CYTOGENETICS OF ABORTERS AND ABORTUSES: A REVIEW

Puteri Jamilatul Noor
Khalid Hassan

Department of Hematology
Institute for Medical Research
Jalan Pahang
Kuala Lumpur
Puteri Jamilatul Noor, B Sc Hons (Mal)
Khalid Hassan, MBBS (S'pore), MRCP (UK), MRC Path, DCP (Lond), DTM & H (B'kok)

SYNOPSIS

The frequency and diversity of chromosome abnormalities make spontaneous abortions an ideal source of material for many types of cytogenetic research. This review concentrates on the more common genetic aspects. Approximately 50 per cent of spontaneous abortuses are chromosomally abnormal. These include autosomal trisomy, monosomy X, triploidy, tetraploidy, marker and ring chromosomes. Studies have also shown that translocation chromosomes carried by aborters are transmitted to their abortuses. There is no difference in reproductive fitness, whether the carrier is male or female.

INTRODUCTION

The explusion of the fetus before the 28th week is known as abortion or miscarriage (1). It is impossible to give an accurate figure of the frequency of abortion because many patients who abort do not seek medical advice. Many abortions occurring early in pregnancy are not diagnosed, because the episode is regarded by the patient as a delayed or abnormal menstrual period and a doctor is not consulted. Also, a patient may conceal the fact that she has had an abortion.

It is generally accepted that about 15% of all recognised pregnancies terminate in spontaneous abortion (2). The proportion of abortions to full time labours is higher in large cities than in rural districts. It is difficult to assess the frequency with which abortion is induced criminally, but in Britain, in spite of the permissive terms of the 1967 Abortion Act and the large number of therapeutic abortions the figure is high, particularly in the large cities. This type of abortion has the highest morbidity and mortality. Local figures are not available. The law is much more stringent here but experience tends to suggest that criminally induced abortion is not uncommon. This review will concentrate on the genetic aspects of the commoner spontaneous abortions.

Spontaneous abortions occur most commonly between the 8th and 13th weeks of gestation. However, doubtless many early cases are unrecognised especially those occurring after only a short period of amenorrhoea. In at least 50 percent of cases a definite cause cannot be determined; even when an association is recognised its mechanism of action is frequently obscure, so that any attempt at classification of the causes of abortion will be theoretical and arbitrary. The causes include gross malformation of the ovum, general disease of the mother, uterine abnormalities, hormonal insufficiency, stimulation of uterine contractions, trauma, fetal death from drugs or irradiation. The causes of abortion can also be considered as fetal or maternal. The commonest cause of spontaneous abortion in early pregnancy is an abnormality of the fetus or chorion which is severe enough to cause death. This may sometimes be shown to be due to a chance chromosomal abnormality, for which either parent may be responsible. It is in most cases a non-recurrent factor and the patient has a 90 percent or greater hope of success in a subsequent pregnancy. If a patient has miscarried once, it is likely that her next pregnancy will be uneventful; only when there has been a sequence of repeated abortions each of the same pattern will the prognosis be bad.

Spontaneous abortion is one of the most common and least understood pathological processes. Because it is so difficult to obtain reliable information about abortion, even the basic facts about its frequency, familial distribution, and relation to parity and parental age are largely unknown or disputable. Such information is needed to provide a basis for advising women with several abortions about their chances of successfully completing another pregnancy, to evaluate measures for the prevention of abortion as well as to provide clues to the aeteology of the condition. Reproductive histories taken by personal interview with a random series of women would probably be the best available data for estimating spontaneous abortion statistics. These should include very early terminations of pregnancy, recognised as spontaneous abortions by the women concerned but not receiving medical attention, which are under represented in any series of consecutive hospital admissions or in consecutive cases from private obstetrical practice. They would allow estimation of abortion risks in women with given numbers of previous abortions and at given ages. Some reservations must be held about the validity of self-diagnosis of early abortion, but this source of error should not affect comparisons within samples.

In general all uterine pregnancies stated by the mother to have terminated at less than 28 weeks gestation are considered abortion. However Javert (3) has suggested that abortion should be defined as a termination of pregnancy before 22 weeks. Difficulties arise from disagreement about when viability is achieved. Twenty eight weeks proved to be a more satisfactory dividing line, since no child reported to be born at less than 28 weeks gestation survived more than a few hours, while several reported to be born at 28 weeks have been known to survive through infancy. Children born alive at 28 weeks to 32 weeks gestation are considered premature, and children born dead at 28 weeks or later are considered stillbirths.

Several studies (4–12) have shown that a high percentage of the abortuses are chromosomally abnormal. The actual frequency of abnormalities is still disputed but if correction is made for selection factors, the figure is close to 50% (13). The estimated incidence of spontaneous abortion varies widely from series to series. Those based on hospital records are invariably low as so many spontaneous abortions occur at home.

In the past few years, cytogenetic studies of human spontaneous abortuses have clearly shown the important role of chromosome anomalies. Great advances in human cytogenetics in recent years are largely due to the discovery of several techniques permitting accurate recognition of each chromosome and its parts. These have included flourescent staining with quanacrine (14), differential staining of repetitive heterochromatin (15) and various methods for producing typical banding patterns with giemsa stain (16–19). The application of these techniques has already led to more accurate identification of several chromosomal abnormalities (20). By means of a modification of one of these techniques based upon the treatment of the fixed chromosomes with trypsin (21), Hirshhorn and colleagues (22) have studied a variety of patients and their families, and have succeeded in identifying precisely a number of different types of chromosomal anomaly.

Human cytogenetic studies before 1970 could only regularly identify chromosomes 1, 2, 3, 16 and 17; the others were classified into groups on the basis of length and centromere position. With the introduction of banding techniques of various kinds, it became possible to identify all the chromosomes in the complement (14) and therefore to accurately detect other chromosomal anomalies and in some instance also their origin which cannot be recognised by standard techniques.

MATERIALS AND METHOD

For most studies on mitotic chromosomes the cytogeneticist requires chromosomes at the metaphase stage, which necessitates the provision of large number of cells in active division. In higher animals mitotic metaphase chromosomes can be prepared either directly from tissues which undergo rapid division *in vivo*, such as cells from the bone marrow or spleen, or from tissues which have been grown and stimulated to divide rapidly in *in vivo* culture.

There have been a number of cytogenetic studies of abortuses (4–13). The specimens selected for study are either abnormal in appearance, consist of incomplete material in which it appeared, from the size of the chorionic sac, that gestation has not progressed beyond 12 weeks, or from women who have had two or more previous spontaneous abortions (miscarriages). Tissues are removed for culture and this usually consist of chorionic or amniotic membrane. Both are embryonic in origin and are much more likely to be viable than tissue from the embryo itself. The latter has often been dead for some weeks, may be very fragmented and is frequently completely absent. In the case of larger fetuses, peritoneum, muscle sheath, and

dura mater are the most suitable sites for tissue sampling.

Advances in tissue culture techniques were supplemented by improved methods for spreading chromosomes. In particular, the use of colchicine to increase the number of cells at metaphase and contract the chromosomes, the use of hypotonic solutions to induce swellings of the cells before fixation, and the introduction of the air-dry spreading technique which greatly facilitated the counting and karyotyping of the large chromosome complements of mammals. This resulted in a dramatic increase in studies on mammalian chromosomes and the demonstration of karyotypes from a wide range of mammals including man.

Stains or dyes used for observing chromosomes and cell structure under the microscope include Feulgen, Orcein, Garmine and Giemsa. Fluochromes such as acridine orange and quinacrine compound render the chromosomes fluorescent under the ultra-violet light. Fluorescence microscopy is a useful technique under some circumstances.

For detailed karyotype studies suitable photomicrographs must be made. Adequate photographic recording is, therefore, more critical with this than with previously available methods of chromosome identification. The detail which can be recorded is determined by the definition and tonal characteristics of the film and the resolving powers of the optical system used.

The chromosomes are mounted in a conventional karyotype. The 22 pairs of autosomes are arranged in descending order of size and in seven groups given in the letters A to G. The grouping is primarily based upon the position of the centromere. The sex chromosomes (gonosomes) are XX in the female and resemble the group C autosomes. The Y chromosome in the male is acrocentric, usually shows characteristic features, and is of variable length.

In addition to chromosome preparations the sex chromatin is studied by some investigators in interphase nuclei either in cultured cells or in a whole mount of amnion, or both. For sex chromatin study the cells are fixed in acetic acid-alcohol mixture and stained.

CHROMOSOME ANOMALIES

Chromosome anomalies which are incompatible with live birth might be associated with intrauterine death and abortion. Penrose and Delhanty (22) were the first to report on a spontaneously aborted fetus with an abnormal karyotype. Since then quite a large number of studies on the cytogenetics of spontaneous abortion have been performed (4–13).

It has been known for many years that a high percentage of embryos and fetuses from spontaneous abortions show structural or numerical abnormalities. Authors vary in their percentage estimates (4–13, 20–75). This is partly due to differences in the ascertainment of cases of study or to different success rates in the culturing of cells from abortuses. It may however also be an expresion of dissimilarities in the genetic structure of the different populations, or of differences in environmental factors.

The earlier the abortion the more likely it is that the conceptus will have a chromosomal abnormality. In spontaneous abortions of the first trimester of pregnancy, the proportion of abortuses with chromosome anomalies ranges from 40 to 65 percent depending on the study (23, 24).Boue and colleagues (25) in a prospective study clearly confirms that abortions occur most frequently during the first 3 months of gestation; in a total of 266 conceptions in which 22 percent aborted, only 1 percent occurred after the third month of pregnancy. In most cases whether a chromosome anomaly results in an abortion or carried through the pregnancy is a matter of degree of viability of the fetus *in utero*. Some unique chromosomal defects may never be found in conceptuses simply because of their lethality in the gametic stage.

Trisomy

On reviewing several large series of chromosomal analysis in spontaneous abortions (4–13, 20–75) it is evident that autosomal trisomies form the most important group of abnormalities detected in the early aborted conceptus. Trisomy is known to be strongly associated with spontaneous abortion. The extra chromosome element is a normal member of the complement, other than Y chromosome, and its presence results in one chromosome being represented three times instead of twice. At least one example of an extra chromosome has been found for each of the seven groups of autosomes. The incidence of the trisomies in different groups varied widely. Creasy and associates (26) in 941 cases found autosomal trisomies to be present in 49.8 percent of conceptuses. When they analysed this group together with those reported in the literature where banding was performed, they reported that among 376 aborted trisomies, trisomy 16 was the most common, accounting for 32 percent. Extra chromosomes 8, 9, 10 and 20 accounted for 2 to 3 percent and chromosomes 7, 3 and 6 approximately 1 percent each.

The frequency of the trisomies by group appeared roughly related to the size of chromosomes making up the group. Trisomies in groups A, B and C were less common than expected on the basis of chance. Whole trisomies in groups D, E and G were more common (27). Group F trisomies were the sole exception in being less common than randomly expected, even though the two chromosome pairs are the second smallest in the complement.

Trisomy 1 is the only one which has not been described. Trisomy X is compatible with normal development and is rarely found in abortuses. Trisomies for chromosomes 13, 18, 21 and X are sufficiently common in liveborn humans for frequencies to be established.

Kajii and colleagues (28), Boue & Boue (25), Lauritsen (29) and Hassold and colleagues (30) suggested that autosomal trisomy mosaicism is rare among spontaneous abortuses because studies with chromosome heteromorphisms proved that the abortuses started as a trisomic zygote and lost one of the trisomic set at the mitotic division, thus giving a diploid cell line. Therefore it is not certain whether the diploid cell line was present *in vivo* or arose *in vitro*.

A family with trisomy 21 mosaicism in 2 successive generations and a Down's child in the third generation has been presented by Werner and colleagues (31). Out of 8 individuals in the family some were found to have marker chromosome 15 ph+ and heteromorphic chromosome 18. The proband was derived from trisomy 21 oogonium by secondary non-disjunction in the mother. It has been known that mosaics for Down's syndrome can develop either from a normal zygote or a trisomy 21 zygote in which mitotic nondisjunction occurs during cleavage division.

An abortus with a 49 XX +2 +5 +8 karyotype reported by Kajii and colleagues (28) is the only example of a triple trisomy.

Studies by Hassold (32) on 40 couples with repeated abortion found that there is a high correlation between the chromosome constitution of the first and second abortions. In each of 21 instances in which the first abortion was chromosomally normal, the subsequent abortions were normal as well. In nine cases the two abortions were chromosomally abnormal and in four of these both abortions were trisomic.

Certain couples are at an increased risk for either repeated chromosomally normal abortions or for repeated trisomic conceptions. The increased risk of trisomy does not seem to be restricted to a particular chromosome and the magnitude of the risk increase appears to be independent of maternal age. The increased likelihood of a spontaneous abortion being trisomic given a previous trisomic abortion cannot be entirely accounted for by increasing maternal age (32)

Most of the cytological information of the origin of human trisomies has come from studies of trisomy 21 in the liveborn population. Maternal first-meiotic division errors are also apparently the most common mechanism leading to liveborn trisomy 21. Carr (33) found that almost 40 percent of all spontaneous abortuses are trisomic for chromosomes that are frequently heteromorphic and associated with increasing maternal age (trisomies 13, 14, 15, 21 and 22) and maternal age independent (trisomies 4, 9, & 16).

Monosomy

The second most common chromosomal anomaly found in the conceptus of an early abortion is monosomy X, i.e. the absence of one sex chromosome. This is the only monosomy which is very common in man. It appears to be true of all studied species that the absence of a chromosome is more damaging to development than an extra element. Although this anomaly is comptible with livebirth, it can be calculated that over 98% of 45 X conceptuses are miscarried, according to Carr (33). This is the same chromosome anomaly as is found in the majority of cases of Turner's Syndrome with gonadal dysgenesis. Some of the 45 X conceptuses result from anaphase lag either during meiosis or mitosis. Anaphase lag is more frequent among young couples (34).

Triploidy

Another condition known as polyploidy is also associated with chromosome anomalies in spontaneous abortions (35). If there are 3 instead of 2 chromosomal sets resulting in a chromosome count of 69 this anomaly is called triploidy. Triploidy occurs in at least 1% of all conceptions in man (36, 37). It is usually lethal during the first three months of intrauterine life although it has been found in a number of viable infants. The longest survival in a case of full triploidy was two months (38).

Three possible sex chromosome complexes in this disorder include XXY, XXX and XYY and examples of each have been encounted (39–41, 46). All studies have shown that 69, XXY is about twice as common as 69, XXX. The 69 XYY triploids are rare, making up less than 5% of the total. The extra chromosome complement in human triploids may come from either parent.

Flourescence marker chromosome analysis in the study of the meiotic mechanisms leading to triploidy was applied for the first time by Uchida & Lin (42) and Jonasson and colleagues (43). Triploidy could arise from non-reduction during the first meiotic division of the father or from dispermy, and from non-reduction during the first meiotic division of the mother respectively. Failure in the 1st meiotic division of the mother might be a much more common cause of triploidy.

The origin of human triploidy was discussed by Neibuhr (44). Using a maximum likelihood analysis, Jacobs & Morton (45) and Beatty (46) derived models for the distribution of chromosome constitutions in triploids, and in this way estimated the frequencies of maternal and paternal origin as well as the frequencies of occurrence of some of the failures leading to triploidy. Thus digyny (maternal origin) was estimated to account for from 7 to 41% of all triploids and diandry (paternal irigin) for 59–93% of all triploids. The estimate of diplospermy (non-reduction during the first or second meiotic division) ranges from zero up to 20% of all triploids (46).

Lauritsen and colleagues (47) in the cases of their series concluded that the extra haploid chromosome set was maternally derived in 42% and paternally derived in 58%. Dispermy and failure in the maternal first meiotic division were the dominant causes. No failures in the second meiotic division were found.

Tetraploidy

Although doubling of the chromosome number or tetraploidy is well known in plants and insects, it is not known to occur in living mammals. It appears to be associated with abortion in man and its occurrence has been described by many workers. Tetraploidy in man is usually so damaging to the conceptus that the embryo is only a stunted, irregular mass of cells. Frequently an embryo is completely lacking and the conceptus consists of an empty chorionic sac. The sex chromosomes are about equally distributed between XXXX and XXYY. This would be expected as tetraploid conceptuses are believed to arise from normal diploid ova in which the chromosomes divide at the first mitotic division after fertilization, but cleavage fails to occur (48).

Translocation

The most common chromosomal abnormality detected in parents with repetitive/habitual abortion is translocation (49–59). Among these D/D translocation accounts for about 50% of anomalies (49, 60, 61). Studies have shown that translocation chromosomes carried by aborters are transmitted to their abortuses (62–64). In early investigations abnormalities in these parents were reported which now seemed to be normal polymorphism.

Stenchever and colleagues (61) studied 28 couples with a history of spontaneous abortions and found that 1 woman out of 3 was a balanced translocation carrier. Stoll (65) found only 6 translocation carriers, besides 2 abnormal karyotypes, in 122 couples. Other workers (66, 67) reported a 2.4% and 10.4% translocation incidence in either one or both parents. A recent study by Davis (68) and co-workers on 100 consecutive couples with repetitive abortions with a

review by them on 1331 couples reported 8 balanced translocation carriers and 82 (6.2%) balanced translocations respectively.

Risk rates for imbalanced segregant liveborn infants are often quoted for specific subgroups of translocations; the assumption made is that these figures apply to cell types of translocation carriers. It is well accepted that there is an empiric risk of 1:10 for maternal carriers and 1:15 for paternal carriers in regard to D/G Robersonian balanced translocation. There is no difference in reproductive fitness whether the carrier is male or female.

Subtr (69) examined 115 couples and observed in 9 (7.8%) the occurrence of reciprocal translocations in regard to spontaneous miscarriage. The study of these reciprocal translocations revealed in a few cases the association of the structural rearrangements with the product of non-disjunction. Kajii and Ferrier (70) found additional trisomy in 3 out of 9 abortuses with reciprocal translocation and considered the possible influences of the balanced translocation in the segregation of other chromosomes in meiosis. They have raised the question of interchromosomal effects.

Stoll and colleagues (65) highlighted the possibility of interchromosomal effects by which the carriage of a balanced rearrangement might interfere with the segregation behavior of other chromosomes not involved in the translocations. They reported from their own experience and from the published reports on 15 cases where a trisomy Mongol child was born to parents carrying translocation which did not involve the G-group chromosomes. The odds of such an event occurring by chance are of the order of one in half a million, but since there is no information as to the size of the population of births from which these examples were drawn the suggested association must be reviewed with caution. The discovery of a reciprocal translocation in a spontaneous abortion raises the question whether it may also lead to the birth of a viable malformed child. The answer would be to some extent related to the relative size of both segments involved in the translocation. If these are very unequal (one being restricted, say to the telomere) then it could be hypothesized that one type of imbalance with the large segment (trisomy or monosomy) may lead to embryonic wastage and the other type to the birth of a malformed child. Conversely if both chromosome segments are large, it is likely that both types of imbalance become lethal. The chromosomes involved in the translocations are not those usually observed in chromosomal aberrations.

Other variants

Extra small chromosomes of unknown origin have been found to be associated with both normal and abnormal phenotypes (71). The association of the extra chromosome with spontaneous abortions is uncertain.

There has been a report (72) of centric fission of chromosome 7 from a female patient who had three previous abortion; the same abnormality was also found in 5 family members and has been transmitted over three generations. Both arms of the broken chromosome 7 looked like telocentric chromosomes. Except for the failure of reproduction, the other effect(s) of this anomaly on, for example, the physical characteristics of affected members could not be evaluated.

Chromosome variants and abnormalities have occasionally been reported in couples having spontaneous abortions. Genest (73) in several studies in the last decade seems to indicate a close association between the presence of the Yq+ variants in male and the risk of spontaneous abortions in the married couples. The incidence of Yq+ found indicates that the risk of spontaneous abortions is increased when the male partner has a very large Y chromosome. The causation of the increase in length of the Y chromosome is not unique but could be the result of either differential spiralization or structural changes. It appears that the adverse effect on fertility and pregnancy of the Yq+ is a characteristic limited only to some large Y chromosomes (74).

Ring chromosomes can also be detected in spontaneous abortions and have been described in a variety of children with varying congenital defects. Ring chromosomes are formed when both ends of the same element are partially deleted and these ends become attached to one another. In view of the fact that the deleted segments may vary considerably in size, it is not surprising that the phenotypic result is inconstant (75).

CONCLUSION

Approximately 50 percent of spontaneous abortuses are chromosomally abnormal and many of the choromosome abnormalities belong to classes not represented among the liveborn. The frequency and diversity of chromosome abnormalities make spontaneous abortions an ideal source of material for many types of cytogenetic research, including the determination of the parental origin of chromosome abnormalities. It is well documented that analysis of chromosome heteromorphisms can be used to specify the mechanism of origin of certain abnormalities; such studies have already been carried out for trisomy 21, trisomy 16 and triploidy.

A number of factors have been associated with pregnancy wastage, including maternal, fetal and placental disorders. Due to the frequency of these problems, the clinical evaluation of couples with recurrent fetal wastage is often not initiated until there has been a specific number of pregnancies lost. The study of parental chromosomes in couples with habitual abortion is usually reserved until other etiologic factors have been excluded. A significant proportion of pregnancy wastage is attributable to fetal chromosome abnormalities; however it is believed that the majority are due to random events.

It is concluded that:

i) Chromosome anomalies in the foetus is a cause of abortion in about half of all first-trimester abortions.
ii) Chromosome anomalies in the parents are generally of minor importance in the aetiology of spontaneous abortion. However, in couples with recurrent abortions of unknown origin cytogenetic analysis of the parents is indicated.
iii) In couples with two consecutive chromosomally abnormal abortuses, amniocentesis may be indicated for prenatal diagnosis in subsequent pregnancies.

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