Cytogenetic Studies of the Leukocytes of Couples with Habitual Abortions

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Karyotypes were prepared from peripheral blood leukocytes in 18 couples with histories of habitual abortions. The standard chromosome analysis and G-banding techniques were studied. The abnormal karyotypes seen were one case with 20% of 45,XX,-14,-15, t(14/15), one case of 46,XY/45,XY,-21 mosaicism, one case of 45,XX,-14,-21,t(14/21), one case of 46,XX/45,XO mosaicism and one case of 46,XYq+. Many other types of chromosomal abnormalities from many reports in couples with spontaneous abortions are discussed.

Key Words: Cytogenetic study, Habitual abortions

The causes of spontaneous abortion are often unknown, but probably multiple, including abnormalities of placentation, infectious or systemic disease, hormonal imbalance, immunological factors, anatomical defects, and genetic errors (Glass and Golbus, 1978). The speculation on the possibility of chromosomal abnormalities in spontaneous abortion were first demonstrated by Penrose and Delhanty (1961). Since then, many cytogenetic studies have clearly demonstrated that a significant proportion of early human spontaneous abortions have a chromosome anomaly (Jacobsen et al., 1963; Bishun et al., 1964) and it is now accepted that chromosomal aberrations involving trisomy, monosomy and translocations are a major cause of early abortion (Carr, 1967; Makino et al., 1967). Because of the possible association between chromosomal abnormalities in the fetus and chromosomal rearrangements in one of its parents, as predisposing to recurrent abortion, cytogenetic investigations on repeated abortions and stillbirths have been started on couples who have experienced repeated spontaneous abortions and/or stillbirths (Schmid, 1962; Carr, 1963; Rowley et al., 1963; Bishun and Morton, 1968; Bhasin et al., 1973; De La Chapells et al., 1973). Since then, there have been many reports seeking chromosome anomalies on couples who suffered from chronic fetal wastage (Yunis et al., 1964; Wingate, 1965; Jacobson et al., 1966; Walzer et al., 1966; McKay et al., 1967; Pergament et al., 1968; Stenchever et al., 1968; Lucas, 1969; Predescu et al., 1969; Wilson, 1969; Hsu et al., 1970; Kadotani et al., 1970; Sparkes and DeChieri, 1970; Grotsky et al., 1971; Hsu et al., 1972; Stenchever and Jarvis, 1971; Kim et al., 1975; Kōener et al., 1975; Tsenghi et al., 1976; Lauritsen, 1976; Byrd et al., 1977; Stenchever et al., 1977; Kajii and Ferrier, 1978; Lancet

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et al., 1978; Mameli et al., 1978; Mannuti et al.,
1978; Neu et al., 1979; Kardon et al., 1980;
(1974) on 17 cytogenetic studies on couples
with a history of recurrent abortions revealed
25 individuals with structural chromosome
abnormalities from a total of 1,793 men and
women karyotypes. Their frequency (1.3%) was
more than four times that in the general
adult population (0.2-0.3%, Jacobs, 1977).
Cytogenetic studies on spontaneous abortuses
also indicated that unbalanced translocations
account for 2 to 4% of all chromosomally
abnormal abortuses (Carr and Gedeon, 1975).
They assumed that aborters with balanced
translocations transmit unbalanced products of
those translocations to their abortuses.
Since chromosome analysis is frequently
recommended for couples who have two or more
episodes of reproductive loss, and since there
are no studies in the Korean population, the
purpose of the present investigation was to
determine the types of chromosome abnormali-
ties in 18 Korean couples with repeated sponta-
neous abortions.

MATERIALS AND METHODS

Chromosome studies were carried out in 18
couples who had a history of two or more
spontaneous abortions or live births with abnor-
malities. All the subjects were from private and
obstetric units of several hospitals in the city of
Seoul. Maternal ages were between 23 and 32
and paternal ages were between 29 and 36.
Cells from peripheral blood were cultured by
a modification of the method of Moorhead et
al (1960) in GIBCO blood culture media. The
slides were made by air-drying and stained with
Giemsa. The trypsin-G-banding preparations
were made by the Seabright (1971) technique.
In most specimens, at least 20 metaphases were
selected for chromosome study. The photo-
micrographs were made using a light green
filter and Kodak high contrast copy film in a
AO photomicroscope.

RESULTS

Among eighteen couples, twelve couples and
six individuals had a modal chromosome number
of 46 and normal female and male chromosome
constitution (46, XX and 46, XY) (Fig. 1). Chromosome abnormalities were noted in three
females and two males in the study (Table 1).
Couple 4. This 30-year-old female was
referred because of a history of four first-tri-
merster abortions. A routine karyotype appeared
with a modal chromosome count of 45. Only
four members of the D group could be identified
but an additional metacentric chromosome was
present (Fig. 2). Although this was assumed to
be a translocation between the 14 and 15 [45,
XX,-14,15,t(14/15)], it was not certain without
the G-banding picture.
Couple 6. This 28-year-old female had a
history of four consecutive first-trimester abor-
tions with no successful pregnancies. Wife's
karyotype was a normal 46,XX. In the hus-
band's karyotype there appeared two kinds of
cells: 80% of the cells had karyotype 46,XY,
and 20% had a 45,XY,-21 (Fig. 3 and 4).
Couple 9. This man and wife were referred
for genetic counseling following three sponta-
neous abortions and a male child with Down's
syndrome. The husband's karyotype was a
normal 46,XY. On the basis of conventional
stain without banding, the 29-year-old wife
revealed 45 chromosomes with balanced trans-
location of 14 and 21 [45,XX,-14-21,t(14/21)]
in 20% of the cells (Fig. 5).
Couple 12. The 32-year-old female had a
history of difficulty in conceiving and three
spontaneous first-trimester abortions. The
husband was found to have a normal 46,XY karyotype. In the wife's culture 30% of the cells were 45,XXO and the remainder had a 46,XX karyotype (Fig. 6).

Couple 14. This couple had five pregnancies, all ending within 10 weeks. The wife's karyotype was normal 46,XX, but the husband who was 34 years old had a clinical diagnosis of secondary hypogonadism. Examination of his chromosomes showed an extended long arm of the Y chromosome (Fig. 7 and 8) in 30% of the cells.

DISCUSSION

In this study, all cases were referred without any information regarding gynecologic examinations, sperm count of the husband, hormone tests or other tests results. They came to us for genetic counseling after they had been seen by obstetric units of hospitals because of consecutive first-trimester abortions. The main problem of concern in the present study was whether chromosomal anomalies found in the couples could be causally related to recurrent spontaneous abortions in the Korean population.

The present study shows chromosome abnormalities in 5 individuals of the 18 couples studied, a frequency of 14%, which is a considerably higher rate than any other reported studies. The frequency of chromosomal abnormalities in couples in the general population examined for repeated spontaneous abortions is 5.4% (Pergament et al., 1968; Wilson, 1969; Kulazhenko et al., 1972; Kiooasaa and Mikelsaar, 1973) and Tsenghi et al. (1976) found 7.79% by the new staining technics. The reported cases of chromosome studies in repeated spontaneous
### Table 2. Major cytogenetic studies in the literature of couples with fetal wastage

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Year</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>Jacobsen et al</td>
<td>1963</td>
<td>46,XX,-13,-13,+13q-,t(13q/13q)</td>
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<tr>
<td>Bishun et al</td>
<td>1964</td>
<td>46,XX/45,XO</td>
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<tr>
<td>Bishun et al</td>
<td>1964</td>
<td>46,XX,Ds+</td>
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<tr>
<td>Pergomet et al</td>
<td>1968</td>
<td>45,XX,-D,-D,t(D/D)</td>
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<tr>
<td>Bishun &amp; Morton</td>
<td>1968</td>
<td>46,XX,18p+</td>
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<tr>
<td>Stenechever et al</td>
<td>1968</td>
<td>46,XX,21p-</td>
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<tr>
<td>Predescu et al</td>
<td>1969</td>
<td>46,XX/45,XO</td>
</tr>
<tr>
<td>Hsu et al</td>
<td>1970</td>
<td>46,XY/47,XY,+D</td>
</tr>
<tr>
<td>Kodotani et al</td>
<td>1970</td>
<td>46,XX,2 inv(p-q), 6 br</td>
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<tr>
<td>Sparkes et al</td>
<td>1970</td>
<td>46,XX,-5,-11,t(5q/11q),+11q-</td>
</tr>
<tr>
<td>Groetsky et al</td>
<td>1971</td>
<td>45,XX,-14,-15,t(14/15)</td>
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<tr>
<td>Stenechever &amp; Jarvis</td>
<td>1971</td>
<td>45,XX,D,-D,t(Dq/Dq)</td>
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<tr>
<td>Lewis and Ridler</td>
<td>1972</td>
<td>45,XY,rob(22;22)</td>
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<tr>
<td>Hsu et al</td>
<td>1972</td>
<td>45,XY/46,XX/47,XXX</td>
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<tr>
<td>Lucas et al</td>
<td>1972</td>
<td>47,XX,+14,t(5/10) (q33;q11)</td>
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<tr>
<td>Schwinger</td>
<td>1973</td>
<td>45,XY,rob(22;22)</td>
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<tr>
<td>Kim et al</td>
<td>1975</td>
<td>46,XX,t(17;19) (q23;p13)</td>
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<tr>
<td>Maeda &amp; Ohno</td>
<td>1976</td>
<td>45,XY,rob(22;22)</td>
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<tr>
<td>Tsenghi et al</td>
<td>1976</td>
<td>46,XY,t(18q/-5p+)</td>
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<td>46,XY/46,XY,t(9q+/-7p-)</td>
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<td>46,XY,t(Yq/-9p+),oqh+</td>
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<td>46,XY,t(7q/-1q+)</td>
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<td>45,XX,-D,-D,+t(D/D)</td>
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<td>46,XX,inv(9q/-p+)</td>
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<td>46,XX,Gp-</td>
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<td>45,XY,rob(22;22)</td>
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aborted since 1977 are summarized in Table 2. Jacobsen (1963) reported a translocation in the 13-15 group as a cause of partial trisomy and spontaneous abortion in the same family. A total of 68 couples and 5 individuals with habitual abortion histories were studied by Stenchever et al. (1968; 1969). They discovered one case of 14/15 translocation carrier. Pergament et al. (1968) also discovered one parent with a D/D translocation, and others including 18p+ and Gp- from 39 couples and four women who experienced repeated spontaneous abortions and/or stillbirths. The findings in the families of the Sparkes and DeChieri’s (1970) report illustrated the relationship of a chromosome translocation to recurrent fetal wastage for some carrier parents. Kodotani et al. (1970), Grotsky et al. (1971) and Lucas et al. (1972) described families with B/C and D/G translocations associated with recurrent abortions. A recent study (Stenchever et al., 1977) of couples with a history of 3 or more spontaneous abortions indicated that a large number of the women (31.2%) were balanced translocation carriers.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>karyotypes</th>
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| Stenchever et al | 1977 | 46,XX,t(2p-;7p+)  
46,XX,t(2q+;8q+)  
46,XX,t(3p-;8q+)  
46,XX,t(1p+;17q-)  
46,XX,t(7q+;10p-)  
46,XX,t(1q-;16q+)  
46,XX,t(1p+;2q+) |
| Byrd et al       | 1977 | 46,XX,t(2;66)(q3;p2)  
46,XY,t(1;9)(p3;q2) |
| Kajii & Ferrier  | 1978 | 45,XY,-13,014,+t(13q;14q)  
46,XX,t(3;21)(5q-;21q+)  
45,XX,t(3;13)(q29;q22)  
45,XX,013,021,t(13q;21q) |
| Mameli et al     | 1978 | 46,XY,t(13q;18q+)  
46,XX,t(10;12)(122;124)  
46,XX,t(13q;14q) |
| Neu et al        | 1979 | 47,XXX |
| Gahmberg et al   | 1980 | 45,XY,-22,t(22/22)  
45,XX,-13,-14,t(13q;14q) |
| Kardon et al     | 1980 | 45,XX,13,-15,t(13/14)  
45,XX,-13,-14,t(13/14) |
| Ward et al       | 1980 | 46,XY,t(8;10)(q24;p11)  
46,XY,t(12;29)(q22;p12)  
46,XX,t(2;6)(q33;q27)  
46,XX,15p+  
46,XX,inv 9(p11q13)  
46,XX,inv 2(p11q13) |
|                  |      | 45,X/46,XX |
Lewis and Ridler (1972), Schwinger (1973), Maeda and Ohno (1976) and Mameli et al. (1978) found 22/22 Robertsonian translocation in couples with recurrent abortions. Bishun et al. (1964) investigated chromosomal mosaicism, such as XX/XO, in a case of repeated abortion. Bishun and Morton (1968) reported an unusually large satellite pair one group D chromosome and mosaicism (45,XO/46,XX) for cultured leukocytes examined among 27 patients, each of whom who had lost the products of conception on two or more occasions. Subfertility and repeated spontaneous abortions have also been described in individuals with sex chromosome anomalies, such as XO/XX, XX/XXX and XXY (Predescu et al., 1969; Stenchever and Jarvis, 1971). Hsu et al. (1972) found triple mosaicism, 45,XO/46,XX/47, XXX in two women with normal phenotype who had histories of fetal wastage. Lauritsen (1976) found three translocation carriers and an XXX woman among the 259 couples and 9 women abortors studied. A consecutive series of 50 couples with a history of fetal wastage were studied cytogenetically with the banding technique (Kim et al., 1975). They found 3 women who were balanced reciprocal translocation carriers (6%) and 1 case with 45,XO/46,XX/47, XXX mosaicism. Kijii and Ferrier (1978) also carried out a cytogenetic survey of 783 abortors. It revealed 4 women and two men as balanced translocation carriers and a woman with an XXX karyotype. Tsenghi et al., (1976) also reported on the frequency of translocations in couples with repeated spontaneous abortions in the Greek population with the banding technique. They found a 3.25% incidence of chromosomal rearrangements in one of the parents. Byrd et al., (1977) reported four balanced translocation carrier parents among 59 couples with histories of recurrent abortions. Neu et al. (1979) reported one woman with a 45,XX, t(13q14q) who had three consecutive first-trimester abortions. Ward et al (1980) reported chromosome variations in six individuals out of 100 couples examined for a history 45,XO/46, XX was found. Others were 46,XX,15ps-, two of 46,XXinv(9)(p11q13), 46,XX,inv(2) (p11q13), and breaks in 16q22 of 13% of cells. Kardon et al. (1980) found a structural rearrangement of chromosomes in couples with a history of having borne a child with multiple congenital abnormalities.

Couples with a history of subfertility and/or multiple spontaneous abortions should be studied cytogenetically not only to detect balanced structural abnormalities but also to search for chromosomal mosaicism. If a chromosome anomaly is found, and precisely identified, a more exact prognosis for future pregnancies can be given and antenatal diagnosis may be offered in suitable cases. Although parental chromosome anomalies are not a frequent cause of recurrent abortion, they occur more commonly than in the general population and it is useful to examine both parents cytogenetically. In all problems of counseling involving cytogenetic abnormalities, if a chromosome anomaly is found, the important thing is to discuss with the patient and the spouse the statistical probabilities involved on the basis of segregation patterns. The patient and spouse then are encouraged to discuss the problem and arrive at their own conclusion regarding future pregnancies. If a pregnancy occurs, it is now possible to utilize amniocentesis and the culture of amniotic fluid cells to make an antenatal evaluation of the fetus.

REFERENCES


Bishun NP, Morton WRM: Chromosome studies on women who have two or more unsuccessful pregnancies. J Obstet Gynec Brit Comm 75:66-70, 1968


Carr DH: Chromosome studies in abortuses and stillborn infants. Lancet 2:603, 1963


Lucas M: Translocation between both members of chromosome pair number 15 causing recurrent abortion. Ann Hum Genet 32:347, 1969


Maeda T, Ohno M: A 22/22 translocation carrier with recurrent abortions demonstrated by a Giemsa
bANDING teCHNIQUE. Hum Genet 31:243-245, 1976
Penrose LS, Delhanty JDA: Triploid cell cultures from macerated foetuses. Lancet 1:1261, 1971
Fig. 1. The normal male chromosome constitution of 46,XY.

Fig. 2. Karyotype of wife, couple 45,XX,-14,-15,t(14/15) (Conventional stain).

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Fig. 3. Karyotype of husband, couple 6: 45,XY,-21 (Conventional stain).

Fig. 4. G-banded karyotype of Fig. 3, 45,XY,-21.
Fig. 5. Wife of couple 9 karyotype showing 45,XX,-14,-21,t(14/21) (Conventional stain).

Fig. 6. Karyotype of wife, couple 12: 45,XO (Conventional stain).
Fig. 7. Karyotype of husband, couple 14: 46,XYq+ (Conventional stain).

Fig. 8. G-banded karyotype of Fig. 7, 46,XYq+.