include whether the eyelids are separated (25-28 weeks) and, using a dissecting microscope, whether the fingerprints are present (10 weeks). I know of no case where the latter have been reported as abnormal in an early fetus, but there is no reason why abnormalities should not be recognised. It is also convenient to examine the palate at this time and to pass a probe into the nose to check the patency of the posterior nares.

Abnormal facies may be diagnostic (Fig. 3) or merely alert the pathologist to the possibility of internal abnormality (Fig. 4a, b).

It is then customary for us to x-ray the fetus using a Faxitron cabinet. This simple device permits rapid, within-department, x-ray pictures to be provided promptly (a Polaroid attachment is available) and will identify bony anomalies (Figs 5 and 6) which are better examined by further x-ray after removal of the viscera.

INTERNAL EXAMINATION

For all fetuses over 100 mm crown-rump length it is my view that a mininicrospy is the best procedure. However, the use of a modified Rokitansky technique is desirable, leaving the kidneys, ureters, and bladder in situ but removing all other viscera en bloc after examining the reflections of the mesentery. This block can then be examined using the dissecting microscope when necessary (Fig. 7).

For smaller fetuses, a modification of the Wilson\textsuperscript{20} free-hand-sectioning method, used extensively on

---

**Fig. 2** Foot length and crown-rump length. Data from Streeter\textsuperscript{13} and Trolle.\textsuperscript{14}

**Fig. 3** Facies in a case of Potter's syndrome (near term).
Fig. 4a
Fig. 4  (a) Facies of fetus with trisomy 16p. (b) Fallot’s tetralogy from the same fetus. Termination at 17 weeks (courtesy of Dr M Becker).

Fig. 4b

Fig. 5  Cerebral anomalies in anencephalic fetus.

Fig. 6  Narrowed chest with distention of abdomen in asphyxiation thoracic dystrophy (courtesy of Dr A Bain).
The examination of embryonic and fetal material in diagnostic histopathology laboratories

rodents in teratological studies, should be employed. Here the trunk of the embryo is cut into slices of approximately 1 cm thickness, and these are examined by dissecting microscopy. This technique is simple and thorough (Fig. 8a, b).

After removal of the viscera the skeleton is again x-rayed. This gives a good picture of the axial skeleton which, apart from specific abnormalities, provides useful information on dating. It is my view that the variability of the time of appearance of other primary centres of ossification makes them of dubious value in this type of exercise. However, vertebral body centres appear in a fixed sequence and with less variability (Fig. 9).

Where any tissue appears abnormal or any organ large or small, a block should be taken (Fig. 10).

Abnormalities of specific systems
When macroscopic anomalies are found the standard technique is often abandoned.

Central nervous system anomalies
In general, these should not be dissected as wet specimens. The cerebrospinal axis may be preserved as a unit and cut in large sections or transversely in a number of blocks (see Fig. 11). Recent reports have emphasised the value of histopathological examination of this type of specimen, which has rarely been performed. Insights into or alternative suggestions for pathogenesis may follow accurate documentation of the changes found. It must be remembered that examination of neurospinal malformations at term will illustrate the effects of injury or abnormality followed by up to eight months of attempted repair and further growth and will then yield little information about the pathogenesis.

Cardiovascular system
Even without an extensive knowledge of the vastly complex field of congenital heart disease a useful assessment of anomalies can be made if the system of Tynan and his colleagues is used. The key to their approach is a 'segmental' description summarised in Table 3. If the heart is classified in this way a description may be of considerable value to those expert in the field or for later checking, in a topic where photography is difficult and where the heart may not always be kept. The scheme shown is greatly simplified. It omits comments on ventricular details and the morphology of outflow tracts but still provides a basis for rational description.

Fig. 7 Horse-shoe kidney in situ.

Fig. 8 (a, b) Slices through lower chest and upper abdomen. Examination of these slices from above and below using a dissecting microscope is an effective way of studying small embryos.
Table 3  *Segmental approach to congenital heart disease*

<table>
<thead>
<tr>
<th>Activity</th>
<th>What to look at</th>
<th>Possible answers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Define atrial situs</td>
<td>Almost always matches visceral situs. If not, remember longest bronchus serves left lung</td>
<td>Situs solitus (normal) Situs inversus Situs ambiguous</td>
</tr>
<tr>
<td>2  Description of atrioventricular junction</td>
<td>If 2 ventricles then 2 atria</td>
<td>May be concordant, i.e., R to R, L to L May be discordant May be ambiguous when atria cannot be defined</td>
</tr>
<tr>
<td>(i) Atrioventricular connections</td>
<td>When both atria connect to single ventricular chamber When one atrium does not communicate with ventricle</td>
<td>Double inlet ventricle</td>
</tr>
<tr>
<td>(ii) Mode of connection</td>
<td>Check anatomy of valve annuli If any rudiments of valve present If no valve</td>
<td>Tricuspid Mitral Right or left better terms Common Imperforate atrioventricular valve Absent L/R atrioventricular connection Right</td>
</tr>
<tr>
<td>(iii) Ventricular morphology</td>
<td>Trabecular pattern (coarse on right, fine on left, with smooth septal area) Rudimentary chambers may be present (see: Tynan et al.)</td>
<td>To right, left, superior, inferior, anterior, posterior Concordant Discordant Double outlet</td>
</tr>
<tr>
<td>(iv) Relation of ventricular chambers</td>
<td>Right ventricles are described relative to left</td>
<td></td>
</tr>
<tr>
<td>3  Ventriculo-atrial junction</td>
<td>Aorta from left Pulmonary artery from right More than half of both the aorta and pulmonary artery arise from chamber. One great artery</td>
<td>Single outlet to: (i) aorta (ii) pulmonary artery (iii) truncus arteriosus</td>
</tr>
<tr>
<td>(i) Arterial connections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii) Arterial relations</td>
<td>Define position of valves in anteroposterior and lateral planes Venous drainage, septal defects, etc.</td>
<td></td>
</tr>
<tr>
<td>4  Describe additional cardiovascular abnormalities</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The examination of embryonic and fetal material in diagnostic histopathology laboratories

Musculoskeletal abnormalities
These may be well defined by x-ray, but valuable data can be obtained by dissection. For example, extra digits can be defined by checking muscle insertions to see whether a 'thumb' is really a 'finger'. Abnormalities of this kind are ill defined but may also occur in association with limb teratogens.24

Urogenital system
After removal of the other viscera, the kidneys and ureters should be freed from the posterior abdominal wall. The pubes are then split and the pelvic viscera removed. Dissection, or fine probing, will then reveal where aberrant ureters drain or whether fistulae exist. Fine polythene catheters, heat-sealed at their ends, make good probes.

Morphological disturbances in chromosomal anomalies: Placenta
Even without an intact fetus some changes can be recognised. Triploidy is associated with changes in the chorionic sac, varying from mild changes in the villi to severe hydatidiform change. Although most triploid embryos are lost spontaneously, collected or therapeutically aborted material may show the changes described above and the presence of large, atypical cells in the villous stroma which may be of value diagnostically (see Ornoy et al.25). Interestingly, triploid cell lines are always associated with an embryo or fetus where cystic disease of the villi has been found, and triploidy is not associated with hyperplastic changes in the trophoblast.26

In a study of spontaneous human abortuses with normal and abnormal karyotypes, Honoré et al.27 were able to correlate placental morphology and the karyotype of the conceptus. Heteroploid abortuses could be differentiated from the diploid. Diploid placentae showed structural uniformity despite zonal differences, evidence of growth, and a coordinated development of trophoblastic epithelium and stroma. Secondary degenerative changes were seen superimposed on this pattern, and their severity depended on the length of time after intrauterine death that the conceptus had been retained. Heteroploid placentae showed disturbed morphology and failure of growth. Cystic villi were common, but

Fig. 10 Cystic change in bile ducts in enlarged liver from case of trisomy 18 (courtesy of Dr JW Keeling). Haematoxylin and eosin × 200.
extensive cyst formation excluded monosomy X and tetraploidy; it was common in triploidy and trisomy. The characteristics described by these authors for various abnormalities are shown in Table 4.

**Metabolic abnormalities**

Prenatal diagnoses are usually made in selected groups. These include women over 35 years who are screened for chromosomal abnormalities and women who have previously given birth to an infant affected by a malformation, for example, neurospinal dysraphism. Metabolic disorders are usually sought only after an affected child has been diagnosed, which emphasises the value of examining abortion material. It is clearly absurd to suggest, however, that all abortions are examined for possible metabolic diseases, so the role of the pathologist is to look out for abnormalities that might be associated with metabolic disease. Foam cells in the placenta or central nervous system suggest lipidosis; some of the skeletal anomalies of the mucopolysaccharidoses are recognisable in fetal material, as are some features of the glycogenoses. Although 17 lipidoses, 11 mucopolysaccharidoses, 22 aminoacidurias or related disorders, 11 defects of carbohydrate metabolism, and 26 miscellaneous disorders have been diagnosed antenatally; a further 7, 7, 17, 8, and 15 disorders are potentially diagnosable in each category, respectively (see Milunsky28).

Alerting a clinical colleague to the possibility of

Table 4 *Changes in the placenta in heteroploid states*

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Placental change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy</td>
<td>Medium size, cystic villi with smooth outline.</td>
</tr>
<tr>
<td></td>
<td>Few trophoblastic buds. Intravillous cystotrophoblastic cells. Hypoplastic trophoblast</td>
</tr>
<tr>
<td></td>
<td>on hypovascular villi</td>
</tr>
<tr>
<td>Triploidy</td>
<td>Diffusely macrocystic (submolar) pattern or, more commonly, some large cysts and some avascular villi. Stromal cells randomly arranged in villi</td>
</tr>
<tr>
<td>Tetraploidy</td>
<td>Decidual and intraplacental haemorrhage common. Hydroptic villi mixed with hypovascular compact villi. Many large immature stromal cells, some hyperchromatic.</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>Grossly hypoplastic. All villi small and hypervascular. Poor trophoblastic growth</td>
</tr>
</tbody>
</table>

Fig. 11 Spinal cord above meningocele. An abnormal central canal can be seen; in lower sections this opens dorsally. Trichrome × 40.
the presence of such a disorder is of major importance in preventive terms.

Conclusions

It is only by the examination of fetal material that we can assess the true frequency and perhaps study the pathogenesis of human congenital defects. Observations on this material may also be valuable in other ways. Busulphan therapy is usually well tolerated in pregnant patients with chronic myeloid leukaemia; 12 patients have delivered normal infants on treatment. The report of myeloschisis in a six-week embryo of a similarly treated woman is thus put in perspective, and the high maternal age noted in this clear report assumes significance. In this way the additive environmental effects which are superimposed upon genetic predisposition to malformation may be identified in susceptible groups. The older woman is more likely to produce abnormal embryos and may thus be more susceptible to weak teratogens. Large-scale reporting of embryonic and fetal data from man will be more valuable than most animal studies.

References

4 Thiersch JB. Therapeutic abortions with a folic acid antagonist, 4-aminopteroylglutamic acid (4-aminopGA) administered by the oral route. Am J Obstet Gynecol 1952;63:1298-304.
27 Honoré LH, Dill FJ, Poland BJ. Placental morphology in spontaneous human abortuses with normal and


**Suggested reading**


Requests for reprints to: Professor CL Berry, Department of Morbid Anatomy, The London Hospital, London E1 1BB, UK.

---

**The March 1980 Issue**

**THE MARCH 1980 ISSUE CONTAINS THE FOLLOWING PAPERS**

The rôle of *Chlamydia trachomatis* in genital-tract and associated diseases. D TAYLOR-ROBINSON AND BJ THOMAS

An evaluation of commercial radioisotope methods for the determination of folate and vitamin B12. DW DAWSON, IW DELAMORE, DI FISH, TA FLAHERTY, AH GOWENLOCK, LINDA P HUNT, K HYDE, JE MACIVER, JANET A THORNTON, AND HM WATERS

Interlaboratory comparison of serum vitamin B12 assay. DL MOLLIN, AV HOFFBRAND, PG WARD, AND SM LEWIS

Collaborative study to recalibrate the International Reference Preparation of Anti-D Immunoglobulin. HH GUNSON, PJ BOWELL, AND TBL KIRKWOOD

Measurement in jejunal biopsies by computer-aided microscopy. GERARD SLAVIN, CHRISTOPHER SOWTER, KENNETH ROBERTSON, SUSAN MCDERMOTT, AND KEITH PATON

Microscopic amyloid deposits in the heart valves: a common local complication of chronic damage and scarring. Y GOFFIN

Acute phase proteins, C9, factor B, and lysozyme in recurrent oral ulceration and Behçet’s syndrome. THOMAS LEHNER AND MATTEO ADINOLFI

Letters to the Editor

Book reviews

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, price £3.00, including postage.
The examination of embryonic and fetal material in diagnostic histopathology laboratories.

C L Berry

doi: 10.1136/jcp.33.4.317

Updated information and services can be found at:
http://jcp.bmj.com/content/33/4/317.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/