CONFINED PLACENTAL MOSAICISM
IN SPONTANEOUS ABORTIONS

by

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B.Sc. Trinity Western University, Canada, 1986

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Genetics Program

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

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Department of Medical Genetics

The University of British Columbia
Vancouver, Canada

Date November, 1991
ABSTRACT

One hundred and eleven early spontaneous abortions were examined for the presence of confined chromosomal mosaicism. For each specimen amnion, chorionic plate, chorionic villi, and umbilical cord were sampled and processed using long term tissue culture techniques. In addition, direct preparation techniques were applied to the chorionic villi. Thus both the cytotrophoblast and the villous stroma fibroblasts of the chorionic villi were assessed. These placental tissues were representative of three developmental cell lineages: (1) trophoblast via cytotrophoblast, (2) extraembryonic mesoderm via chorionic villous stroma and chorionic plate, and (3) embryonic ectoderm via amnion and umbilical cord.

Fifteen metaphases per tissue were cytogenetically analyzed. Forty spontaneous abortions yielded cytogenetic results, and consisted of 11 karyotypically normal and 29 abnormal specimens. The chromosomal anomalies observed were trisomy, triploidy, structural anomalies, monosomy X, and confined mosaicism involving trisomic and tetraploid cell lines.

Ten percent of the studied specimens revealed a confined mosaic constitution suggesting that this chromosomal defect is common in the spontaneous abortion population. Segregation or cleavage errors are thought to have taken place within 3 diploid and 1 trisomic conceptus. The timing of the mitotic error can be estimated from the affected placental tissues.

Although the contribution of confined chromosomal mosaicism to the etiology of pregnancy loss is not clearly understood, any alteration of the genomic content of developmental cell lineages may effect the function of the resulting tissue(s) and therefore the survival or demise of the conceptus.
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to my parents, for their encouragement

to my sister, for her patience

to my love, for his support

x
SECTION 1
INTRODUCTION

1.1 Spontaneous Abortion and Pregnancy

1.1 a. Definitions

The term spontaneous abortion has been defined as the premature natural expulsion of the products of conception (Dorland, 1981). Included within this definition are those abortions thought to have been imminent, incomplete or missed, which required medical intervention for the removal of the nonviable conceptus. An abortion occurs prior to 20 weeks of gestation; after 20 weeks, pregnancy loss is referred to as a premature delivery of a liveborn or a stillbirth. Embryonic or early loss occurs prior to the 8th week of development or 10 gestational weeks. Fetal loss occurs between 10 to 20 weeks of gestation and has been described as a late abortion. The term recurrent or habitual abortion has been defined as 2 or more abortions per couple.

1.1 b. Incidence

The estimated rate of human pregnancy loss is 15-20 %; the highest rate occurs during the first trimester and the rate rapidly declines as pregnancy proceeds (Warburton and Fraser, 1964; Shepard and Fantel, 1979; Jacobs and Hassold,
1987) (Figure 1.1). This estimate is based on the clinical recognition of a pregnancy, that is, the survival of the conceptus until the second developmental week to cause a missed menstrual period (Shiota, 1984). Sixty percent of reproductive loss occurs prior to clinical recognition, due to failure of zygotic cleavage and of implantation (Hertig, 1967; Shepard and Fantel, 1979; Shiota, 1984).

Figure 1.1
Decline of reproductive loss during the gestational period (Hertig, 1967; Jacobs and Hassold, 1987).
Since 75% of human conceptions result in spontaneous abortion, the probability of a successful pregnancy is estimated as 25% per menstrual cycle (Wimmers and Van der Merwe, 1988). Although the loss of a conceptus is a common event, its etiology is not fully understood.

1.2 Factors Contributing to the Spontaneous Abortion Event

A number of maternal and embryonic/fetal factors are thought to contribute to the loss of a pregnancy.

1.2 a. Maternal Factors

Among identified maternal factors of spontaneous abortion are: infections, immunological rejection, abnormalities of the uterine environment, endocrinological disorders, maternal diseases, and environmental factors. Infection has been associated with one third of fetal abortions. The infection may originate either by the ascension of vaginal microflora or in the maternal circulatory system. The microorganisms are thought to directly cause fetal disease (Rushton, 1985). Immunological rejection of the fetus is thought to occur by one of the following mechanisms: (1) the maternal organism sets up a direct response to the conceptus, (2) graft-versus-host reaction via maternal cells entering the embryonic/fetal tissues, or (3) the cells of the conceptus enter the maternal tissues causing an antibody response (Rushton, 1985). A correlation between habitual aborters and ABO
incompatibility between the mother and the conceptus has been observed (Gill, 1983). Fifteen percent of recurrent fetal loss and premature labour has been attributed to anatomically abnormal uterine environments (Glass and Golbus, 1978; Rushton, 1985). An alteration of placental hormonal homeostasis, such as declining levels of progesterone, human chorionic gonadotrophin, or luteal relaxin has also been associated with recurrent abortions (Rushton, 1984). Other maternal factors, such as gastrointestinal, cardiovascular, respiratory, or renal disease and environmental factors, such as alcohol consumption and smoking have been suggested as causative agents for spontaneous abortions (Rushton, 1985; Goulet and Theriault, 1987).

1.2 b. Embryonic/Fetal Factors

Although maternal and fetal factors are not completely independent of one another, two features - morphology and chromosomal anomalies - have been cited as embryonic/fetal factors.

1.2 b (i). Morphological Factors

Some investigators have suggested that abnormal anatomical morphology of the embryo or fetus participates in the initiation of the abortive event. Although spontaneous abortions have a high incidence of malformation, many fetuses with
serious anomalies, such as neural tube defects survive to term (Byrne et al., 1985; Kalousek et al., 1987; Kalousek et al., 1990).

1.2 b (ii). Chromosomal Factors

The factor having the greatest influence on the survival of the conceptus is chromosomal anomalies (Gilbert et al., 1987; Jacobs and Hassold, 1987).

One of the earliest cytogenetic surveys of abortuses and stillborns was reported by Carr (1963), who showed an association between abnormal chromosomal constitution and spontaneous abortion. Since the 1970s, a large number of spontaneous abortions have been studied cytogenetically by using trypsin-Giemsa banding techniques (Boue et al., 1975; Creasy et al., 1976; Warburton et al., 1964, 1980; Hassold et al., 1980a; Kajii et al., 1980; Lauritsen, 1982). These studies identified frequency and types of chromosomal abnormalities associated with spontaneous abortions.

Chromosomal abnormalities account for approximately 60 % of all early recognized spontaneous abortions (Hassold et al., 1980a; Noor and Hassan, 1984; Jacobs and Hassold, 1987). The incidence of chromosomal abnormalities in conceptuses declines to 6 % after the 12th week of gestation and to 0.58 % in livebirths (Lauritsen, 1982; Boue et al., 1985; Kalousek et al., 1988; Wimmers and Van der Merwe, 1988) (Figure 1.2). Some studies have suggested that the incidence of chromosomal abnormalities is even higher in pre-recognized pregnancies (Gilbert et al., 1987).
Spontaneous abortions have been associated with all types of chromosomal anomalies; these cytogenetic anomalies have been divided into 5 categories: trisomy, monosomy, polyploidy, structural rearrangement, and other miscellaneous anomalies (Figure 1.3).

Figure 1.2
Percentage of cytogenetic abnormalities associated with spontaneous abortions during the gestational period (Craver and Kalousek, 1987; Gilbert et al., 1987).
At a frequency of 56% of spontaneous abortuses, trisomy is the most common chromosomal abnormality observed (Jacobs and Hassold, 1987). Trisomies have been identified for every autosomal chromosome, with the exception of chromosome 1 (Kalousek, 1987). Table 1.1 lists the frequency of occurrence for the trisomies observed in 7 spontaneous abortion surveys (Jacobs and Hassold, 1987). The most common trisomy is an additional chromosome 16, followed by 22, 21, 15, 13, 2, and 18 (Jacobs and Hassold, 1987).
If nondisjunction were a random event, the expected frequency of individual trisomies for each chromosome would be 4%. The discrepancy between observed and calculated trisomic frequencies may be attributed to: (1) nonrandom nondisjunction, (2) relative lethality of chromosomes in trisomic form, and (3) selection bias for tissues which fail to grow in culture.

Table 1.1
Frequencies of individual trisomies observed within the spontaneous abortion population. (Jacobs and Hassold, 1987).

<table>
<thead>
<tr>
<th>CHROMOSOME</th>
<th>PERCENTAGE</th>
<th>CHROMOSOME</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.9</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>14</td>
<td>4.3</td>
</tr>
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<td>4</td>
<td>2.4</td>
<td>15</td>
<td>7.2</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>16</td>
<td>31.1</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>17</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>4.2</td>
<td>18</td>
<td>4.8</td>
</tr>
<tr>
<td>8</td>
<td>3.9</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>2.7</td>
<td>21</td>
<td>9.5</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>22</td>
<td>10.4</td>
</tr>
<tr>
<td>11</td>
<td>0.3</td>
<td>X</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Collectively, polyploidy is the second largest contributor to abnormal chromosomal constitution in spontaneous abortions. This category is divided into two groups: triploidy (3n), which comprises 15% and tetraploidy (4n), which comprises 5%.
There are 3 proposed mechanisms for triploidy: (1) dispermy fertilization, (2) maternal or paternal nonreduction in either the first or second meiotic division, and (3) the incorporation of the second polar body into the zygote (Jacobs and Hassold, 1987). The most common triploid sex complement is XXY, followed by XXX and XYY (Carr, 1970).

Tetraploidy is thought to occur either by trispermy fertilization, or failure of zygotic cleavage after the first mitosis (Carr, 1970; Szulman et al., 1981). All tetraploids observed thus far have either been 92,XXYY or 92,XXXX. Polyploids greater than 4n do not occur.

The third most frequent (18 %) abnormality is monosomy X (Jacobs and Hassold, 1987). Monosomy for autosomal chromosomes have not been reported in the literature, with the exception of chromosome 21 (Ohama and Kajii, 1972; Abeliovich et al., 1979). A 45,Y constitution has also never been detected from abortus tissues. These types of monosomies are thought to perish prior to the clinical recognition of pregnancy (Jacobs and Hassold, 1987).

The final 2 categories are that of structural rearrangements (4 %) and other miscellaneous chromosomal anomalies, including chromosomal mosaicism (2 %) (Jacobs and Hassold, 1987). Structural anomalies usually result in the duplication and/or deletion of portions of a chromosome.

It has been suggested that cytogenetically abnormal conceptuses are selectively lost during the first trimester of pregnancy (Lauritsen, 1982). Tetraploidy, triploidy, autosomal monosomy and most trisomy conceptuses rarely survive to term.
(Jacobs and Hassold, 1987). Structural anomalies, monosomy X, and trisomies for chromosomes 13, 18, and 21 are chromosomal aberrations most often seen in liveborns (Table 1.2). The mechanisms involved in the loss of some types of chromosomally abnormal embryos and the survival of others is poorly understood. It has been suggested that the survival of a small proportion of trisomy 13 and 18 conceptuses is related to the presence of confined placental mosaicism (Kalousek et al., 1989). The survival of trisomy 21 remains unexplained.

Table 1.2
The frequency and distribution of chromosomal abnormalities during pregnancy (Jacobs and Hassold, 1987).

<table>
<thead>
<tr>
<th>Population</th>
<th>Total Abnormal</th>
<th>Trisomy</th>
<th>Monosomy X</th>
<th>Triploidy</th>
<th>Tetraploidy</th>
<th>Structural Anomaly</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous Abortion</td>
<td>48%</td>
<td>56%</td>
<td>18%</td>
<td>15%</td>
<td>5%</td>
<td>4%</td>
<td>2%</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>6%</td>
<td>67%</td>
<td>4%</td>
<td>11%</td>
<td>0%</td>
<td>7%</td>
<td>11%</td>
</tr>
<tr>
<td>Liveborns</td>
<td>0.6%</td>
<td>53%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>44%</td>
<td>4%</td>
</tr>
</tbody>
</table>

1.3 Chromosomal Mosaicism

Chromosomal mosaicism has been defined as the presence of two or more different chromosomal cell lines in the same individual (Kalousek and Dill, 1983). The most common mosaic constitution consists of a mixture of a normal diploid cell line and an aneuploid cell line. Mosaicism originates from a post-zygotic chromosomal mutation via nondisjunction, anaphase lag, or structural rearrangement.
The resultant mosaic pattern within the conceptus is dependent upon the timing of the chromosomal mutation, the cell lineage affected, and the viability of the mutation itself. If the chromosomal mutation affects the progenitors of both the embryo and placenta, the resulting mosaicism is generalized throughout the tissues of the conceptus. However, if the mutation affects only one type of progenitor, the resulting mosaicism is limited to specific tissues. Hence in the confined chromosomal mosaic conceptus, the chromosomal complement of the embryo and placenta are not necessarily the same.

1.3 a. Generalized Mosaicism

In the generalized mosaic conceptus all tissues are affected. This type of mosaicism has been described for most autosomal trisomies and for both sex chromosome monosomy and trisomy, within prenatal and neonatal populations (Nielsen and Sillesen, 1975; Warburton, 1978; Knight et al., 1984; Jacobs and Hassold, 1987) (Table 1.3).
Table 1.3
The frequency of occurrence for mosaicism within various cytogenetically abnormal populations (Nielsen and Sillesen, 19751; Warburton, 19782; Knight et al., 19843; Jacobs and Hassold, 19874).

<table>
<thead>
<tr>
<th>SAMPLE POPULATION</th>
<th>PERCENTAGE</th>
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<tr>
<td>Liveborns1</td>
<td>0.02*</td>
</tr>
<tr>
<td>Stillbirths1</td>
<td>0.5*</td>
</tr>
<tr>
<td>Amniotic Fluid Cell Cultures3</td>
<td>0.26</td>
</tr>
<tr>
<td>Chorionic Villus Samples4</td>
<td>2</td>
</tr>
<tr>
<td>Spontaneous Abortions2</td>
<td>10*</td>
</tr>
</tbody>
</table>

* estimated from mosaic trisomies

Mosaicism in spontaneous abortions was initially observed in long term tissue culture studies (Table 1.4). The majority of these mosaic cases consisted of a mixture of normal and trisomic cell lines, consequently the frequency of mosaicism has been reported as a percentage of trisomic abortuses. The frequency of mosaicism among trisomic abortuses was under much debate; frequencies as low as 0-1.9 % (Boue et al., 1975; Lauritsen, 1976; Hassold et al., 1977; Kajii et al., 1980) and as great as 4.3-9.7 % (Cresy et al., 1976; Warburton et al.; 1978) have been reported. Kajii et al. (1980) suggested that mosaic trisomies were induced in vitro since the mosaic tissues were in culture for a longer period of time than the non-mosaic trisomies. Other investigators have shown that when more than 10 metaphases per specimen were examined, the frequency of observed mosaicism increased (Warburton et al., 1978; Hassold et al., 1980a). In addition, Hassold et al. (1980a) showed that the aneuploid cell line was of meiotic origin, by
examining informative chromosomal heteromorphisms. It is now thought that 10% of all spontaneously aborted trisomies exist in mosaic form (Warburton et al., 1978; Hassold et al., 1980a; Bernert, 1988; Eiben et al., 1990).

Diploid/tetraploid mosaicism detected by long term tissue culture techniques have been attributed to in vitro clonal growth of abnormal cell lines (Hunt and Jacobs, 1985a,b). Polyploid metaphases are thought to arise from placental tissues as an inherent feature of tissue culturing (Hunt and Jacobs, 1985a).

Table 1.4
Summary of the mosaic spontaneous abortion cases identified by long term tissue culture studies.

<table>
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<tr>
<th>STUDY</th>
<th>TOTAL NO.</th>
<th>ABNORMAL SPECIMENS</th>
<th>NO. OF CELLS</th>
<th>MOSAICISM:</th>
<th>?</th>
<th>TOTAL</th>
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<td>2n/2n + 1</td>
<td>2n + 1/2n + 2</td>
<td>2n/4n</td>
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<tr>
<td>Creasy et al., 1976</td>
<td>941</td>
<td>287 (30%)</td>
<td>4</td>
<td>-</td>
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<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>(1.3%)</td>
<td></td>
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<tr>
<td>Boue et al., 1975</td>
<td>1498</td>
<td>921 (61%)</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>(0.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warburton et al., 1978</td>
<td>592</td>
<td>195 (33%)</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>(1.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hassold et al., 1980a</td>
<td>999</td>
<td>462 (46%)</td>
<td>3 &amp; 10</td>
<td>10</td>
<td>3</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td>(1.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kajii et al., 1980</td>
<td>447</td>
<td>241 (54%)</td>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>(0.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>4477</td>
<td>2106 (47%)</td>
<td>-</td>
<td>21</td>
<td>3</td>
<td>2</td>
</tr>
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Legend:

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<th>= number</th>
<th>2n+1</th>
<th>= trisomy</th>
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<td>2n+2</td>
<td>= double trisomy</td>
</tr>
<tr>
<td>2n</td>
<td>= diploidy</td>
<td>4n</td>
<td>= tetraploidy</td>
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</tbody>
</table>

13
Eiben et al. (1986; 1987; 1990) and Bernert et al. (1988) have studied spontaneous abortion specimens cytoogenetically by processing chorionic villous tissue by the direct preparation technique. Twenty-five (2.5 %) specimens of a total of 1023 abortuses were identified as mosaic conceptuses. The majority (84 %) of these specimens were diploid/tetraploid mosaics.

1.3 b. Confined Chromosomal Mosaicism

The concept of mosaicism confined to specific tissues in humans was initially discussed by Warburton et al. (1978), as an explanation to the high frequency of mosaicism observed in autosomal trisomy abortuses. Confined placental mosaicism in humans was not documented until 1983 by Kalousek and Dill. In their study, 2 of 9 placentas from pregnancies with unexplained intrauterine growth retardation (IUGR) demonstrated confined chorionic mosaicism (Table 1.5). In both cases, a mixture of normal and aneuploid cell lines were identified within the chorion and only normal cells were found in amnion and cord blood.
Table 1.5
Confined chorionic mosaicism in two placentas from pregnancies with IUGR (Kalousek and Dill, 1983).

<table>
<thead>
<tr>
<th>TISSUES</th>
<th>NORMAL</th>
<th>ANEUPLOID</th>
<th>KARYOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>case 1</td>
<td>chorion</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>amnion</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>cord blood</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>skin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>case 2</td>
<td>chorion</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>amnion</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>cord blood</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

With the development of chorionic villus sampling, a number of investigators reported cytogenetic discordance between placental and embryonic/fetal tissues (Callen et al., 1988). This discordance has been associated with the cytogenetic differences observed between direct preparations and long term tissue cultures of chorionic villi, and has resulted in false first trimester prenatal diagnoses (Simoni et al., 1985; Eichenbaum et al., 1986; Heim et al., 1986; Jackson, 1986; Linton and Lilford, 1986; Martin et al., 1986; Pergament and Verlinsky, 1986; Kalousek et al., 1987; Mikkelsen et al., 1987; Simoni, 1987; Schwinger et al., 1989). These discrepancies have been attributed to confined placental mosaicism, caused by the presence of different chromosomal cell lines within the sampled placental tissues. Confined placental mosaicism occurs at a frequency of 1-2 % in first trimester viable pregnancies.
Confined chorionic mosaicism may have also interfered with the interpretation of cultured amniotic fluid samples. The discrepancies associated with amniocentesis, which can not be explained by cultural artifact, generalized mosaicism, or maternal cell contamination are thought to be due to the contamination of amniotic fluid with chorionic cells. The chorion is thought to be derived from a different cell lineage than the amnion, and therefore may differ in its cytogenetic constitution (Kalousek et al., 1987).

In addition to second trimester placentas, third trimester and/or term delivery placentas have also exhibited confined mosaicism. Kalousek et al. (1989) has observed mosaicism confined to the cytotrophoblast for all trisomy 13 and 18 placentas examined. Three of 73 placentas associated with fetal IUGR have also exhibited confined mosaicism (Kalousek and Dill, 1983; Kalousek et al., 1989).

1.3 c. Difficulties in Studying Confined Mosaicism

Traditionally, only single tissue samples of either embryonic or placental origin have been analyzed in spontaneous abortion studies (Boue et al., 1975; Creasy et al., 1976; Hassold, 1980a; Kajii et al., 1980; Eiben et al., 1987; Bernert et al., 1988; Eiben et al., 1989). In both long term tissue culture and direct preparation studies, mosaicism detected has been assumed to be generalized and therefore representative of the whole conceptus. Thus confined mosaicism has not yet been documented in the spontaneous abortion population.
Confined placental mosaicism can only be observed in studies where more than one gestational tissue per specimen has been cytogenetically analyzed. With the exception of one study, the protocol used to examine spontaneous abortions has been inadequate for the observation of confined mosaicism. This study analyzed spontaneous abortion specimens with both long term tissue culture and direct preparation techniques simultaneously (Yu et al., 1987). Five discrepancies were observed in 27 of these specimens. Three cases were attributed to maternal cell contamination. Two cases consisted of normal diploid karyotypes for the direct preparation technique and tetraploid cells for the long term tissue cultures. These diploid/tetraploid mosaic cases were attributed to in vitro tissue culture artifact (Yu et al., 1987).

1.4 The Cell Lineage of the Conceptus

Development of the conceptus proceeds by a number of mechanisms: compaction, cleavage, cell polarization, position effects, cell death, and cell adhesion (Pijnenborg et al., 1985; Pedersen, 1986; Herdecke, 1987). Each of these mechanisms contributes to the alteration of functional information within the descendants of the zygote. Cell differentiation of multipotent cells limits developmental potential and gives rise to specific patterns of development (Rossant, 1986).
Some of these developmental patterns have been identified in invertebrates, such as *Drosophila melanogaster* and *Caenorhabditis elegans*. For *Caenorhabditis elegans*, the cell lineages from zygote to adult worm have been identified and mapped (Sulston et al., 1983; Johnson, 1986). In the *Drosophila melanogaster* the germinal discs which give rise to compartments and eventually appendages have also been identified (Rossant, 1986). However, for both these systems the detailed developmental molecular mechanisms are not yet fully understood.

For mammals, the developmental processes are even less understood. In mammalian studies two forms of cell lineage research have been pursued: (1) the morphological examination of early primate embryos, and (2) the use of genetic markers in rodents. Together, these investigations have suggested cell lineage trees composed of progenitors which contribute to the extraembryonic tissues, as well as the embryonic tissues of a conceptus.

Two sets of synonymous terms have been used to describe primate and rodent cell lineages (Table 1.6).

**Table 1.6**

Synonymous terms in rodent and primate cell lineage studies.

<table>
<thead>
<tr>
<th>RODENTS</th>
<th>PRIMATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner cell mass</td>
<td>Embryoblast</td>
</tr>
<tr>
<td>Primitive endoderm</td>
<td>Hypoblast</td>
</tr>
<tr>
<td>Trophoderm</td>
<td>Trophoblast</td>
</tr>
</tbody>
</table>
1.4 a. The Primate Cell Lineage

The primate cell lineage has been developed by the morphological examination of presomite human and rhesus monkey embryos from the Carnegie Collection (Herdecke, 1987; Luckett, 1978). This is a static experimental system in which cellular movement and/or differentiation has been interpreted from the identification of cell types and cell placement in serial histological sections of embryos.

Figure 1.4 is a schematic representation of the major cell lineages in primate development. The initial segregation of lineages takes place during blastogenesis, when the cells of the blastocyst differentiate into either trophoblast or embryoblast. With the exception of the trophoblast, all extraembryonic tissues are derived entirely from the embryoblast. Upon re-examination of the Carnegie collection, Luckett (1978) cites the hypoblast, as the progenitor of the yolk sac and the primitive streak, as the source of all extraembryonic mesoderm including the chorionic plate, chorionic villi and body stalk. However, there remains much controversy with respect to the origin of the cells which contribute to the chorionic plate and yolk sac (Herdecke, 1987). Human amnion is derived from primary ectoderm without additional growth from the trophoblast (Herdecke, 1987).
Figure 1.4
Primate cell lineage as envisioned by serial sections of early embryos (Luckett, 1978; Herdecke, 1987).

1.4 b. The Rodent Cell Lineage

A large contribution to the mammalian cell lineage has been derived from the studies of rodent models (Ansell, 1975; Gardner and Papaioannou, 1975; Graham and Deussen, 1978; Market and Petters, 1978; Copp, 1979; Johnson and Ziomek, 1981; Balakier and Pedersen, 1982; Papaioannou, 1982; Johnson, 1986; Dyce et al.,
Figure 1.5 represents the major cell lineages in the preimplantation and postimplantation mouse embryo. Many studies have concentrated their efforts on the timing and distinction of the inner cell mass and the trophectoderm (Ansell, 1975; Gardner, 1978; Graham and Deussen, 1978; Johnson and Ziomek, 1981; Balakier et al., 1982; Pedersen, 1986; Dyce, 1987). These investigators have described the initial differentiation step to commence at or during early cleavage, and give rise to polarized inner and outer cells of the morula (Graham and Deussen, 1978; Johnson, 1981). The outer cells are thought to be the progenitors of trophectoderm and the inner cells are the exclusive progenitors of the inner cell mass (Ansell, 1975; Balakier et al., 1982). Mintz (1970) and Markert and Petters (1978) have demonstrated that only a small number of cells of the inner cell mass contribute to the development of the embryo proper. The remaining inner cell mass is thought to give rise to extraembryonic tissues (Mintz, 1970; Markert and Petters, 1978). Much controversy remains as to the origin of extraembryonic mesoderm and extraembryonic ectoderm, and therefore the chorionic plate and amnion respectively (Gardner, 1986; Dyce et al., 1987).
1.4 c. Derivation of Sampled Tissues

1.4 c (i). Amnion

Amnion is thought to be derived from either (1) delamination and differentiation of the cytotrophoblastic tissue (Hamilton and Mossman, 1978) or (2) through a cavitative event on the dorsal side of the embryonic knot (Herdecke, 1987). Much of the data which had suggested the differentiative relationship between cytотrophoblast and amnion was derived from the Carnegie Hall collection of human
specimens (Hamilton and Mossman, 1978; Herdecke, 1987). The original interpretations have been criticized by Luckett (1978) who has suggested that amnion is derived from the epiblast. Further studies, with the use of mouse models appear to support Luckett’s (1978) observations (Markert and Peters, 1978; Herdecke, 1987). Hence, amnion is thought to be the tissue which is most representative of the embryo proper, being derived from the embryonic ectoderm cell lineage (Gardner, 1986; Gardner, 1978).

1.4 c (ii). Chorionic Plate and Chorionic Villi

Chorion has been defined as extraembryonic mesenchyme and subdivided into chorionic plate and chorionic villi. The chorionic plate is the condensed extraembryonic tissue located below amnion, to which chorionic villi are attached. Traditionally, chorion was thought to consist of both extraembryonic mesenchyme and trophoblast (Hamilton and Mossman, 1978; Gardner, 1978). The chorionic plate is now thought to represent a mesodermally derived tissue, with both extraembryonic and embryonic origins (Markert and Petters, 1978; Herdecke, 1987). Two cell lineages, the trophoblast and the extraembryonic mesenchyme are present in the chorionic villi.

Embryonic mesoderm is derived from the central portion of the primitive streak, as ectodermal cells migrate between the ectoderm and the endoderm (Hamilton and Mossman, 1978). During the formation of this third germ layer there is an intermingling of cells at the periphery of the embryo and extraembryonic tissue.
Thus through cell migration embryonic mesoderm contributes to the formation of both the chorionic plate and chorionic villous stroma (Luckett, 1978; Herdecke, 1987). Several hypotheses have been suggested to explain the origin of extraembryonic mesoderm: (1) a delaminative process from the trophoblast prior to the formation of the primitive streak, (2) derivation from the caudal portion of the primitive streak, and (3) directly from the inner cell mass (Mintz, 1970; Gardner, 1978; Hamilton and Mossman, 1978; Luckett, 1978; Markert and Petters, 1978; Herdecke, 1987). It has been estimated from mouse chimera studies that only 5-7 inner cell mass cells contribute directly to the formation of the embryo proper, and the remaining cells give rise to portions of the extraembryonic supportive tissues (Mintz, 1970).

1.4 c (iii). Cytotrophoblast

Both the human and mouse studies agree that the cytotrophoblast is derived from the trophoblast cell lineage (Gardner, 1978; Luckett, 1978; Papaioannou, 1982; Gardner, 1986). This delineation is obvious at the blastocyst stage of development, but may have taken place as early as the morula stage. Thus the trophoblast is the first cell lineage to delineate from the conceptus. Therefore the tissue(s) which originate from the trophoblast are least genetically similar to the embryo proper.

The trophoblast is the progenitor to two cell types, the cytotrophoblast and the syncytiotrophoblast. The syncytiotrophoblast is the terminal derivation of the cytotrophoblast and is no longer capable of undergoing mitosis. The cytotrophoblast is capable of undergoing mitosis and was cytogenetically examined in this study.
1.4 d. Summary

From the compiled data previously discussed, the mammalian cell lineage assumed for this study is as follows (Figure 1.6).

Figure 1.6

Mammalian cell lineage demonstrating the derivation of the placental tissues sampled in this study.

Note that three cell lineages of the conceptus were represented by the tissue samples analyzed. These cell lineages include (1) embryonic ectoderm via amnion, (2) extraembryonic mesoderm via chorionic plate and chorionic villus stroma, and (3) trophoblast via cytotrophoblast.
1.5 Purpose of the Study

The purpose of this study is to document the existence of confined chromosomal mosaicism within the early spontaneous abortion population. Confined chromosomal mosaicism has never been reported in the tissues of spontaneous abortions. The documentation of confined mosaicism requires the cytogenetic analysis of multiple tissues. Placental tissues are consistently present in the spontaneous abortion specimen and represent three developmental cell lineages. The cytogenetic analysis of these cell lineages and the interpretation of the results may reveal some of the dynamics of the mammalian placental and embryonic development.

Furthermore, documentation of confined mosaicism within spontaneously aborted conceptions may lead to a greater understanding of spontaneous abortion mechanisms. A better understanding of the distribution of different cell lineages and details of the early embryonic developmental process may be achieved through the documentation of different types of confined mosaicism.
SECTION 2
METHODS AND MATERIALS

2.1 Spontaneous Abortion Specimens

2.1 a. Source of Specimens

The spontaneously aborted conceptions were obtained from 3 sources: (1) Children’s Hospital Embryofetopathology Laboratory, (2) Shaughnessy Hospital Surgical Ward, and (3) Vancouver General Hospital, Anatomical Pathology Department. The Embryofetopathology Laboratory receives specimens from the 2 hospitals already listed, as well as all the other hospitals in British Columbia.

2.1 b. Nature of Specimens

The age of the aborted conceptus was defined by both the gestational and developmental age. The gestational age has been defined as the period of time elapsed from the last known menstrual period. The developmental age commences from the day of fertilization. Intrauterine retention following embryonic death was calculated as follows: Intrauterine Retention = Gestational Age - (Developmental Age + 2 weeks). Emphasis was placed upon the collection of consecutive embryonic specimens, however, several previable fetal abortuses were also examined.
2.1 c. Morphological Description of Specimens

The spontaneous abortion specimens were classified according to morphological description: ruptured chorionic sac, embryo, or fetus. A ruptured chorionic sac is an open or fragmented gestational sac, without an identifiable embryo/fetus. An embryonic loss consists of a gestational sac and recognizable embryo proper. A fetal loss consists of a placenta and fetus. The categories of embryonic specimens were further subdivided into 4 types of growth disorganized embryos.

A type 1 growth disorganized embryo is a chorionic sac without an embryo present, which may or may not contain amniotic tissue. Type 2 is a gestational sac containing an undifferentiated lump of embryonic tissue. Type 3 is as a poorly developed embryo having retinal pigmentation at its cranial pole. Type 4 is an embryo with disharmonious limb bud development for its size, within a gestational sac (Kalousek et al., 1990).

The chorionic villi were classified into 3 groups on the basis of their morphological structure observed under a dissection microscope. The normal chorionic villi were cylindrically shaped with branched villous stalks and referred to as having a “fluffy” appearance. These chorionic villi always contained vascular components. The second type of chorionic villi observed was described as stringy, that is, they were rather elongated cylinders with reduced branches and buds.
These villi were either vascular or avascular. The third type of villous morphology was described as cystic, that is, distal portions tended to be swollen or hydropic in nature.

2.1 d. Tissues Sampled

Four tissues were sampled from each specimen: amnion, chorionic plate, chorionic villi, and umbilical cord. In conceptions without identifiable amnion and umbilical cord, chorionic plate and chorionic villi were sampled.

2.2 Tissue Culture

The supplies used for tissue culturing, harvesting, and analysis have been summarized in Table 2.1a and 2.1b.

Table 2.1a

Instruments used for tissue culture, harvesting, and staining procedures.

<table>
<thead>
<tr>
<th>Incubators</th>
<th>Forma Scientific either water-jacketed and/or Model 3028 (Ohio, USA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar Flow Hood</td>
<td>Western Scientific, Model 1000 (USA)</td>
</tr>
<tr>
<td>Microscopes - Dissecting</td>
<td></td>
</tr>
<tr>
<td>- Inverted</td>
<td>Nikon SM-1 (Japan)</td>
</tr>
<tr>
<td>- Phase Contrast</td>
<td>Nikon TMS (Japan)</td>
</tr>
<tr>
<td></td>
<td>either Nikon Optiphot HFX-11 (Japan) and/or Zeiss Photomicroscope II</td>
</tr>
<tr>
<td>Slide Warmer</td>
<td>Labline Instruments (Illinois, USA)</td>
</tr>
</tbody>
</table>
Table 2.1b
Chemicals and Solutions used for tissue culture, harvesting, and staining procedures.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Chloride</td>
<td>1 % solution (BDH Chemicals, Canada)</td>
</tr>
<tr>
<td>Colcemid</td>
<td>10mcg/ml of colcemid rehydrated with Hank’s balanced salt solution - with (Gibco Laboratories, Grand Island, USA)</td>
</tr>
<tr>
<td>Culture Medium</td>
<td>consisted of MEM, 30 % by volume of FBS, 1 % by volume of PSF, + 1 % by volume L-glutamine (Gibco)</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum (Gibco)</td>
</tr>
<tr>
<td>Fixative A Solution</td>
<td>composed of 3:1 methanol (Baker analyzed, New Jersey, USA) + glacial acetic acid (BDH)</td>
</tr>
<tr>
<td>Fixative B Solution</td>
<td>composed of a mixture of 96 % 3:2 acetic acid + distilled water and 4% methanol</td>
</tr>
<tr>
<td>Fixative C Solution</td>
<td>composed of 3:1:1 parts of acetic acid, distilled water, and methanol</td>
</tr>
<tr>
<td>Giemsa Stain</td>
<td>composed of 2 ml of Giemsa stain (BDH) within 50 ml Gurr’s buffer (BDH)</td>
</tr>
<tr>
<td>Gurr’s Buffer</td>
<td>phosphate buffer, pH 6.8 at 20°C (BDH)</td>
</tr>
<tr>
<td>Gluteraldehyde</td>
<td>BDH Chemicals</td>
</tr>
<tr>
<td>Hank’s Solution</td>
<td>balanced salt solution with or without calcium chloride, magnesium chloride and magnesium sulphate and contains a phenol red indicator (Gibco)</td>
</tr>
<tr>
<td>MEM</td>
<td>minimum essential medium (Gibco)</td>
</tr>
<tr>
<td>PSF</td>
<td>Antibiotic-Antimycotic solution which consisted of 10,000 units/ml penicillin G sodium, 10,000 mcg/ml streptomycin sulphate, + 25mcg/ml amphotericin B (Fungizone) (Gibco Laboratories)</td>
</tr>
<tr>
<td>Saline Solution</td>
<td>0.9 % solution (BDH Chemicals) + 2% PSF (Gibco)</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>1 % by weight solution (BDH)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Trypsin - tissue culture</td>
<td>1 ml of either 2.5 % trypsin (Gibco) or trypsin-EDTA (0.05 % trypsin + 0.53 mM EDTA) (Gibco), diluted in 9 ml of Hank’s balanced salt solution - without calcium chloride, magnesium chloride and magnesium sulphate (Gibco)</td>
</tr>
<tr>
<td>Trypsin Solution - banding</td>
<td>1 ml of rehydrated Bacto trypsin (Difco, Michigan, USA) within 50 ml of 0.9% saline solution; Difco trypsin was rehydrated with 10 ml of Hank’s balanced salt solution</td>
</tr>
<tr>
<td>Transport Medium</td>
<td>a suspension of MEM and 4% by volume of PSF</td>
</tr>
</tbody>
</table>

### 2.2 a. Collection and Growth of Tissues

The tissue biopsies were collected in the hospitals and placed in transport medium warmed to room temperature. Using a dissecting microscope, contaminants such as, maternal decidua were removed from the tissues. The tissue biopsies were set up according to the following methodologies.

### 2.2 a. (i). Direct Preparation

The chorionic villi sample was divided into 2 portions, for both direct preparation and long term tissue culture.

Within a laminar flow hood, the chorionic villi were rinsed several times in sterile transport medium. The cleaned sample was divided into 2 or 3 petri dishes.
containing 5 ml of culture medium. The chorionic villi were incubated for 24 to 48 hours at 37°C and 5 % CO₂.

2.2 a. (ii). Long Term Tissue Culture

Amnion, chorionic plate, chorionic villi and umbilical cord were set up for long term tissue culture. Tissues of some specimens were shared between this research laboratory and the Children’s Hospital Service Cytogenetics Laboratory.

Following microscopic examination, the tissue samples were aseptically rinsed several times in either transport medium or saline plus PSF. The cleaned sample was divided into 2 or 3 petri dishes and grossly minced with sterile scissors. 1.5 ml of culture medium was added to each petri dish and incubated at 37°C and 5 % CO₂.

After the first 48 hrs of incubation, 1 ml of culture medium was added to each petri dish. The cultures were examined bi-weekly with an inverted microscope. Table 2.2 describes a number of tissue culture maintenance procedures.
Table 2.2
Tissue culture procedures for the maintenance of long term cultures.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADDING Culture Media</td>
<td>1ml of culture medium: to initial cultures after 48 hrs of incubation, to old/sick cultures, or to reestablished trypsinized tissue cultures</td>
</tr>
<tr>
<td>EXCHANGE Media</td>
<td>half of the medium is replaced with fresh culture medium</td>
</tr>
<tr>
<td>FEEDING Tissue Cultures</td>
<td>complete replacement of medium with 3 ml of fresh culture medium</td>
</tr>
<tr>
<td>SUPERNATANT Cultures</td>
<td>the supernatant of the original tissue cultures were poured into petri dishes, thus allowing remaining tissue fragments to seed onto the dishes</td>
</tr>
<tr>
<td>TRYPsinATION of Tissue Cultures</td>
<td>to generate multiple clonal tissue cultures, the petri dish with confluent growth is rinsed twice with Hank's balanced salt solution and a trypsin solution added, when cells begin to lift off of the bottom of the petri dish 2-3 ml of culture medium is added and the mixture is equally divided into 2-3 petri dishes</td>
</tr>
</tbody>
</table>

2.2 b. Harvesting and Slide Making

2.2 b. (i) Direct Preparation

0.2 ml of a colcemid solution was added to the petri dish of chorionic villi and reincubated for 1 to 2 hrs. For longer colcemid exposure, the volume of colcemid was reduced by half.

The tissue culture medium was removed with a pasteur pipette under a bench top fume hood. 10 mls of a sodium citrate solution, prewarmed to 37°C was added to the chorionic villi for 20 to 30 minutes. The sodium citrate solution was removed and fixative A solution was added in a drop-wise manner until the villi were completely
covered. The chorionic villi were exposed to fixative A for 3 consecutive 10 minute periods. The chorionic villi were sealed in a petri dish with parafilm and stored in a -20°C freezer for 2 hours prior to slide making.

For slide making, the harvested chorionic villi were warmed to room temperature. Fixative A solution was removed and 10 drops of fixative B solution was added to the villi for 3 to 5 minutes. The supernatant was removed from the chorionic villi and gently dropped onto several slides warmed to 37-40°C. Upon evaporation of fixative B, the slides were examined with phase contrast microscopes for the presence of metaphases.

2.2 b. (ii) Long Term Tissue Cultures

When a sufficient number of cells were observed, 0.1 ml of a colcemid solution was added to the culture medium and reincubated for a period of 45 to 60 minutes. For a longer colcemid exposure, the volume of colcemid was reduced by half.

The tissue culture supernatant was decanted under the bench top fume hood and 10 ml of a prewarmed (37°C) sodium citrate solution was added for a period of 23 to 30 minutes. 0.5 ml of fixative A solution was added directly into the sodium citrate solution for 5 minutes. Half the volume of citrate/fixative mixture was removed from the petri dish and 1 ml of fixative A solution was added for 5 minutes. The citrate/fixative mixture was completely removed and 5 ml of fixative A solution was added onto the culture for a minimum 10 minutes.
To release the chromosomes from cytoplasm, fixative A solution was removed and fixative C solution was poured directly into the petri dish for 15 to 25 seconds. Fixative C was removed and the culture rinsed 3 times with Fixative A solution. The petri dish was gently tapped and air-dried to spread the metaphase chromosomes. An inverted microscope was used to confirm the presence of metaphases on the tissue culture dishes.

2.3 Analysis

2.3 a. Staining and Banding of Chromosome Preparations

2.3 a. (i) Solid Staining

The majority of the direct preparation metaphases were solid stained (Appendix I). The slides were immersed in Giemsa staining solution at room temperature for 90 seconds, rinsed with distilled water and dried for microscopic analysis.

2.3 a. (ii) Trypsin/Giemsa Banding

All of the long term culture metaphases were trypsin/Giemsa banded (Appendix I). The trypsin solution was poured into the tissue culture dish for 15 to 25 seconds. The petri dish was rinsed with a calcium chloride solution twice for a total of a 1 minute exposure. The dish was rinsed with distilled water, and stained at room
temperature for 90 to 150 seconds with a Giemsa solution.

Following Giemsa staining, the petri dish was rinsed with distilled water, dried, cut and mounted on large glass slides for microscopic analysis.

2.3 b. Chromosome Analysis

Fifteen metaphases were cytogenetically analyzed for every tissue. Incomplete metaphases, less than 44 chromosomes or unspread chromosomes were not analyzed. When possible, original explant dishes, not trypsinized culture dishes were used for analysis.

Direct preparation metaphases were grouped A to G. The metaphases from long term tissue cultures were banded and analyzed according to chromosome banding patterns.

2.3 c. Documentation of Mosaicism

Mosaicism was determined when 3 or more of 15 metaphases had an identical karyotype which differed from the rest of the cells analyzed. To exclude cultural artifacts, multiple long term dishes were examined in each mosaic case and the second chromosomal complement was demonstrated to exist in more than one dish or seeded explant. Metaphases from trypsinized tissue cultures dishes were avoided when possible. For the direct preparations, 2 sets of 8 slides were made from different portions of the fixed chorionic villi. The diagnosis of mosaicism was accepted only when both sets revealed the same karyotypes.
2.4 Photography

For each tissue analyzed, karyotypes were made of the primary and secondary cytogenetic cell lines, as well as any other unusual findings. Representative photographs of the three categories were taken of chorionic villi stored in gluteraldehyde at $4^\circ$ C. Negatives and photographic prints were processed in the laboratory.

2.5 Flow Cytometric Analysis

2.5 a. Amnion Study

Ten samples of fresh amniotic tissue from euploid pregnancies were submitted to the Immunology Laboratory at Children's Hospital for flow cytometric analysis. These tissues were rinsed in saline and frozen in a $-70^\circ$ C freezer until their subsequent processing. The tissue was thawed, disaggregated by collagenase digestion, filtered, and processed for DNA staining using propidium iodide. Angela Tsang processed and analyzed these tissues with an EPIC - C flow cytometer (Coulter Electronics, U.S.A.).

In addition to flow cytometric analysis, the amniotic tissue samples were processed by long term tissue culture methods. Ten metaphases per sample were cytogenetically analyzed. The tissue culture techniques, banding, and analysis were consistent with those procedures previously described.
2.5 b. Tetraploid Verification

Samples of chorion and chorionic villi from a cytogenetically diagnosed diploid/tetraploid mosaic case were submitted for flow cytometric analysis. These tissue samples were fixed in formalin and kept at room temperature prior to their analysis.
3.1 Surveyed Population of Spontaneous Abortion Specimens

One hundred and eleven spontaneously aborted conceptuses were collected and examined for the presence of confined chromosomal mosaicism. In addition to chromosomal studies, information was collected on the gestational age, maternal age, and number of pregnancy losses.

3.1 a. Gestational Ages of Specimens

The gestational ages ranged from 5 to 20 weeks (Figure 3.1). In 7 (6.3 %) cases, the age of the specimen was not available. Seventy (63 %) of the specimens represented first trimester abortions; the majority of these spontaneous abortions took place during the 10th through 12th weeks of gestation. The remaining thirty-four (30.7 %) specimens were represented by second trimester spontaneous abortions.
Figure 3.1
Distribution of gestational ages of the spontaneous abortions studied.

<table>
<thead>
<tr>
<th>Gestational Age (weeks)</th>
<th>Number of Spontaneous Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-6</td>
<td>5</td>
</tr>
<tr>
<td>7-8</td>
<td>5</td>
</tr>
<tr>
<td>9-10</td>
<td>10</td>
</tr>
<tr>
<td>11-12</td>
<td>15</td>
</tr>
<tr>
<td>13-14</td>
<td>10</td>
</tr>
<tr>
<td>15-16</td>
<td>5</td>
</tr>
<tr>
<td>17-18</td>
<td>2</td>
</tr>
<tr>
<td>19-20</td>
<td>2</td>
</tr>
<tr>
<td>NA</td>
<td>2</td>
</tr>
</tbody>
</table>

3.1 b. Distribution of Maternal Age and Spontaneous Loss of Recurrence

The maternal ages ranged from 14 to 43 years of age, with the majority of specimens from mothers 30 to 39 years of age (33.3 %), followed 20 to 29 years of age group (21.6 %), the above 40 years of age group (14 %) and the 10 to 19 years of age group (7 %) (Figure 3.2abcd and 3.4b).

The majority of spontaneous abortions were first time losses. However, the recurrence of a spontaneous loss varied from the second loss to the sixth lost pregnancy. No paternal information was available.
The numbers of spontaneous losses for each maternal age group.
3.1 c. Morphological Description of Specimens

3.1 c. (i). Gross Morphology of Specimens

Of the 111 specimens examined 45% were ruptured chorionic sacs, 35% were embryonic losses, and 17% were fetal losses (Table 3.1). The embryonic losses were subdivided into 3 groups: complete embryos (11%), incomplete embryos (11%), and growth disorganized embryos (14%). In 2 of the 111 cases the morphological description was not available.

Table 3.1
The classification of spontaneous abortion specimens based on gross morphology.

<table>
<thead>
<tr>
<th>MORPHOLOGICAL DESCRIPTION</th>
<th>NUMBER OF SPECIMENS</th>
<th>CYTOGENETICALLY ANALYZABLE</th>
<th>ABNORMAL KARYOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruptured Chorionic Sac</td>
<td>51</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Embryonic Loss:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>12</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Incomplete</td>
<td>12</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Growth Disorganized</td>
<td>15</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fetal Loss:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>14</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Incomplete</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Not Available</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total: Number</td>
<td>111</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>Percent</td>
<td>100%</td>
<td>37%</td>
<td>73%</td>
</tr>
</tbody>
</table>
3.1 c. (ii). Gross Morphology of Chorionic Villi

In 40% of the specimens collected the chorionic villous morphology was described as stringy, followed by cystic (25%), and fluffy (25%) (Table 3.2). In 11 cases the chorionic villous morphology was not available. Figure 3.3abc are pictorial representations of these 3 classes.

Table 3.2
The classification of chorionic villi based on gross morphology.

<table>
<thead>
<tr>
<th>CLASSES OF CHORIONIC VILLI</th>
<th>DESCRIPTION OF CHORIONIC VILLI</th>
<th>TOTAL NO. OF SPECIMENS</th>
<th>CYTOGENETICALLY SUCCESSFUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic</td>
<td>swollen, hydropic</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>Stringy</td>
<td>elongated, long</td>
<td>44</td>
<td>13</td>
</tr>
<tr>
<td>Fluffy</td>
<td>branched, buds</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>Not Available</td>
<td>------</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>------</td>
<td>111</td>
<td>40</td>
</tr>
</tbody>
</table>
Figure 3.3  Pictorial representations of the three classes of chorionic villi. (Ruler increment = 1 mm)

a. **Cystic**: all specimens were avascular

b. **Stringy**: both vascular and avascular chorionic villi
3.2 Successfully Analyzed Spontaneous Abortion Population

3.2 a. Gestational Ages of Specimens

The gestational ages ranged from 5 to 19 weeks, with the majority (80%) of the abortions taking place between the 10th and 12th weeks of gestation (Figure 3.1). Only 7 (17.5%) specimens were successfully analyzed from the second trimester of gestation.
3.2 b. Distribution of Maternal Age and Spontaneous Loss of Recurrence

Maternal ages of the successfully analyzed specimens ranged from 14 to 44 years, with the majority (63%) of specimens from mothers between the ages of 30 and 39.

Forty eight percent of the successfully analyzed spontaneous abortion specimens represented first time losses, 22.5% were second losses, 12.5% were third losses, 10% were fourth losses and 7.5% were the fifth spontaneous loss.

3.2 c. Morphological Description of Specimens

3.2 c. (i). Karyotypically Normal Specimens

11 of 40 specimens were cytogenetically normal (Table 3.4a). These specimens were represented by both early and late spontaneous abortions (Table 3.3). The embryonic specimens consisted of ruptured chorionic sacs and incomplete embryos. One incomplete embryo and 2 fetal specimens were retained in the uterus for 1-5 week periods prior to their expulsion. From previous large cytogenetic studies, as great as 40% of studied spontaneous abortion specimens have a normal cytogenetic constitution (Hassold et al., 1980a; Noor and Hassan, 1984; Jacobs and Hassold, 1987).
Table 3.3
Morphology and presumed etiology of the spontaneous abortions among karyotypically normal specimens.

<table>
<thead>
<tr>
<th>CASE NO.</th>
<th>R.P. weeks</th>
<th>DEVELOPMENTAL ANOMALY</th>
<th>ETIOLOGY OF ABORTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Ruptured Chorionic Sacs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87-20*</td>
<td>--</td>
<td>hyperplastic villi</td>
<td>unknown</td>
</tr>
<tr>
<td>88-07*</td>
<td>--</td>
<td>none for gestational sac</td>
<td>unknown</td>
</tr>
<tr>
<td>89-07*</td>
<td>--</td>
<td>none for gestational sac</td>
<td>unknown</td>
</tr>
<tr>
<td>B. Embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87-23*</td>
<td>2.5</td>
<td>major embryonic anomalies</td>
<td>abnormal morphology</td>
</tr>
<tr>
<td>88-41</td>
<td>--</td>
<td>type 1 growth disorganized</td>
<td>abnormal morphology</td>
</tr>
<tr>
<td>C. Fetuses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87-41*</td>
<td>5</td>
<td>none identified in fetal parts</td>
<td>unknown</td>
</tr>
<tr>
<td>87-42*</td>
<td>0</td>
<td>none identified in fetal parts</td>
<td>unknown</td>
</tr>
<tr>
<td>87-51</td>
<td>0</td>
<td>minor fetal anomalies</td>
<td>amniotic infection syndrome</td>
</tr>
<tr>
<td>87-54</td>
<td>0</td>
<td>none</td>
<td>amniotic infection syndrome</td>
</tr>
<tr>
<td>87-63</td>
<td>0</td>
<td>none</td>
<td>immunological factors</td>
</tr>
<tr>
<td>89-01</td>
<td>1</td>
<td>minor fetal anomalies</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Legend:

* incomplete specimens
R.P. intrauterine retention period
3.2 c. (ii). Karyotypically Abnormal Specimens

The 29 cytogenetically abnormal specimens were embryonic losses consisting of ruptured chorionic sacs, and complete, incomplete, and growth disorganized embryos (Table 3.4b). Although the gestational ages of some specimens would suggest a more advanced development, examination of the embryo proper and placenta revealed that the demise of the conceptus took place during the first trimester. No one type of cytogenetic abnormality was associated with one morphological defect (Table 3.4b).

3.2 d. Cytogenetic Analysis of Specimens

Fourty of the 111 spontaneous abortion specimens examined, fulfilled the requirements of this study, that is, both the direct preparation of chorionic villi and a long term tissue cultures were analyzable. Twenty-nine of these 40 specimens were cytogenetically abnormal (Tables 3.4a and 3.4b).

The cytogenetic abnormalities observed in this study have been summarized in table 3.5, and include trisomy, triploidy, monosomy X, structural rearrangements, and tetraploidy. Three cases of trisomy 16, 3 cases of trisomy 21, 2 cases of trisomy 22, and one case each of trisomy 4, 8, 10, and 14 were observed (Table 3.5). The trisomy 4 case had an additional abberation, a chromosome 2 inversion. A double trisomy involving chromosomes 16 and 20 was identified. The triploid cases revealed all three types of sex chromosome constitutions, namely, XXX, XYY and XXY. In one case, triploidy was associated with an additional chromosome 7, for a 70,XXY,+7
karyotype. The structural rearrangements observed in this sample of abortuses included translocations, deletions and isochromosomes. Two cases each of sex chromosome monosomy (45,X) and tetraploidy (92,XXYY) were detected.

Table 3.4a Tissues analyzed for the karyotypically normal specimens.

<table>
<thead>
<tr>
<th>CASE NO.</th>
<th>KARYOTYPE</th>
<th>DPV</th>
<th>LTV</th>
<th>CH</th>
<th>AM</th>
<th>CO</th>
<th>NO. CELLS</th>
<th>G.A. weeks</th>
<th>CATEGORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>87-20</td>
<td>46,XX</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>33</td>
<td>8</td>
<td>RCS</td>
</tr>
<tr>
<td>87-23</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>10</td>
<td>RCS</td>
</tr>
<tr>
<td>87-41</td>
<td>46,XY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>47</td>
<td>16</td>
<td>FETUS-I</td>
</tr>
<tr>
<td>87-42</td>
<td>46,XY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>56</td>
<td>14</td>
<td>FETUS-I</td>
</tr>
<tr>
<td>87-51</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>51</td>
<td>18</td>
<td>FETUS-C</td>
</tr>
<tr>
<td>87-54</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>45</td>
<td>15</td>
<td>FETUS-C</td>
</tr>
<tr>
<td>87-63</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>56</td>
<td>16</td>
<td>FETUS-C</td>
</tr>
<tr>
<td>88-07</td>
<td>46,XY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>5</td>
<td>RCS</td>
</tr>
<tr>
<td>88-41</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>46</td>
<td>6</td>
<td>RCS</td>
</tr>
<tr>
<td>89-01</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>52</td>
<td>12</td>
<td>FETUS-C</td>
</tr>
<tr>
<td>89-07</td>
<td>46,XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>10</td>
<td>RCS</td>
</tr>
</tbody>
</table>

Legend:

<table>
<thead>
<tr>
<th>DPV</th>
<th>LTV</th>
<th>CH</th>
<th>AM</th>
<th>CO</th>
<th>G.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>direct preparation villi</td>
<td>long term culture villi</td>
<td>chorion</td>
<td>amnion</td>
<td>umbilical cord</td>
<td>gestational age</td>
</tr>
<tr>
<td>RCS</td>
<td>I</td>
<td>C</td>
<td>NA</td>
<td>NO.</td>
<td></td>
</tr>
<tr>
<td>ruptured chorionic sac</td>
<td>incomplete specimen</td>
<td>complete specimen</td>
<td>not available</td>
<td>number</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.4b  Tissues analyzed for all of the karyotypically abnormal specimens.

<table>
<thead>
<tr>
<th>CASE NO.</th>
<th>KARYOTYPE</th>
<th>DPV</th>
<th>LTV</th>
<th>CH</th>
<th>AM</th>
<th>CO</th>
<th>NO. CELLS</th>
<th>G.A. weeks</th>
<th>CATEGORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>87-04</td>
<td>48,XY,+16,+20</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>22</td>
<td>9</td>
<td>GD1</td>
</tr>
<tr>
<td>87-05</td>
<td>46,XX/47,XX,+4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>67</td>
<td>10</td>
<td>RCS</td>
</tr>
<tr>
<td>87-06</td>
<td>47,XX,+14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>85</td>
<td>7</td>
<td>EMBRYO-C</td>
</tr>
<tr>
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<td>47,XX,+16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>43</td>
<td>8</td>
<td>NA</td>
</tr>
<tr>
<td>87-15</td>
<td>69,XXY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>62</td>
<td>16</td>
<td>RCS</td>
</tr>
<tr>
<td>87-16</td>
<td>69,XYY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>41</td>
<td>11</td>
<td>RCS</td>
</tr>
<tr>
<td>87-28</td>
<td>69,XXY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>33</td>
<td>11</td>
<td>EMBRYO-C</td>
</tr>
<tr>
<td>87-29</td>
<td>69,XXX</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>63</td>
<td>19</td>
<td>EMBRYO-I</td>
</tr>
<tr>
<td>87-45</td>
<td>47,XY,-9, + der(9), + der(8)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>53</td>
<td>12</td>
<td>RCS</td>
</tr>
<tr>
<td>87-48</td>
<td>45,X</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>85</td>
<td>10</td>
<td>EMBRYO-I</td>
</tr>
<tr>
<td>87-66</td>
<td>46,XY/92,XXYY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>83</td>
<td>10</td>
<td>GD2</td>
</tr>
<tr>
<td>88-01</td>
<td>47,XX,+8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>60</td>
<td>12</td>
<td>GD2</td>
</tr>
<tr>
<td>88-03</td>
<td>47,XX,+4,inv(2)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>40</td>
<td>12</td>
<td>RCS</td>
</tr>
<tr>
<td>88-04</td>
<td>47,XX,+21</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>71</td>
<td>11</td>
<td>EMBRYO-I</td>
</tr>
<tr>
<td>88-11</td>
<td>47,XY,+10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>44</td>
<td>15</td>
<td>GD1</td>
</tr>
<tr>
<td>88-12</td>
<td>47,XY,+21</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>45</td>
<td>9</td>
<td>RCS</td>
</tr>
<tr>
<td>88-13</td>
<td>46,XY/92,XXYY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>40</td>
<td>12</td>
<td>RCS</td>
</tr>
<tr>
<td>88-20</td>
<td>70,XXY,+7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>12</td>
<td>RCS</td>
</tr>
<tr>
<td>88-23</td>
<td>47,XX,+21</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>70</td>
<td>10</td>
<td>RCS</td>
</tr>
<tr>
<td>88-24</td>
<td>46,XY,-18, +i(18q)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>60</td>
<td>8</td>
<td>EMBRYO-C</td>
</tr>
<tr>
<td>88-26</td>
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<td>+</td>
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<td>47,XY,+22</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>60</td>
<td>11</td>
<td>EMBRYO-I</td>
</tr>
<tr>
<td>89-02</td>
<td>69,XYY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>NA</td>
<td>RCS</td>
</tr>
<tr>
<td>89-03</td>
<td>47,XY,+20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>11</td>
<td>RCS</td>
</tr>
<tr>
<td>89-05</td>
<td>45,X</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>42</td>
<td>9</td>
<td>EMBRYO-I</td>
</tr>
<tr>
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<td>47,XX,+22</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>57</td>
<td>11</td>
<td>EMBRYO-I</td>
</tr>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>59</td>
<td>9</td>
<td>EMBRYO-C</td>
</tr>
<tr>
<td>89-11</td>
<td>47,XY,+16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>10</td>
<td>RCS</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>75</td>
<td>9</td>
<td>RCS</td>
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</tbody>
</table>
Table 3.5
Summary of the observed chromosomal anomalies in the spontaneous abortion population.

<table>
<thead>
<tr>
<th>CYTOGENETIC ANOMALY</th>
<th>KARYOTYPE</th>
<th>NO. CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy:</td>
<td>47,XX, +4,inv(2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XX/47,XX, +4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XX, +8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XY, +10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XX, +14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XX, +16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XX/47,XX, +16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XY, +20</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XX, +21</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>47,XY, +21</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>47,XX, +22</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>48,XY, +16, +20</td>
<td>1</td>
</tr>
<tr>
<td>Triploidy:</td>
<td>69,XXX</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>69,XXY</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>69,XXY</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>70,XXY, +7</td>
<td>1</td>
</tr>
<tr>
<td>Structural Rearrangement:</td>
<td>46,XY,-18, +i(18q)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XX,-13, +t(13; 14)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>47,XY,-9, +der(9), +der(8)</td>
<td>1</td>
</tr>
<tr>
<td>Monosomy:</td>
<td>45,X</td>
<td>3</td>
</tr>
<tr>
<td>Tetraploidy:</td>
<td>46,XY/92,XXYY</td>
<td>2</td>
</tr>
</tbody>
</table>
3.2 d. (i). Cytogenetic Analysis of Mosaic Cases

Four cases of confined mosaicism were identified within the 40 cytogenetically analyzed spontaneous abortion specimens (Tables 3.5 and 3.6). The abnormal chromosomal constitutions associated with normal cell lines were trisomy 4, 16, and tetraploidy (Table 3.6).

Table 3.6

Summary of the mosaic spontaneous abortion specimens. Figures within the brackets refer to the number of metaphases analyzed.

<table>
<thead>
<tr>
<th>CASE NO.</th>
<th>KARYOTYPE</th>
<th>DIRECT VILLI</th>
<th>CULTURED VILLI</th>
<th>CHORION</th>
<th>AMNION</th>
<th>CORD</th>
</tr>
</thead>
<tbody>
<tr>
<td>87-05</td>
<td>46,XX/47,XX,+4</td>
<td>46,XX (6)</td>
<td>47,XX,+4 (11)</td>
<td>46,XX (20)</td>
<td>46,XX (15)</td>
<td>46,XX (15)</td>
</tr>
<tr>
<td>87-66</td>
<td>46,XY/92,XXYY</td>
<td>46,XY (16)</td>
<td>92,XXYY (20)</td>
<td>92,XXYY (16)</td>
<td>92,XXYY (31)</td>
<td>---</td>
</tr>
<tr>
<td>88-13</td>
<td>46,XY/92,XXYY</td>
<td>46,XY (17)</td>
<td>92,XXYY (12)</td>
<td>92,XXYY (3)</td>
<td>92,XXYY (8)</td>
<td>---</td>
</tr>
<tr>
<td>89-09</td>
<td>46,XX/47,XX,+16</td>
<td>47,XX,+16 (15)</td>
<td>---</td>
<td>47,XX,+16 (15)</td>
<td>46,XX/47,XX,+16 (13)</td>
<td>---</td>
</tr>
</tbody>
</table>
Mosaic Case 87-05:

Case 87-05 was the first spontaneous loss of a 39 year old mother with an unremarkable reproductive history (Gravida 4 and Para 3). The specimen was a ruptured chorionic sac of 10 weeks gestation with stingy avascular chorionic villi. These first trimester villi were characteristically irregularly developed with scalloped trophoblast borders. Abnormal amnion/chorion fusion was identified and suggestive of chromosomal heteroploidy.

A normal chromosomal constitution was observed in a total of 36 metaphases from the cytotrophoblast, amnion, and umbilical cord. A pure trisomy 4 constitution was identified from 31 metaphases of the chorionic villous stroma and chorionic plate (Table 3.6).

Mosaic Case 87-66:

This specimen was the first pregnancy of a 34 year old mother. The specimen was 10 weeks of gestation and consisted of an intact chorionic sac containing a growth disorganized embryo. The chorionic villi were cystic and avascular with proliferative buds. Histological examination of the chorionic sac revealed the presence of well developed amniotic membrane and chorionic plate. The decidua showed focal haemorrhage and inflammation.

All of the 20 cytotrophoblast derived metaphases were cytogenetically normal. In each of the long term tissue cultures tetraploidy was observed. Fifteen metaphases from three explant colonies on one tissue culture dish was used to
assess the amnion. Twenty-eight metaphases from two culture dishes containing a total of 10 explant colonies of chorion were analyzed. A total of 29 metaphases from 2 culture dishes containing 7 explant colonies of chorionic villous stroma were analyzed (Table 3.6).

**Mosaic Case 88-13:**

This specimen was diagnosed as a missed abortion by ultrasound and had been medically removed from the uterus. It was the second spontaneous loss of a 25 year old mother (Gravida 4 and Para 2). The specimen was a ruptured chorionic sac of 12 gestational weeks. Although the chorionic villi appeared cystic and avascular, histological examination identified stromal vessels and embryonic blood cells. The decidua showed focal necrosis and inflammation.

A normal chromosomal constitution was observed in all 17 metaphases of the cytotrophoblast. In each of the long term tissue cultures a tetraploid constitution was identified. Eight analyzable metaphases were observed for 3 amnion explant colonies. Only 3 metaphases from the chorionic plate were analyzable. Three culture dishes containing a total of 11 explant colonies of chorionic villous stroma had 12 analyzable metaphases (Table 3.6).
Mosaic Case 89-09:

This specimen was the third spontaneous loss for a 36 year old mother (Gravida 5 and Para 2). It consisted of a ruptured sac with an abnormal embryo of 30 days of developmental age. Abnormal amnion/embryo attachment was observed to extend caudally from the anterior mid section of the embryo to the tail. The embryo lacked normal spinal curvature and the branchial arches and limbs had not yet developed. The spine and associated somites, and optic placodes were visible. No umbilical cord was identified. This specimen was retained within the uterus for 1 month prior to its evacuation. No gross morphological description of the chorionic villi was available. Histological examination revealed typical first trimester chorionic villi morphology with hydropic degenerative changes.

Cytogenetic evaluation was based on the cytotrophoblast and the long term culture of both amnion and chorion (Table 3.6). A single cell line of trisomy 16 was observed in the 15 metaphases of cytotrophoblast and 15 metaphases from 3 explants of chorion. A mixture of diploid and trisomic 16 metaphases were identified in both explant dishes and trypsinized dishes of amnion. In total 13 diploid metaphases and 26 trisomy 16 metaphases were identified in the amnion of this specimen.
3.3 Flow Cytometric Analysis

3.3 a. Amnion Study

Ten samples of amnion from pregnancies ranging from 11 to 42 weeks of gestational age were simultaneously analyzed by cytogenetic and flow cytometric techniques for the presence of tetraploid cells. The purpose of this amnion study was to determine whether or not a correlation between cytogenetic and flow cytometric findings exist. Eight of the samples submitted for flow cytometric analysis were successfully analyzed. Dr. Leslie Mitchell’s interpretations have been summarized in Table 3.7.

Ten metaphases were cytogenetically analyzed per tissue sample and found to correspond to the flow cytometric results. In each of the 2n\4n cases, only one of the 10 metaphases examined was tetraploid.

Table 3.7
A comparison of cytogenetic and flow cytometric analysis of 10 amnion samples.

<table>
<thead>
<tr>
<th>ANALYSIS:</th>
<th>2n</th>
<th>2n\4n</th>
<th>Failed</th>
<th>Cells Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYTOGENETIC</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>EPIC FLOW CYTOMETRIC</td>
<td>6</td>
<td>2**</td>
<td>2</td>
<td>14,830*</td>
</tr>
</tbody>
</table>

* average number (ranged from 8,246-20,000)
** >15 % in G2+M peak
3.3 b. Diploidy/Tetraploidy Mosaicism Verification

This specimen, a ruptured chorionic sac was the second loss for a 42 year old mother with unremarkable reproductive history (Gravida 4, Para 2). The chorionic villi were processed by both long term tissue culture and direct preparation techniques by Irene Barrett of the Research Centre Cytogenetic Laboratory. The chorion was processed and analyzed by the Children's Hospital Cytogenetic Laboratory. The cytogenetic results produced in 2 different laboratories have been summarized in Table 3.8.

Table 3.8

Cytogenetic analysis of the diploid/tetraploid mosaic conceptus.

<table>
<thead>
<tr>
<th>TISSUE:</th>
<th>NO. OF CELLS:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2n</td>
</tr>
<tr>
<td>Cytotrophoblast(^1)</td>
<td>12</td>
</tr>
<tr>
<td>Villous Stroma(^1)</td>
<td>-</td>
</tr>
<tr>
<td>Chorion(^2)</td>
<td>-</td>
</tr>
</tbody>
</table>

NO. = Number
1 = Research Centre Cytogenetic Laboratory
2 = Children's Hospital Cytogenetic Laboratory

Chorion and chorionic villi samples were also submitted for flow cytometric analysis. Both the villi and chorion were diagnosed as containing tetraploid populations, as 14.9 % and 34.9 % of the cells (30,000 per tissue) were observed in the G2+M peak respectively.
4.1 Documentation of Confined Mosaicism

Among 40 studied spontaneously aborted specimens, 29 were cytogenetically abnormal and consisted of a variety of complete trisomies, polyploidies, structural rearrangements, and monosomies of chromosome X. The frequency of occurrence of these aneuploid and polyploid constituents were similar to those observed in previous spontaneous abortion studies (Boue et al., 1975; Creasy et al., 1976; Warburton et al., 1978; Hassold et al., 1980a; Kajii et al., 1980). Of the 6 most commonly observed trisomies in previous abortus studies (16, 22, 21, 15, 14, 18), 4 were observed in this study, with the most prevalent being chromosome 16, followed by 21, 22, and then 14. Relatively rarer trisomies, namely 4, 8, 10, 20, and one double trisomy were also observed in this study.

Confined placental mosaicism was detected in 4 of the 40 early spontaneous abortion specimens studied; 2 of these involved chromosomal trisomy and 2 tetraploidy, representing 15% of the total number of trisomic specimens and 100% of the total number of tetraploid specimens observed in this study. No generalized mosaicism was detected in this population.
This study was designed to cytogenetically differentiate between confined versus generalized mosaicism by examining multiple tissues for each abortus specimen. In all previous cytogenetic investigations of spontaneous abortion specimens, the mosaicism detected was assumed to be generalized throughout the conceptus. The accepted frequency of mosaicism in the spontaneous abortion population has been 1.1 % of the 4477 abortuses examined by long term tissue culture techniques (Boue et al., 1975; Creasy et al., 1976; Warburton et al., 1978; Hassold et al., 1980; Kajii et al., 1980) and 2.5 % of the 1023 abortuses examined by direct preparation techniques (Eiben et al., 1986; 1987; 1990; Bernert et al., 1988). However, these studies made no distinction between confined and generalized forms of mosaicism, since only one tissue per specimen had been analyzed. When a 95 % confidence interval was applied to this study's data, the frequency of confined mosaicism for spontaneous abortions ranged from 1-19 % (Appendix I). Thus the frequency of mosaicism observed in this relatively small population was not unlike that observed in previous spontaneous abortion studies, with the exception of the type of mosaicism observed.

The chorionic villi sampled (CVS) pregnancies represent a control group population for the frequency of confined mosaicism in viable conceptuses of similar gestational age to the group of spontaneous abortions examined in this study.
The frequency of confined mosaicism observed in these specimens has been 1-2 % which is consistent with the observed frequency in this study. A variety of autosomal trisomies, monosomy X, tetraploidies, and structural rearrangements have been observed in mosaic form with euploid cell lines in CVS samples (Schwinger et al., 1989).

The relatively large ratio of confined mosaic specimens to the total population examined may be due to a number of unavoidable selection biases. For example, the types of specimens sent to the Embryofetalpathology Laboratory were often associated with gross morphological abnormalities and older mothers with poor reproductive histories. Specifically, the specimens shared between this study and the Clinical Cytogenetic Laboratory were subject to bias, since many of these specimens were associated with known trisomic morphology and/or with habitual aborters. Thus one would expect an unusually high percent of chromosomally abnormal specimens from habitual aborters. This was not the case, as the 73 % overall chromosomal abnormality ratio falls within the previously observed range of chromosomal anomalies for spontaneous abortions (Boue et al., 1975; Creasy et al., 1976; Warburton et al., 1978; Hassold et al., 1980a; Kajii et al., 1980). In addition, half of the specimens observed in this study were first time losses and only 30 % of the
specimens were the third or greater spontaneous loss. A bias exists for the selection of abortuses form older mothers, since the majority (63 %) of the specimens were from mothers between the ages of 30 to 39. It has been well documented that an increase in maternal age is associated with a greater frequency of nondisjunction resulting in trisomic constitutions (Hassold et al., 1980b). The degree to which an increased maternal age affected the results of this study is difficult to determine. Although a number of selection biases have influenced this data, they have not been unlike the selection biases experienced by previous spontaneous abortion studies.

4.1 a. Diploid/Trisomy Mosaics

The most common form of mosaicism observed in traditional long term tissue culture studies of spontaneous abortions was diploid/trisomy mosaics. Trisomic constitutions of chromosomes 2, 4, 3, 8, 10, 16, 18, 20, and 22 have been previously documented in association with diploid cell lines (Warburton et al., 1978; Hassold et al., 1980a; Kajii et al., 1980). Four cases of double trisomy involving chromosomal combinations of 2 and 16, 2 and 22, 16 and 20, and 21 and 22 have been associated with trisomy cell lines of 16, 22, 16, and 21 respectively (Warburton et al., 1978; Hassold et al., 1980a).
Both of the confined placental diploid/trisomic mosaics observed in this study had a female karyotype and they involved chromosomes 4 and 16. In the 46,XX/47,XX,+4 specimen, 100% of the metaphases analyzed were trisomic in the cultured villi and 100% of the metaphases analyzed were diploid for the cytotrophoblast, chorion, amnion, and cord. Hence the trisomic cell line was confined to the chorionic villi. In the 46,XX/47,XX,+16 specimen, 100% of the metaphases analyzed were trisomic for the cytotrophoblast and chorion. The mosaicism was confined to the amnion, which consisted of 33.3% diploid and 66.6% trisomic cells.

Maternal contamination has often been sited as a source of error when documenting female diploid/trisomic mosaic cases. In this study, the probability of this error was reduced by the examination of each of the tissues by a dissection microscope for the presence of decidua. The euploid and aneuploid karyotypes were subjectively compared for the heteromorphic regions of Giemsa banded chromosomes and found identical. Since only the cytotrophoblast is cytogenetically analyzable after short term incubation, its diploid karyotype reduces the possibility for maternal contamination for the 46,XX/47,XX,+4 case.

4.1 b. Diploid/Tetraploid Mosaics

Although diploid/tetraploid mosaics have been frequently observed in cultures from spontaneous abortion specimens, these tetraploid metaphases have been attributed to in vitro tissue culturing artifact (Hunt and Jacobs, 1985a). These
culturing artifacts may include the induction of tetraploidy with (1) the use of colchicine in the harvesting procedure and (2) advanced in vitro aging prior to the harvesting of metaphases (Kaji and Matsuo, 1981; Chamla et al., 1980). Chamla et al. (1980) observed an enhanced rate of tetraploidy in fibroblast cultures with the application of 0.4 g/ml of colchicine for 2.5 hours prior to harvesting. It is unlikely that the addition of colchicine for 2.5 hours would be capable of inducing an endoreduplicated event, since the replication cycle of fibroblasts is 36 hours in length (Laila, 1988). It is equally unlikely that in vitro aging could account for the chromosomal abnormalities observed in this study, since the human fibroblast remains euploid for approximately 50 generations, before the fibroblast culture undergoes senescence and death (Freshney, 1987). Since the cell cycle of the human fibroblast has been estimated to be 36 hours (Laila, 1989), the maximum number of generations to elapse in tissue culture prior to harvesting in this laboratory was fourteen.

Since the introduction of chorionic villus sampling for first trimester prenatal diagnosis, the direct preparation technique has also been applied to the chorionic villi of spontaneous abortions. Similar cytogenetic abnormalities as seen with traditional long term tissue culture methods have been observed, with the exception of the types of mosaics identified. The majority (84 %, 21/25) of the identified mosaic specimens consisted of diploid/tetraploid constitutions, where the tetraploid cell line
was greater than 20% of the metaphases analyzed (Eiben et al., 1986; 1987; 1990; Bernert et al., 1988). Cultural artifact is an unlikely explanation for this diploid/tetraploid mosaicism, since the culturing duration was approximately 24 hours (Eiben, et al., 1990). These authors have suggested that the occurrence of diploid/tetraploid conceptuses may be more frequent than previously suggested (Eiben et al., 1990).

In this study 2 karyotypically male diploid/tetraploid mosaics were observed. In both cases 100% of the metaphases analyzed of the cultured villi, chorion, and amnion were tetraploid and 100% of the cytotrophoblast metaphases analyzed were diploid. In order to confirm these findings and to rule out culture artifact, the flow cytometric studies were initiated. I have compared the frequency of tetraploidy in cultured amnion versus uncultured amnion. The tetraploidy was found in 10% of the metaphases from cultured amnion samples, which corresponded well with the identified low level of tetraploidy observed by flow cytometry. Tetraploidy was also confirmed by flow cytometric analysis in a diploid/tetraploid specimen observed by Irene Barrett of the Research Centre Cytogenetic Laboratory. In this diploid/tetraploid specimen, 20% of the analyzed metaphases of the cytotrophoblast were tetraploid and 100% of the analyzed metaphases of the chorionic villi and chorion were tetraploid. Unfortunately, this type of comparison was not available for the two diploid/tetraploid specimens observed in this study, as no tissue for flow cytometry was available.
4.1 c. Difficulties Studying Confined Mosaicism

The observation of an additional cell line at the frequency of 20 % or greater has been diagnostic for mosaicism. This frequency however does not necessarily represent the true frequency of mosaicism for the tissue being analyzed. The true frequency of mosaicism may be masked by a number of factors such as (1) the assumption that a relatively small piece of tissue is representative of the whole, (2) the selection for or against aneuploid cells in tissue culture, and (3) the assumption that the 15 analyzed metaphases are truly representative of the total metaphases available. Hook (1977) had developed a table of confidence limits which excludes mosaicism within a tissue on the basis of the number of cells analyzed. For example, when 15 cells are analyzed, as in this study, and are found to be identical, the level of mosaicism of more than 19 % can be excluded with 95 % confidence (Hook, 1977). Hence low levels of mosaicism in sampled tissue may be unobserved.

The documentation of confined mosaicism requires the analysis of multiple placental tissues. As a result, only those cases which yielded cytogenetic results for both the cytotrophoblast and at least 2 long term cultured tissues were accepted into
this study. Thus the cytogenetic data available for the 71 incomplete specimens was not analyzed. In all previous spontaneous abortion studies only one representative tissue was analyzed, with exception of one investigation (Yu et al., 1987). In addition, the origin and/or type of sampled tissue was often not identified, and varied from actual fetal biopsies to pieces of the placental membrane (Warburton et al., 1978; Hassold et al., 1980a). In many of these spontaneous abortion surveys, a cytogenetic diagnosis was made on as few as 3 cells (Boue et al., 1975; Creasy et al., 1976; Hassold et al., 1980a). When less than 4 cells are analyzed, a level of mosaicism less than or equal to 50% cannot be excluded from the analyzed tissue (Hook, 1977).

4.2 Etiology of Confined Mosaicism

The origin of chromosomal mosaic confinement to specific tissues must relate back to the derivation of the tissues themselves, and ultimately the developmental process involved in the generation of the embryo proper and placenta from the zygote. The mosaic constitution results from a chromosomal mutation early during development giving rise to 2 or more cell lines with different chromosomal complements (Kalousek and Dill, 1983; Kalousek, 1988). The resulting pattern of mosaicism is dependent upon (1) the cell lineages involved and therefore the timing of the mutational event and (2) the toleration of the mutation and therefore the viability.
of the affected tissues. For example, some trisomic constitutions are thought to be more lethal than other constitutions, as seen by the differences in their frequency of occurrence (Jacobs and Hassold, 1986). Thus the observation of confined mosaicism through cytogenetic analysis of multiple tissues may reflect some of the processes involved in mammalian development.

4.2 a. Origins for Confined Mosaicism in the Specimens Observed

The confinement of chromosomal mosaicism to specific tissues requires an attempt to explain its limited presence. In this study the simplistic cell lineage discussed in the introduction was used.

4.2 a. (i). 46,XX/47,XX,+4 Mosaic Specimen

The trisomic cell line was confined to the chorionic plate and villous stroma; the remaining tissues were diploid. Thus the nondisjunctional event leading to the occurrence of the trisomic cell line in a diploid conceptus most likely took place prior to or during the delineation of the inner cell mass precursor cells to the extraembryonic mesoderm tissues. The cells giving rise to the embryonic ectoderm have remained unaffected, as confirmed by the diploid amnion (Figure 4.1).

The observation of purely trisomic villous stroma fibroblasts was unexpected. The stroma is thought to be derived mainly from extraembryonic mesoderm, but also has a small contribution from the migration of cells from the primitive streak.
The diagnosed bias toward the trisomic cell line in chorion may be explained by (1) the site of villous sampling relative to the site of the primitive streak, (2) failure to identify mosaicism due to sampling error, and (3) the inability of the diploid cells to migrate into the trisomic cell environment.

Since the cytotrophoblast tissue was also diploid, the progenitor cells of the trophoblast cell line must have been unaffected by the mitotic error. Hence the conceptus is most likely to have arisen from a diploid zygote.

**Figure 4.1**
The suggested location for the mutation during development giving rise to confined mosaicism in the 46,XX/47,XX,+4 specimen.
4.2 a. (ii). 46,XY/92,XXYY Mosaic Specimens

In both of these specimens, only diploid cells were identified in the cytotrophoblast and only tetraploid cells were identified for each of the long term tissues. This suggests that an early cleavage error took place during development, affecting all the cells of the inner cell mass of a diploid zygote (Figure 4.2).

Figure 4.2
The suggested location for the mutation during development giving rise to the two confined mosaic 46,XY/92,XXYY specimens.

This form of confined mosaicism had been previously observed by Yu et al. (1987), who described the long term tissue cultured chorionic villi as tetraploid and the direct preparation of the chorionic villi as diploid. Yu et al. (1987) suggested that
the tetraploidy observed in a diploid/tetraploid conceptus resulted from culturing artifact. This interpretation is unlikely for our specimens, since all of the original explant dishes of the long term tissues manifested the identical alteration and a similar specimen studied after the closure of my study showed the presence of tetraploid cells in uncultured chorionic villi and chorionic plate.

4.2 a. (iii). 46,XX/47,XX,+16 Mosaic Specimen

All 3 major cell lineages of the conceptus were represented by the cytogenetic analysis of amnion, chorion, and cytotrophoblast. A pure trisomic 16 cell line was present in both the cytotrophoblast and the chorion. The amniotic tissue itself was mosaic, having both diploid and trisomic 16 cell lines.

In this case, the mitotic error producing the diploid cell line in the amnion likely occurred in the embryonic progenitor cells to affect the embryonic ectoderm. However, not all of the embryonic progenitor cells were affected, thus resulting in a mixture of diploid and trisomic cells within the amnion (Figure 4.3).
4.3 Significance of Confined Mosaicism

The purpose of this study was to assess both frequency and types of confined mosaicism in the spontaneous abortion population. In this study, 10% of studied specimens revealed a confined mosaic constitution suggesting that this chromosomal defect is common in spontaneous abortion specimens. In addition, the observation of diploid/tetraploid confined mosaic specimens suggests that tetraploidy may be a more common cytogenetic defect in the spontaneous abortion population than previously reported in the literature.
The detected mosaicism is confined to a specific cell lineage, such as trophoblast, extraembryonic mesenchyme or embryonic ectoderm. Thus for each of the mosaic cases the approximate timing of the mutational event during early embryonic development can be implied based on the involvement of specific developmental cell lineage(s) and under the assumption that the viability of the mutation is the same in all embryonic tissues.

The detailed analysis of cytogenetic findings of the mosaic cases indicates that among the 4 mosaic spontaneous abortion specimens, chromosomal mutation occurred in 3 diploid zygotes and 1 trisomic zygote. In the 46,XX/47,XX,+4 mosaic specimen nondisjunction resulted in the formation of a trisomic cell line; in the 46,XX/47,XX,+16 mosaic specimen either nondisjunction or anaphase lag resulted in the formation of a diploid cell line. The remaining two 46,XY/92,XXYY mosaic specimens resulted from cleavage errors.

The resulting cell lineages affected by a mutation were the extraembryonic mesoderm (46,XX/47,XX,+4), the complete inner cell mass (46,XY/92,XXYY) and the embryonic ectoderm (46,XX/47,XX,+16). Thus both confined placental mosaicism as well as, confined embryonic mosaicism was demonstrated.

Although the contribution of confined chromosomal mosaicism to the etiology of pregnancy loss is not clearly understood, any alteration of the genomic content of developmental cell lineages may effect the function of the resulting tissue(s) and therefore the survival or demise of the conceptus.


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Roberts CJ and Lowe CR, "Where have all the Conceptions gone?" Lancet, March 1, 1975.


APPENDIX I

Figure 1  Trypsin Giemsa banded long term tissue culture chromosomes.

Figure 2  Giemsa solid stained direct preparation chromosomes.
<table>
<thead>
<tr>
<th>STAGE</th>
<th>DESCRIPTION</th>
<th>AGE (days)</th>
<th>DEVELOPMENTAL PROCESSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>zygote</td>
<td>-</td>
<td>fertilization; 1st cleavage</td>
</tr>
<tr>
<td>2</td>
<td>morula</td>
<td>1-1.5</td>
<td>cleavages; compaction; differentiation into inner &amp; outer cells</td>
</tr>
<tr>
<td>3</td>
<td>free blastocyst</td>
<td>4-4.5</td>
<td>secretion by trophoblastic cells; development of intercellular clefts, confluence into blastocyst cavity</td>
</tr>
<tr>
<td>4</td>
<td>attached blastocyst</td>
<td>5.5-6</td>
<td>adhesion to endometrium</td>
</tr>
<tr>
<td>5</td>
<td>implanting blastocyst</td>
<td>7</td>
<td>Implantation = differentiation into cytotrophoblast &amp; syncytiotrophoblast, proliferation of extraembryonic endoderm, development of primary yolk sac, endodermal reticulum, &amp; amniotic cavity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 development of trophoblastic lacunae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11 circulation in trophoblastic lacunae</td>
</tr>
<tr>
<td>6</td>
<td>embryonic disc</td>
<td>13</td>
<td>proliferation of extraembryonic mesoderm, development of chorionic cavity &amp; villi; transformation of primary yolk sac into secondary; development of primitive streak &amp; formation of embryonic mesoderm</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>15</td>
<td>development of notochordal process, connecting stalk, allantoic diverticulum, blood &amp; blood vessels</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>17</td>
<td>development of primitive pit, chordal canal, &amp; canalis neurentericus</td>
</tr>
<tr>
<td>9</td>
<td>embryo proper</td>
<td>19</td>
<td>development of neural folds, somites 1-3; onset of folding process of yolk sac</td>
</tr>
</tbody>
</table>
Figure 3  Flow Cytometric Analysis Data

a. Normal Diploid Tissue:

b. Tetraploid Chorionic Villi:

c. Tetraploid Chorionic Plate:
Statistical Analysis Applied to Data:

Equation 1
Confidence interval for the frequency of confined mosaicism in the spontaneous abortion population (Walpole, 1968).

\[(1 - \alpha)100\% = \hat{p} - z_{\alpha/2} \cdot \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}\]

where \(\hat{p} = \frac{x}{n}\)

\(\hat{q} = 1 - \hat{p}\)

\(\alpha/2 = 1.96\) for 95 % confidence interval

\(x = 4\) confined mosaic cases

\(n = 40\) analyzed specimens

The frequency of confined mosaicism is 1-19 % of the total spontaneous abortion population, with a 95 % confidence interval.