INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

WILFON, SUSAN GAIL

CHROMOSOMAL STUDIES OF RECURRENT SPONTANEOUSLY ABORTING COUPLES

THE UNIVERSITY OF ARIZONA M.S. 1984

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages __√__
2. Colored illustrations, paper or print __√__
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages _____
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

University
Microfilms
International

CHROMOSOMAL STUDIES OF RECURRENT SPONTANEOUSLY ABORTING COUPLES

by

Susan Gail Wilfon

A Thesis Submitted to the Faculty of the

COMMITTEE ON GENETICS

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCES

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 8 4

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations form this thesis are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Susan Gail Wilfox

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

J. R. DAVIS
Dept. of Pathology

April 24, 1984
Date

DEDICATION

I would like to thank Dr. Davis and the ladies at the Cytogenetics Laboratory for all of their assistance. I could not have finished without them.

Mostly, I want to thank Duane for his love and encouragement, and my parents for their patience and support.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABSTRACT

Many researchers have suggested that chromosomal abnormalities may be related to couples with recurrent spontaneous abortions. One cause of these abnormalities could be nondisjunction of the chromosomes due to satellite associations.

Fifteen couples with various reproductive histories, along with thirty individuals of unknown fertility were the subjects of this study. Fifty cells for each individual were examined under a microscope and any chromosomal abnormalities and satellite associations were noted. Two control subjects and one infertile male had an abnormality in one cell out of fifty. Three of the thirty infertile individuals had a sex chromosome variation in a few cells. One of these could be a case of low mosaicism, the others are probably spurious aberrations. An increase in satellite associations was found in one individual per infertile couple with two exceptions.

This information along with other studies can be used in counselling of couples with recurrent spontaneous abortions.

CHAPTER 1

INTRODUCTION

Extensive research has been conducted on couples who have had recurrent spontaneous abortions. Many causes have been cited which may lead to spontaneous abortion, which is the natural termination of a fetus prior to the stage in which it is viable. Uterine abnormalities, systemic diseases, hormonal imbalances, immunologic factors, anatomical defects and genetic errors are some of the well documented causes of spontaneous abortions (Ward, Henry, and Robinson 1980, Byrd, Askew, and McDonough 1977), but their effects on recurrent fetal wastage are not known. It is well documented, however, that chromosomal abnormalities and irregular disjunction are major contributing factors to both single and recurrent spontaneous abortions (Blumberg et al. 1982, Stoll 1981).

According to several studies (Ward et al. 1980, Byrd et al. 1977, Blumberg et al. 1982) approximately forty to sixty percent of first trimester spontaneously aborted fetuses have a chromosomal abnormality. Conflicting evidence from the Geneva Conference states that only nineteen percent of spontaneous abortions are

due to chromosomal abnormalities (Byrd et al. 1977). However most are probably due to random events occurring in meiosis or early mitosis (Bhasin, Forester, and Fuhrmann 1972). Some of these abnormalities are due to chromosomal anomalies occurring in one of the parents, such as balanced translocations and inversions. In one study it was suggested that there is an occurrence during meiosis in which a chromosome carrying a structural abnormality produces an anomaly which involves a different pair of chromosomes. This could help explain the increased frequency of chromosomal abnormalities among couples with repeated spontaneous abortions (Stoll, Flori, and Beshara 1978, Kardon et al. 1980). However, many researchers believe that interchromosomal interference is simply a myth.

Chromosomal Abnormalities

A number of studies have been conducted in order to determine the cause of recurrent spontaneous abortions. Heritage et al. (1978) studied thirty-seven couples with histories of two or more spontaneous abortions, and found two translocation carriers and a trisomy X female. In another paper, Neu, Entes, and Bannerman (1979), found one case of a translocation carrier after examining thirty couples and two individuals. Ward et al. (1980) did a study of one hundred couples, six of whom had a

chromosomal variation, but none of which had a translocation. In a previously mentioned report one balanced translocation carrier and an individual with a pericentric inversion were found (Bhasin et al. 1972). Davis et al. (1982), studied one hundred couples with various abortion histories. Eight of these individuals had a balanced translocation, two had a structural variant and one had a poly-X mosaicism. In comparing the results of these studies, it can be shown that there is an increased frequency of chromosomal abnormalities in couples with recurrent spontaneous abortions. However, the percent of these couples with an abnormality ranges from 2 to 18.75 percent (Osztovics, Toth, and Wessely 1982).

One of the recent studies (Fitzsimmons, Naper, and Jackson 1983) showed an increase in the frequency of chromosomal abnormalities with an increase in the number of spontaneous abortions. 1.8 percent of the couples with two consecutive abortions had a chromosomal abnormality as compared with 2.3 percent of the couples with three or more abortions. They also found that couples with first trimester losses had an increased frequency of chromosomal aberrations (Fitzsimmons et al. 1983). In another study, Husslein et al. (1982), examined the chromosomes of one hundred and fifty couples with recurrent fetal wastage and found 4.7 percent were chromosomally abnormal.

They also found that the chances of carrying an infant to term become reduced with an increase in miscarriages. In contrast to this, Osztovics et al. (1982), found that there was no significant correlation between the number of miscarriages and the proportion of chromosomal abnormalities in the individuals studied. Another important point to note is that in Osztovics' et al. (1982) study, there was no instance of both partners of a couple having an abnormality. After carefully examining all of these reports, it can clearly be shown that there is a strong correlation between repeated spontaneous abortions and chromosomal abnormalities.

Nondisjunction and Satellite Associations

Researchers have also studied the causes and effects of nondisjunction and how they relate to spontaneous abortions. Nondisjunction is the failure of chromosomes to disjoin or move to separate poles during anaphase of either the first or second meiotic division or during a mitotic division. If nondisjunction occurs during mitosis the result will be a mosaic cell line. However, if nondisjunction occurs during meiosis it can yield either a monosomic zygote or a trisomic zygote which will either fail to implant, or may implant but later abort. Most nondisjunctions will lead to some form of aneuploidy such as trisomy, or monosomy. Many studies

have shown that balanced translocations may lead to abnormal disjunction of other chromosomes during meiosis or early mitosis. This could explain the high association of balanced heterozygous translocations with repeated spontaneous aborters (Tsenghi et al. 1976, Kardon et al. 1980, Husslein et al. 1982, Stoll et al. 1978). Other minor abnormalities such as inversions could also predispose chromosomes to nondisjunction (Husslein et al. 1982). The consequences of nondisjunction may lead to the birth of a live trisomic infant, but more often will result in a chromosomally abnormal, spontaneously aborted fetus (Fitzsimmons et al. 1983).

It is very possible that satellite associations may also lead to nondisjunction. The proximal region of satellites include the loci for the production of ribosomal DNA. It is in this region that the nuceolar material forming a nucleolus is synthesized during early interphase. The nucleoli of the satellite chromosomes may associate, and the association may persist during cell division (Zankl and Zang 1974). These satellite associations may possibly be a predisposing factor to abnormal disjunction of the satellite chromosomes, leading to trisomies involving the D and G group chromosomes (Hansson 1970, Mattei et al. 1976, Zankl and Zang 1974).

In Hansson's study (1970), numerous other reports were mentioned in which an increase in satellite associations was found among mongoloids and their mothers. Zankl and Zang's paper of 1974 showed an increase in the percentage of acrocentric chromosomes which had variant satellites. They also showed that those chromosomes with enlarged satellites associated more frequently than those with normal or small satellites. Mattei et al. (1976), showed that the number of associations did not depend on sex, but did however, increase with age, though not significantly until after the age of thirty-three. Therefore it is important to note satellite associations when studying those individuals with fertility problems.

Objectives

The objective of this thesis was to study and test the hypotheses that chromosomal abnormalities and/or satellite associations, could be contributing factors to the cause of recurrent spontaneous abortions. This was attempted by examining the chromosomes and satellite associations of fifty cells from each partner of an infertile couple and comparing the findings with those found in the same number of control subjects. According to research already conducted on this topic, an increased number of chromosomal abnormalities and/or an increased

number of satellite associations in the infertile couples as compared to the control subjects was expected. This would help to explain the recurrent spontaneous abortions associated with these couples.

CHAPTER 2

MATERIALS AND METHODS

In the course of clinical studies in the Cytogenetics Laboratory at the Arizona Health Sciences Center, samples of blood from couples with fertility problems are received. These infertile couples have various reproductive histories, however, most have had two or more spontaneous abortions. Fifteen consecutive couples were selected to be studied, some of which had normal or stillborn children in addition to a history of spontaneous abortions. One couple had not yet been able to become pregnant. Therefore, unlike other studies, the precise reproductive histories will be given only minor emphasis.

During this study, control subjects were selected by matching their age and sex to one of the partners in an infertile couple. Most of the control subjects were of unknown fertility, since only a few of them had had pregnancies. These individuals were all selected at random.

The procedures, methods and reagents used in this study were identical for the infertile couples as well as for the normal individuals. Approximately five to seven milliliters of blood were drawn from each individual

into a sodium heparin tube. This was then set up in four tubes per individual of RPMI plus phytohaemagglutinin. Two tubes were incubated for seventy-two hours and then harvested. The two remaining tubes were incubated for a total of ninety-four hours. At the end of seventy-two hours, 0.05 milliliters of methotrexate was added to each tube. Seventeen hours later at the end of eighty-nine hours of incubation, the cells were washed in unsupplemented RPMI and resuspended. 0.05 milliliters of thymidine was then added and the cultures were incubated for an additional four and a half hours and harvested. Slides were made within twenty-four hours after harvesting and placed on a slide warmer overnight. The slides were then banded and stained. Fifty cells were scored on each individual and any chromosomal abnormalities and satellite associations were noted. For those individuals in which an abnormality was found, an attempt was made to score a total of one hundred cells, however no abnormalities were found in the second group of fifty cells for any of the individuals with an abnormality. Pictures were taken of two cells per individual in the couples and one cell per individual in the controls, and any cells containing an abnormality, in order to construct karyotypes. In addition to these cells, approximately five others per individual were karyotyped. Of the fifty cells examined some were methotrexate and some were routinely processed

depending on the mitotic index for each culture. The following procedures adapted from the protocols of the Cytogenetics Laboratory at the Arizona Health Sciences Center, were used.

Protocol 2: Peripheral Blood for Chromosome Analysis

Procurement

Five to seven milliliters of blood was drawn from each individual into a sodium heparin (green stopper #3204-KA) vacutainer tube.

Cultivation

1. The tube of blood was gently inverted to mix. Six to seven drops of blood were added to two five milliliter centrifuge tubes of medium which were previously warmed to 37^0C. The tubes were then placed, slightly tilted, in a CO_2 incubator at 37^0C for seventy-two hours.

2. One hour before harvesting, two drops of colcemid were added to the tubes, inverted to mix, and placed back in the incubator.

3. At the end of seventy-two hours, the tubes were once again inverted to mix and centrifuged for five minutes at 1200 rpms.

4. The supernatant was poured off and the cells resuspended in five milliliters of 37^0C hypotonic and placed in the incubator for an additional fifteen minutes.

5. At the end of the incubation time, the tubes were centrifuged for five minutes at 1200 rpms, the supernatant was poured off and the cells were resuspended in five milliliters of cold fixative, making sure that all of the cells were suspended as to avoid clumping.

6. The tubes were then placed in the freezer for at least two hours.

7. The tubes were then centrifuged and the old fixative was removed. The cells were then resuspended in new fixative and the procedure repeated two more times.

8. The cells were then resuspended and slides were made.

Slide Preparation

1. Slides were cleaned using distilled water and the excess was shaken off. Approximately six drops of cell suspension were dropped onto a slide from one inch above. If the chromosomes were not spreading well, the slides were steamed immediately after the solution was dropped. If the chromosomes were spreading too much, hot water was used to clean the slides rather than distilled. Other alternations were made in the technique depending on the level of humidity. The slides were then examined on a phase microscope and checked for spreading of the chromosomes and the mitotic activity. The slides were then labeled with the individual's number, the date and the

slide number.

2. The slides were then placed on the warmer overnight and removed the following day.

Analysis and Karyotyping

1. The slides were then banded (see Protocol 8).

2. Fifty cells in metaphase were found on low power (10X) and then observed under oil (100X). The position of the cell on the slide was recorded. All of the chromosomes in every spread were counted and five to eight cells were karyotyped. All abnormalities, aneuploidies and satellite associations were recorded.

3. Two cells for each infertile individual and one cell for each of the controls was selected for photographing.

4. The chromosomes were then cut from the pictures and karyotyped.

Protocol 8: Giemsa-Trypsin Stain Modification for Banding

1. Pancreatin solution diluted in Hank's BBS was used for banding at a 1:40 or 1:20 dilution depending on banding. The solution was kept at room temperature or at 37^{0}C. The slides were dipped in the solution for varied amounts of time, but no longer than two seconds. If the time, temperature or strength of the solution were increased, the banding also increased.

2. The slides were then rinsed immediately in tap water and stained in Giemsa for two and a half to three minutes. The slides were then air or blown dried.

3. The dry slide was then dipped in Xylene and mounted onto a coverslip using coverbond.

4. The dry slide was then scanned for metaphases.

Protocol 11: Methotrexate Treatment of Peripheral Blood Cultures to Yield Long Chromosomes for High Banding Resolution

1. When the blood was received, two additional five milliliter tubes were set up and incubated for seventy-two hours, as described in Protocol 2.

2. After seventy-two hours of incubation, one drop of methotrexate at 10^{-5}M concentration was added to each tube to make a final concentration of 10^{-7}M.

3. Seventeen hours later the cultures were centrifuged and washed once with five milliliters of unsupplemented RPMI. The supernatant was then poured off and an additional five milliliters of RPMI was added. One drop of thymidine at 10^{-3}M was also added to the cultures and they were inverted to mix.

4. The tubes were then returned to the incubator and allowed to grow for an additional four and a half hours. Two drops of colcemid were added fifteen minutes before the time was up.

5. The cultures were then harvested as in Protocol 2.

Reagents

RPMI-1640 (Gibco)

Fetal Bovine Serum (Gibco)

Penicillin-Streptomycin (Gibco)

Phytohaemagglutinin, lyophilized in five milliliters of sterile distilled water

Colcemid (Gibco), resuspend in ten milliliters of sterile distilled water

Hypotonic, 0.075M KCl at 37°C

Fixative, 3 parts absolute methanol:1 part glacial acetic acid

Pancreatin, 4xN.F. (concentration 10X) Gibco

Gurr's buffer tablets

Coverbond

Giemsa stain (1 milliliter in 49 milliliters of buffer)

Methotrexate sodium NDC 005-4556-26, one drop of 10^{-5}M

Thymidine, one drop of 10^{-3}M

CHAPTER 3

RESULTS

Of the sixty individuals studied, six were found with some type of chromosomal abnormality in at least one of their cells. Four of these individuals were infertiles and two were controls. All of their abnormal cells, along with one of their normal cells were karyotyped and photographed and can be seen in Figures 1 through 15.

In Table I the reproductive histories of the fifteen infertile couples are presented. This information was sent to our clinic by the personal physicians of the couples and then obtained for use in this study. Tables II and III list the karyotypes of the infertiles and the control subjects respectively. In no instance were more than four abnormal cells found in an individual.

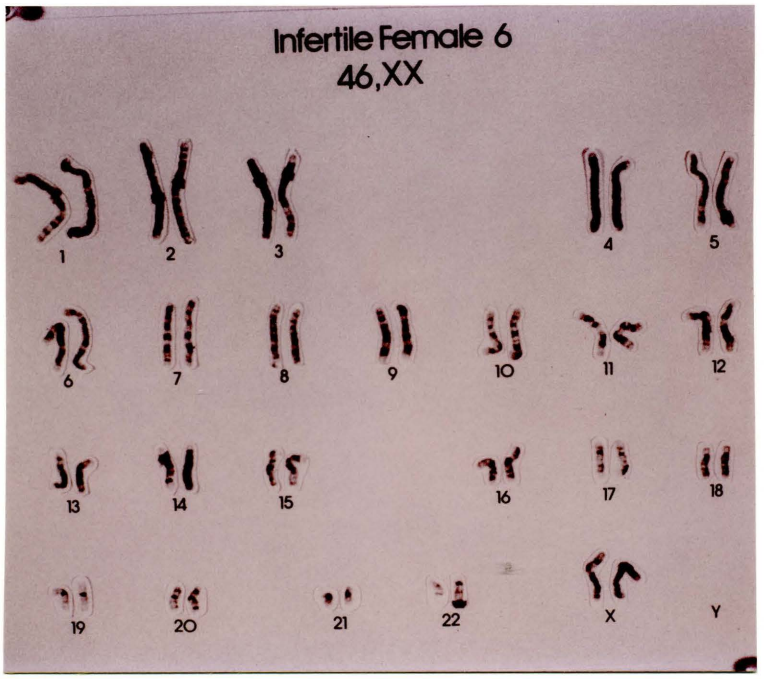
Tables IV through VII represent the total number of satellite associations found in the sixty individuals. When originally scored, the associations ranged from having two chromosomes involved in association to five. In cases where three, four or five chromosomes were found to be in association, these were broken down to represent three, six or ten pairs of chromosomes in association in order to make the presentation of the data and the

calculations more simplistic. (For example, when chromosomes 13/14/15/21 were scored as being in association, when presenting the data in the tables, this was broken down to represent pairs 13/14, 13/15, 13/21, 14/15, 14/21 and 15/21. The total number of satellite associations per individual is tallied at the bottom of each table. This represents the total number of associations in fifty cells per individual. However, in most cases less than thirty cells per individual contained a satellite association.

Table VIII shows the chi square and contingency tests for the study.

Figure 1. Infertile Female 6. Normal cell, 46,XX.
96 out of 100 cells.

Figure 2. Infertile Female 6. Abnormal cell, 47,XXX.
4 out of 100 cells.


Infertile Female 6
46,XX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

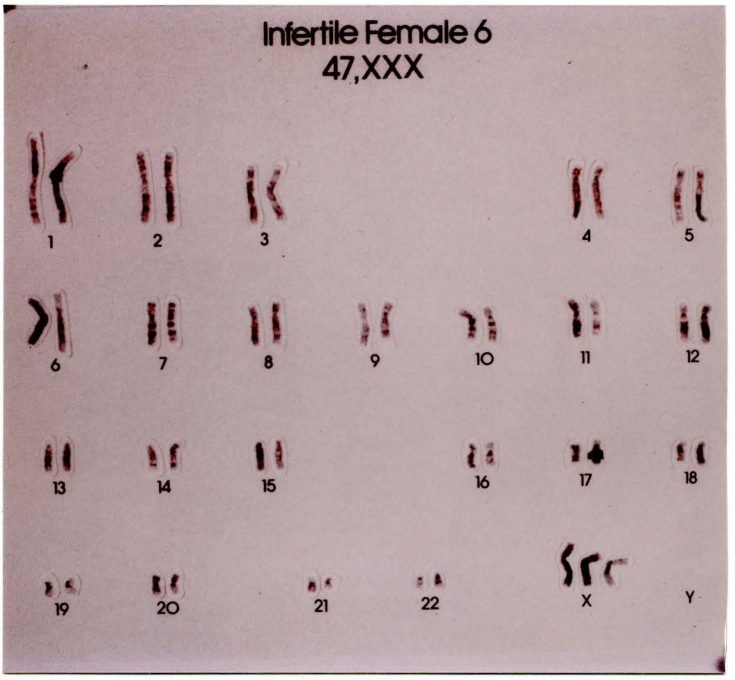
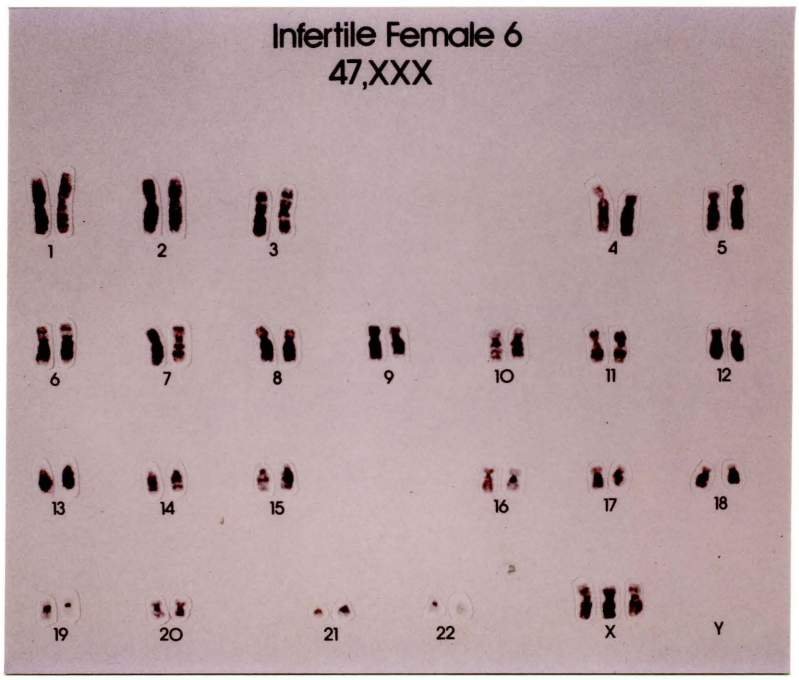

Infertile Female 6
47,XXX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 3. Infertile Female 6. Abnormal cell, 47,XXX. 4 out of 100 cells.

Figure 4. Infertile Male 12. Normal cell, 46,XY. 97 out of 100 cells.


Infertile Female 6
47,XXX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

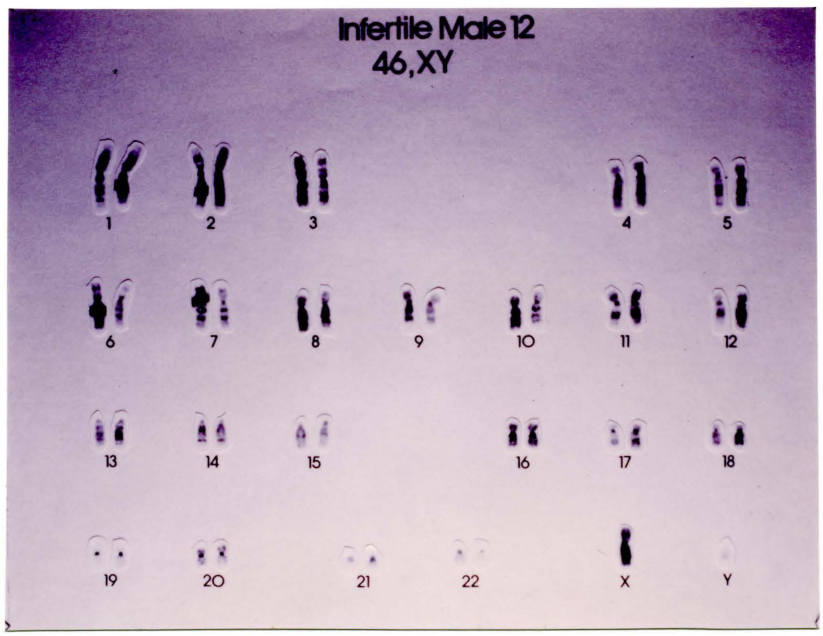
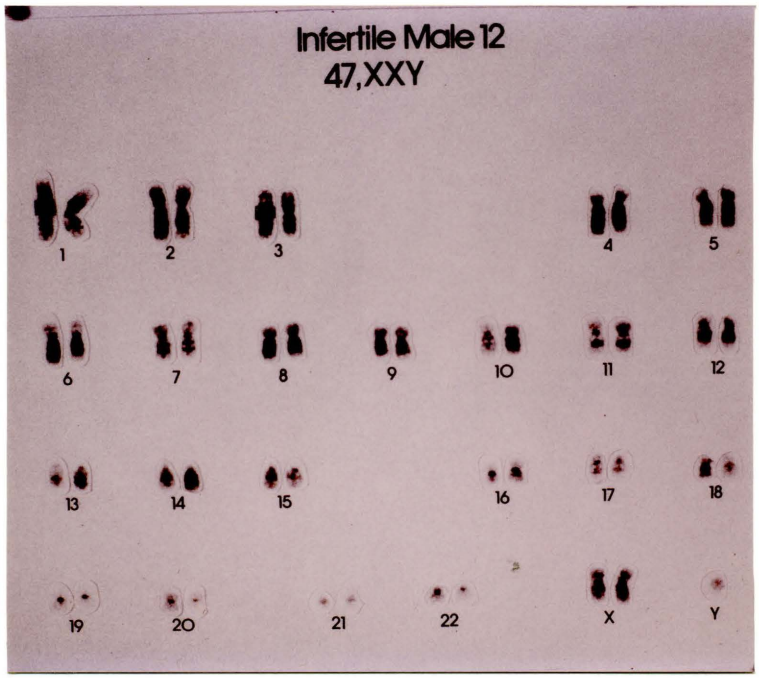

Infertile Male 12
46,XY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 5. Infertile Male 12. Abnormal cell, 47,XXY.
2 out of 100 cells.

Figure 6. Infertile Male 12. Abnormal cell,
45,XXXYY,-3,-15,-16,-17,-19,-21,+mar1,+mar2.
1 out of 100 cells.


Infertile Male 12
47,XXY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

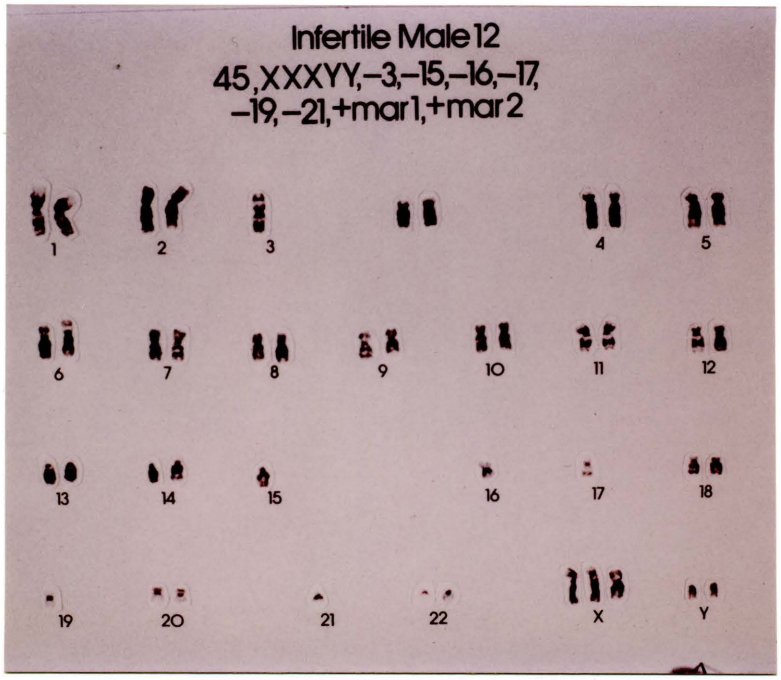
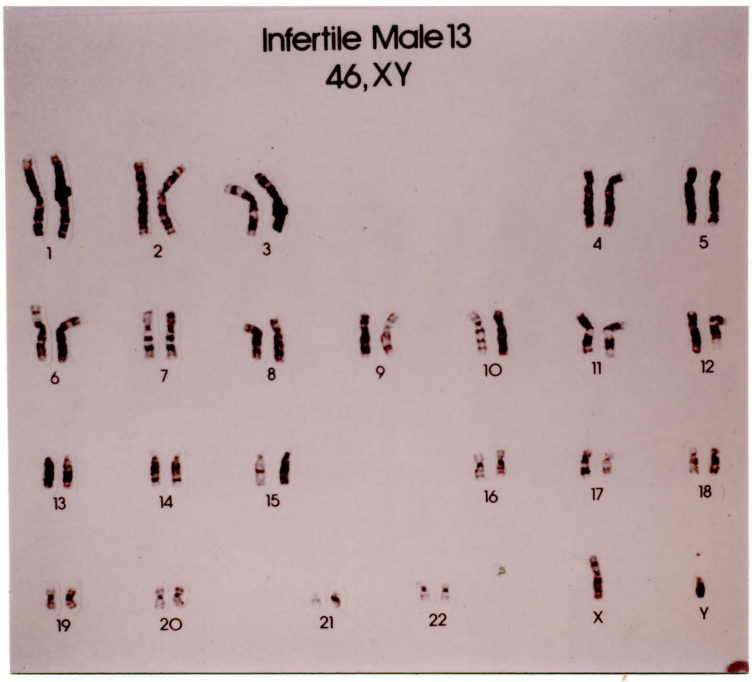

Infertile Male 12
45,XXXYY,-3,-15,-16,-17,
-19,-21,+mar1,+mar2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 7. Infertile Male 13. Normal cell, 46,XY.
49 out of 50 cells.

Figure 8. Infertile Male 13. Abnormal cell,
46,XY,t(2;14;22).
1 out of 50 cells.


Infertile Male 13
46, XY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

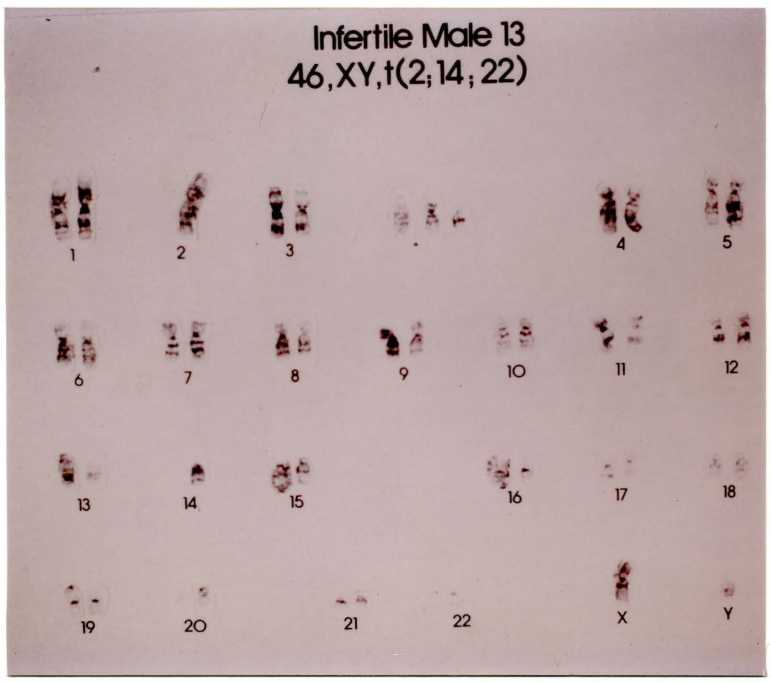
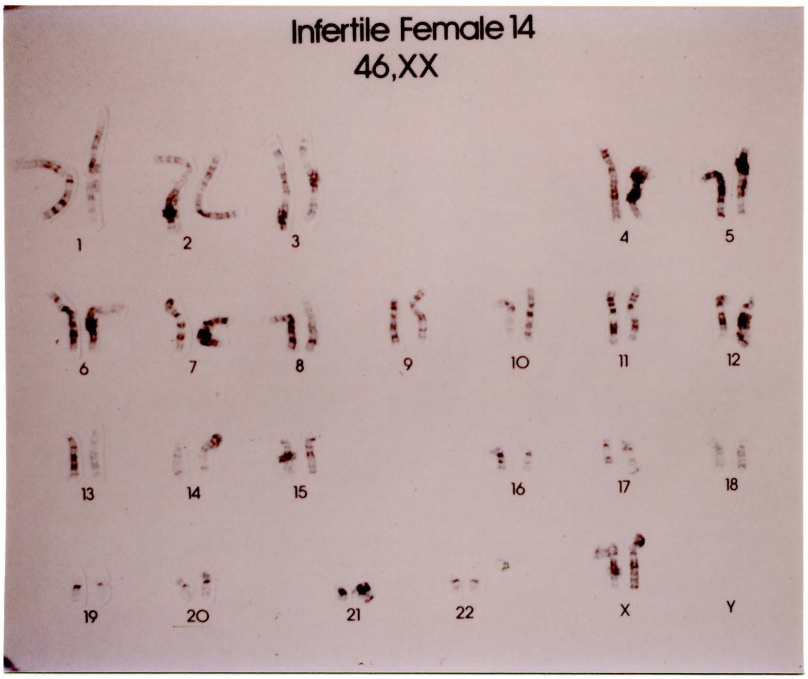

Infertile Male 13
46, XY, t(2;14;22)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 9. Infertile Female 14. Normal cell, 46,XX.
48 out of 50 cells.

Figure 10. Infertile Female 14. Abnormal cell, 46,XX5p-.
1 out of 50 cells.


Infertile Female 14
46,XX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

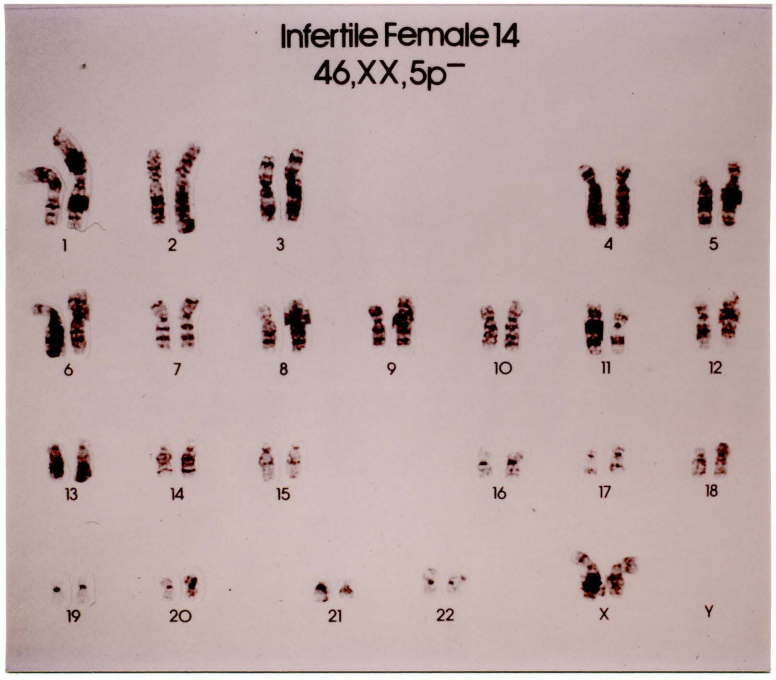

Infertile Female 14
46,XX,5p⁻
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 11. Infertile Female 14. Abnormal cell, 47,XXX. 1 out of 50 cells.

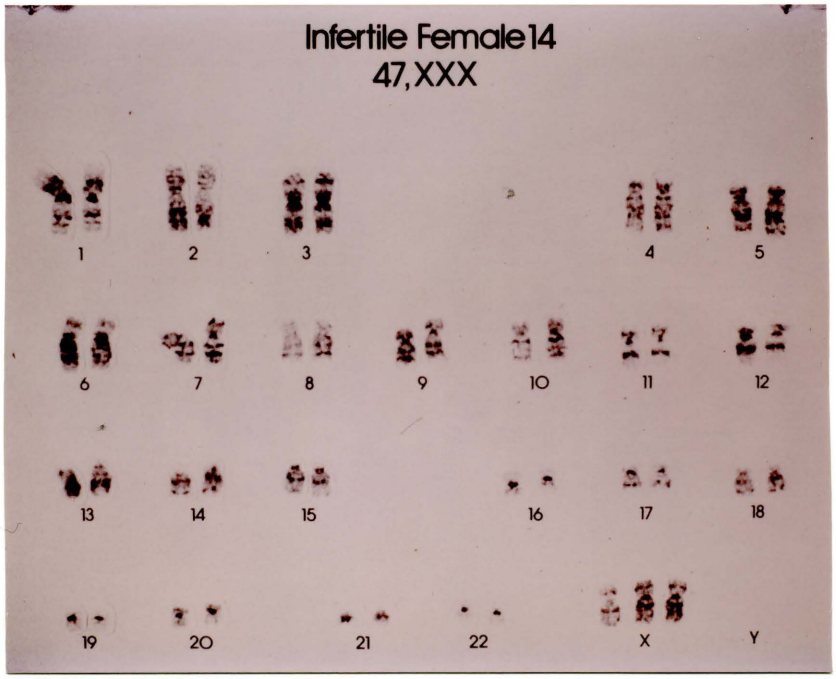
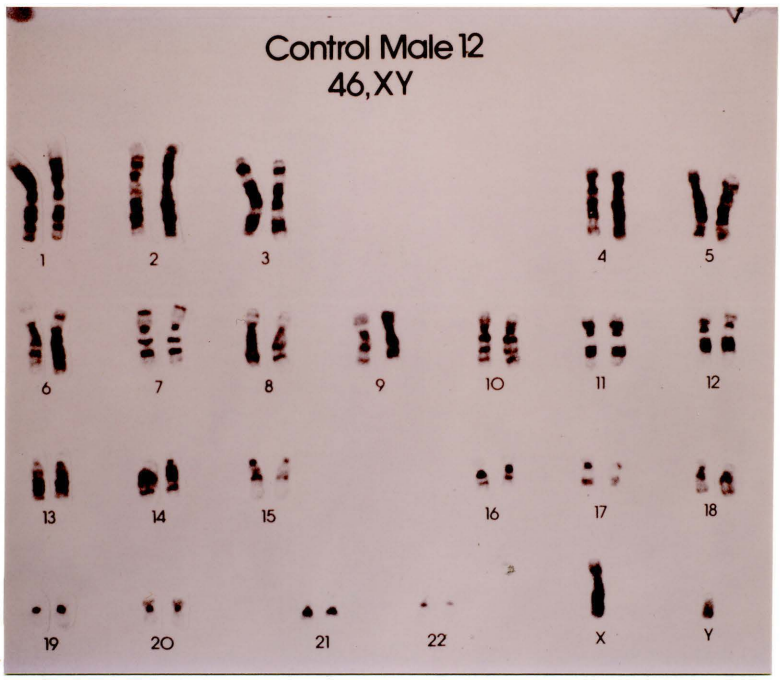

Infertile Female14
47,XXX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 12. Control Male 12. Normal cell, 46,XY.
49 out of 50 cells.

Figure 13. Control Male 12. Abnormal cell, 46,XY,+1p.
1 out of 50 cells.


Control Male 12
46,XY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

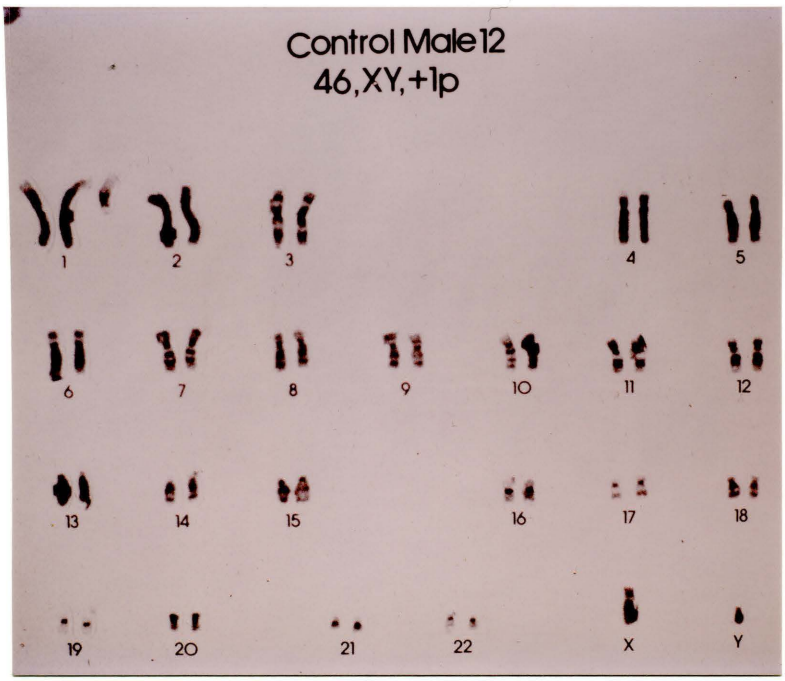
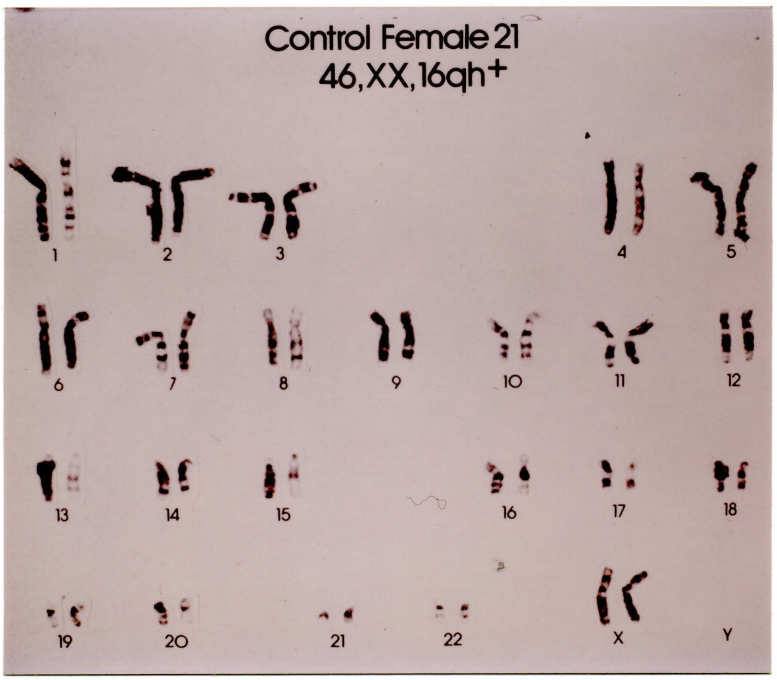

Control Male 12
46,XY,+1p
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 14. Control Female 21. Normal cell, 46,XX,16qh+.
49 out of 50 cells.

Figure 15. Control Female 21. Abnormal cell,
46,XX,16qh+,t(2;10).
1 out of 50 cells.


Control Female 21
46,XX,16qh+
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

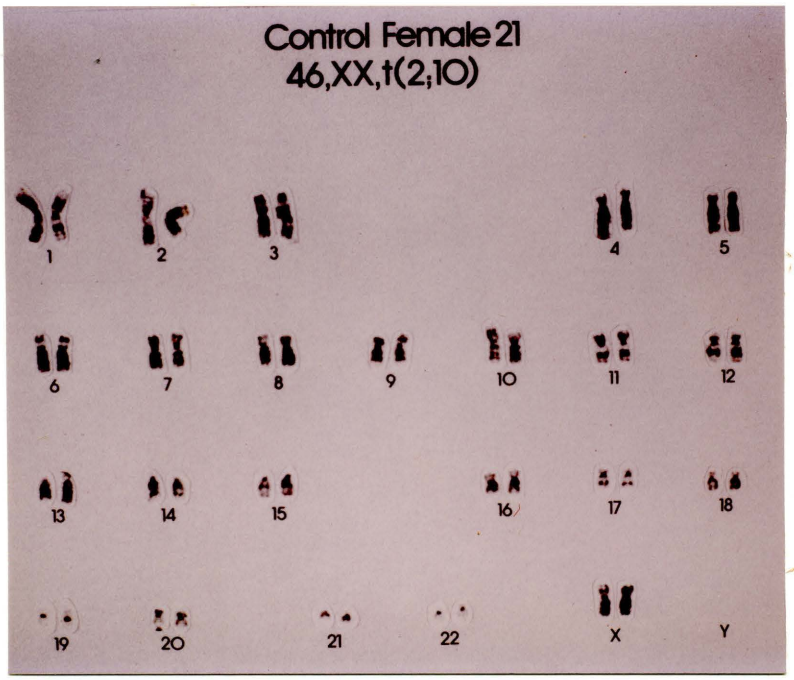

Control Female 21
46,XX,t(2;10)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

TABLE I

REPRODUCTIVE HISTORIES OF INFERTILE COUPLES

Infertile Couple 1	3 Spontaneous Abortions
Infertile Couple 2	2 Spontaneous Abortions
Infertile Couple 3	2 Spontaneous Abortions 1 Stillbirth
Infertile Couple 4	5 Spontaneous Abortions 1 Normal Child
Infertile Couple 5	Habitual Abortions
Infertile Couple 6	2 Missed Abortions
Infertile Couple 7	Habitual Miscarriages
Infertile Couple 8	Never Been Pregnant Husband Had Chemotherapy
Infertile Couple 9	Unknown Infertility 1 Normal Living Child
Infertile Couple 10	2 Spontaneous Abortions 1 Normal Child In Between
Infertile Couple 11	Habitual Abortions
Infertile Couple 12	Habitual Abortions
Infertile Couple 13	3 Miscarriages Wife Had Chemotherapy For Ovarian Cancer
Infertile Couple 14	3 Documented Miscarriages
Infertile Couple 15	Pregnant 5 Times With 3 Spontaneous Abortions

TABLE II

KARYOTYPES OF INFERTILE COUPLES

	Males	Females
Infertile Couple 1	46,XY	46,XX
Infertile Couple 2	46,XY	46,XX
Infertile Couple 3	46,XY	46,XX
Infertile Couple 4	46,XY	46,XX
Infertile Couple 5	46,XY	46,XX
Infertile Couple 6	46,XY	46,XX(96 cells) 47,XXX(4 cells)
Infertile Couple 7	46,XY	46,XX
Infertile Couple 8	46,XY	46,XX
Infertile Couple 9	46,XY	46,XX
Infertile Couple 10	46,XY	46,XX
Infertile Couple 11	46,XY	46,XX
Infertile Couple 12	46,XY(97 cells) 47,XXY(2 cells) 45,XXXYY,-3,-15, -16,-17,-19,-21, +mar1,+mar2(1 cell)	46,XX
Infertile Couple 13	46,XY(49 cells) 46,XY,t(2;14;22) (1 cell)	46,XX
Infertile Couple 14	46,XY	46,XX(48 cells) 46,XX,5p- (1 cell) 47,XXX(1 cell)
Infertile Couple 15	46,XY	46,XX

TABLE III

KARYOTYPES OF CONTROL SUBJECTS

Males		Females	
Control 2	46,XY	Control 1	46,XX
Control 4	46,XY	Control 3	46,XX
Control 5	46,XY	Control 7	46,XX
Control 6	46,XY	Control 8	46,XX
Control 9	46,XY	Control 10	46,XX
Control 12	46,XY(49 cells) 46,XY,+1p(1 cell)	Control 11	46,XX
Control 13	46,XY	Control 14	46,XX
Control 17	46,XY	Control 15	46,XX
Control 24	46,XY	Control 16	46,XX
Control 25	46,XY	Control 18	46,XX
Control 26	46,XY	Control 19	46,XX
Control 27	46,XY	Control 20	46,XX
Control 28	46,XY	Control 21	46,XX,16qh+ 46,XX,16qh+, t(2;10) (1 cell)
Control 29	46,XY	Control 22	46,XX
Control 30	46,XY	Control 23	46,XX

TABLE IV

SATELLITE ASSOCIATIONS OF INFERTILE MALES

Sat. Assoc.	Couple # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
13/13	2	1	-	-	-	2	-	1	5	1	-	1	-	2	-	15
13/14	8	2	1	-	1	5	2	2	2	1	2	4	-	-	1	31
13/15	4	5	4	2	1	1	4	6	-	-	1	3	2	1	1	35
13/21	4	2	7	7	1	1	2	2	2	-	1	3	-	-	-	32
13/22	5	10	5	-	2	1	8	5	2	-	-	2	2	5	1	48
14/14	-	-	-	-	1	-	1	-	-	-	2	-	-	-	-	4
14/15	4	4	2	-	1	3	-	1	2	6	9	3	4	4	3	46
14/21	9	2	1	2	1	3	3	2	5	5	10	2	2	3	4	54
14/22	4	2	4	2	-	1	3	1	-	2	1	3	5	2	-	30
15/15	-	5	-	-	-	2	1	-	4	-	-	1	-	-	-	13
15/21	3	3	5	1	-	-	3	1	3	2	3	2	1	2	3	32
15/22	8	11	3	1	-	1	4	5	-	3	3	2	3	2	2	48
21/21	4	2	4	3	1	-	-	3	2	-	-	-	-	2	-	21
21/22	5	5	2	3	3	1	1	1	2	5	2	4	2	1	2	39
22/22	-	3	-	-	-	1	-	-	-	-	-	2	-	-	-	6
Total	60	57	38	21	12	22	32	30	29	25	34	32	21	24	17	454

TABLE V

SATELLITE ASSOCIATIONS OF INFERTILE FEMALES

Sat. Assoc.	Couple # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
13/13	1	1	-	-	-	-	-	-	-	1	-	-	1	-	-	4
13/14	9	4	4	1	2	4	3	4	2	3	2	2	3	2	1	46
13/15	2	5	5	-	1	-	-	1	2	-	2	1	1	2	1	23
13/21	3	6	4	1	-	2	3	-	5	1	2	3	1	2	6	39
13/22	5	10	2	-	1	1	-	3	4	1	-	2	2	2	-	33
14/14	1	1	4	-	1	-	-	2	-	-	2	-	-	-	-	11
14/15	8	4	5	5	5	6	2	3	-	6	3	3	3	4	5	62
14/21	7	4	14	5	4	2	3	-	-	7	4	2	1	1	1	55
14/22	5	1	6	2	3	-	2	-	6	4	8	1	1	1	1	41
15/15	3	-	-	-	-	-	-	-	-	1	-	-	1	-	-	5
15/21	6	5	6	10	1	3	1	2	1	4	3	2	1	3	3	51
15/22	6	5	2	3	1	4	4	2	-	1	2	1	2	-	4	37
21/21	1	1	2	3	2	-	1	-	1	1	-	3	-	1	1	17
21/22	2	3	6	4	3	6	7	-	2	5	6	6	2	1	4	57
22/22	2	3	1	-	-	1	1	-	2	-	2	1	-	-	-	13
Total	61	53	61	34	24	29	27	17	25	35	36	27	19	19	27	494

TABLE VI

SATELLITE ASSOCIATIONS OF CONTROL MALES

Sat. Assoc.	Control # 2	4	5	6	9	12	13	17	24	25	26	27	28	29	30	Total
13/13	1	3	-	-	-	-	-	1	1	-	-	-	-	1	2	9
13/14	2	4	2	3	3	1	-	3	3	-	1	2	3	8	3	38
13/15	3	6	1	2	4	1	4	3	6	1	3	1	1	1	2	39
13/21	-	5	1	3	5	1	4	6	5	4	2	-	4	9	5	54
13/22	5	3	2	2	5	2	2	1	1	-	2	-	3	-	1	29
14/14	1	-	2	1	1	2	-	-	-	-	1	-	1	-	2	11
14/15	-	3	1	-	1	4	4	2	2	1	3	4	1	1	6	33
14/21	2	1	2	3	4	3	3	3	4	-	3	2	5	7	5	47
14/22	5	1	1	1	4	1	-	-	2	-	1	3	3	2	-	24
15/15	-	2	-	2	-	2	4	-	1	-	1	1	-	-	1	14
15/21	2	-	2	6	3	1	5	2	3	2	1	5	2	2	3	39
15/22	4	-	2	6	-	-	2	1	2	1	1	2	1	-	1	23
21/21	-	-	1	3	1	-	2	2	1	4	-	-	-	-	-	14
21/22	1	2	4	4	4	3	2	2	-	2	1	2	1	-	1	29
22/22	2	-	5	1	-	1	-	1	-	2	-	1	1	-	-	14
Total	28	30	26	37	35	22	32	27	31	17	20	23	26	31	21	417

TABLE VII

SATELLITE ASSOCIATIONS OF CONTROL FEMALES

	Control #															
	1	3	7	8	10	11	14	15	16	18	19	20	21	22	23	
Sat. Assoc.																Total
13/13	-	-	1	3	-	1	-	-	-	-	-	2	-	2	-	9
13/14	1	3	3	2	-	-	2	9	2	1	-	-	3	-	1	27
13/15	8	3	-	4	1	1	2	6	-	4	1	-	2	1	2	35
13/21	2	6	-	2	6	1	3	4	2	7	4	4	5	4	2	52
13/22	3	1	-	1	1	-	5	6	2	1	-	3	-	4	-	27
14/14	1	2	-	1	1	-	-	-	2	1	1	-	-	-	1	10
14/15	3	2	2	5	3	-	3	1	5	4	1	-	-	-	4	33
14/21	5	6	1	-	5	1	3	-	2	8	-	1	2	1	2	37
14/22	1	1	-	1	3	-	5	3	1	-	4	-	3	-	3	25
15/15	-	-	1	1	-	2	2	-	-	-	1	-	-	-	-	7
15/21	6	3	-	2	1	5	4	-	2	3	1	1	2	1	7	38
15/22	4	1	-	2	-	1	3	2	4	2	1	2	1	3	2	28
21/21	4	3	-	1	2	-	1	-	1	1	1	-	1	-	2	17
21/22	6	2	-	1	1	-	6	2	2	1	7	5	3	3	-	39
22/22	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	2
Total	44	33	8	26	24	12	40	33	25	33	22	19	22	19	26	386

TABLE VIII

STATISTICAL INFORMATION

	# of individuals with abnormalities	# of individuals w/o abnormalities	Total
Infertiles	4	26	30
Controls	2	28	30
Total	6	54	60

For Entire Group $X^2=\frac{(4x28-2x26-\frac{1}{2}x60)^2x60}{(30)x(6)x(30)x(54)}=0.185$

P=0.95 or greater

	# of individuals with abnormalities	# of individuals w/o abnormalities	Total
Infertiles	4	22	26
Controls	2	28	30
Total	6	50	56

In Couples With Two or More Spontaneous Abortions

$X^2=\frac{(4x28-2x22-\frac{1}{2}x56)^2x56}{(26)x(6)x(30)x(50)}=0..382$

P=0.90 to 0.95

TABLE VIII (CONTINUED)

13/13	$\frac{(37-116.7)^2}{116.7}=54.4$	14/22	$\frac{(120-116.7)^2}{116.7}=0.1$
13/14	$\frac{(142-116.7)^2}{116.7}=5.5$	15/15	$\frac{(39-116.7)^2}{116.7}=51.7$
13/15	$\frac{(132-116.7)^2}{116.7}=2.0$	15/21	$\frac{(160-116.7)^2}{116.7}=16.1$
13/21	$\frac{(177-116.7)^2}{116.7}=31.2$	15/22	$\frac{(136-116.7)^2}{116.7}=3.2$
13/22	$\frac{(137-116.7)^2}{116.7}=3.5$	21/21	$\frac{(69-116.7)^2}{116.7}=19.5$
14/14	$\frac{(36-116.7)^2}{116.7}=55.8$	21/22	$\frac{(164-116.7)^2}{116.7}=19.2$
14/15	$\frac{(174-116.7)^2}{116.7}=28.1$	22/22	$\frac{(35-116.7)^2}{116.7}=57.2$
14/21	$\frac{(193-116.7)^2}{116.7}=49.9$	Total	$X^2=397.4$ P=much less than 0.001

13	$\frac{(662-700.4)^2}{700.4}=2.1$	21	$\frac{(832-700.4)^2}{700.4}=24.7$
14	$\frac{(701-700.4)^2}{700.4}=0.0$	22	$\frac{(627-700.4)^2}{700.4}=7.7$
15	$\frac{(680-700.4)^2}{700.4}=0.6$	Total	$X^2=35.1$ P=much less than 0.001

CHAPTER 4

DISCUSSION

Chromosomal Abnormalities

It has been shown that chromosomal abnormalities may be related to the cause of recurrent spontaneous abortions. Therefore it is considered beneficial to perform cytogenetic observations on couples with fetal wastage. It has recently been reported that there is a five to six percent frequency of chromosomal anomalies found in couples with repeated pregnancy loss (Fyrns et al. 1984). This conclusion was reached after examining a large number of studies. However, this figure was obtained by examining only couples with two or more spontaneous abortions, or with one spontaneous abortion and one stillbirth in a very large sample population.

In this study, there was no incidence of a chromosomally abnormal cell line, however, chromosomal abnormalities were noted in six individuals. Of these six individuals, four were infertiles and two were control subjects. The only case where there could be an instance of low mosaicism is Infertile Female 6 in which four out of fifty cells were 47,XXX. However, in a repeat sample of blood, no abnormal cells were found among fifty

counted. Also in a prior cervical cytology smear performed, only single barr bodies were found. In three of the four infertiles a sex chromosome aneuploidy was found. None of the controls showed any sex chromosome abnormalities. In a study by Hassold in 1980, two women were reported in which a low percentage of cells were 47,XXX. One woman had had two spontaneous abortions and the other had had four miscarriages. In this study all four of the infertiles with abnormal cells had two or more spontaneous abortions. Stoll (1981) also reported a study in which one 47,XXX woman was found. In 1982, Davis found a woman with poly-X mosaicism. It has been previously documented that an occasional occurrence of cells with an extra X chromosome can be seen in women that are otherwise normal. According to Stoll (1981) this effect may be age related. In this study Infertile Female 6 was thirty-six years old. Infertile Male 12 was thirty-five and Infertile Female 14 was thirty-one.

In Control Female 21 it is important to note that her number 16 chromosome had extra heterochromatin. Extra heterochromatin has been reported as leading to improper segregation of the chromosomes during meiosis (Tsenghi et al. 1976) due to interchromosomal interference mentioned previously.

None of the chromosomal abnormalities found in this study can be considered as major anomalies. However, it is possible that they may be more than spurious rearrangements and mutations. Looking back at prior cases in the laboratory, in eighty-two couples with various reproductive histories, three were found to have translocations, fourteen had normal variants and eleven were found to have random abnormalities in only a few cells. These random abnormalities (16.7%) were similar to those observed in this study where 13.3% of the infertile individuals and 10.0% of the entire sample population had random abnormalities. When we look only at the couples with two or more spontaneous abortions, 15.4% had a random chromosomal abnormality as compared to 6.6% in the controls alone. However, statistically this is not a significant difference.

Although there were no major chromosomal abnormalities seen in this study, the random aberrations seen are still important to note. The fact that no translocations or inversions were found could be due to the small sample size used in this study. If five to six percent of the couples were expected to have an abnormality, only 1.5 of the infertile individuals studied would have shown an abnormal cell line. Statistically, this study is still considered significant. Presently no other studies have

mentioned whether or not they found random abnormal cells. Oftentimes they are considered spurious and therefore are not recorded, but if they occur as frequently in other studies as in this study, they might be of some significance, and therefore should be presented in the data.

Satellite Associations

According to Miller (1977) satellite associations involve chromosomes which participated in organizing a nucleolus. Also the material which is sometimes seen stretched between the satellite regions is thought to be the remains of the nucleolus. These bridges, along with the satellite regions are known to consist of rDNA which codes for rRNA. Miller also showed that the length of the satellite stalk and the amount of silver stain is correlated with the frequency of satellite associations. Only nucleolar organizing regions (NOR) that produce rRNA in the previous interphase are stained with silver and these active NOR's along with longer satellite stalks, increase the frequency of satellite associations.

It has been suggested (Hansson 1970) that since the number of satellite associations varies among individuals, those with a higher frequency of associations would have a greater risk of nondisjunction. It has also been mentioned that the frequency of satellite

associations and nondisjunction increase with age (Mattei 1976). In this study an increase in the number of satellite associations over the control counterparts, was found in at least one member of each infertile couple with the exception of Infertile Couples 6 and 13. Looking only at those individuals in the population study who had a random chromosomal abnormality, Infertile Female 6, Infertile Male 13, Control Male 12 and Control Female 21 all had a decreased number of satellite associations as compared to their counterparts, while Infertile Male 12 and Infertile Female 14 had an increased number of associations. As far as the age factor, no pattern was noted in this study; however, Mattei (1976) did not observe an increase in the frequency of satellite associations until after the age of thirty-three, and in the present report only twelve individuals were older than thirty-three. The results were also compared separately for males and females. There was no age effect seen in either case and there was also no significant difference between the number of associations seen in females as compared to males.

Patil and Lubs (1971) and Schmid, Krone and Vogel (1974) agreed that the most frequent chromosome in association is the 14, followed by the 21, 13, 22 and 15. According to the current study, they occurred in the

following order; 21, 14, 15, 13, 22. However, the range was only from 23.76 to 17.90 percent. In computing a X^2 test for a 1:1:1:1:1 segregation, the findings were not significant due to an excess of chromosome 21 in association. It was also obvious in this study that the homologous associations were much less frequent than nonhomologous associations. These findings are in accordance with those of Patil and Lubs (1971). In a study by Jacobs, Mayer and Morton (1976) an excess of 21/22 pairs and a deficiency of 14/22 pairs was found. In Patil's paper the 14/21 was the most common pair while the 15/22 was the least. The findings in this report are in agreement with both of these.

It is important to note that in this study the chromosome 21 was in association more frequently than the other chromosomes. This is also the most common trisomy found among individuals. These acrocentric chromosomes are randomly associating as can be seen in the X^2 test.

Overall, there seems to be an increase in the frequency of satellite associations in the infertile individuals, with a few exceptions. Since satellite associations may be a predisposing factor to nondisjunction, this could explain the abnormalities normally found among recurrent spontaneous aborters.

In addition the abnormal cells found in this study could also be explained by nondisjunction. Although not all of the individuals with an abnormal cell had a high frequency of satellite associations, they were all slightly older, and according to aforementioned studies, nondisjunction increases with age.

CHAPTER 5

CONCLUSION

Fifteen couples with various abnormal reproductive histories, along with thirty control subjects were studied for cytogenetic evaluation. Chromosomal abnormalities and satellite associations were noted for each of the sixty individuals. Six individuals were seen with some type of chromosomal aberration; however only one, two three or four cells per individual were abnormal. Three of these six had a sex chromosome aneuploidy, often found among older people. These unusual anomalies can be best explained by improper disjunction of the chromosomes during mitosis or meiosis. In one case an extra load of heterochromatin could have caused an interchromosomal effect predisposing to nondisjunction. In the other cases, the cause of this nondisjunction could be age related or due to some other unknown cause.

An increase in satellite associations was observed in at least one individual per couple with two exceptions. Since satellite associations may lead to improper disjunction, this could help to explain the increased rate of recurrent spontaneous abortions among these individuals. A fetus with one extra or one less chromosome (aneuploidy)

usually will not survive until term (aborted or stillborn) or will fail to implant (infertility).

Overall, there were no major chromosomal abnormalities seen among the recurrent spontaneous aborters used in this study. Random abnormalities did occur however, and should be noted since they may be of some significance. It can not be concluded whether or not the reproductive problems in these couples are due to chromosomal effects. However the sample size was small and perhaps had some effect on the results. It is still important to note any type of abnormalities when working with these couples, since five to six percent of recurrent spontaneous aborters do have a chromosomal abnormality.

REFERENCES

Bhasin MK, Forester W, Fuhrmann W: A cytogenetic study of recurrent abortion. Humangenetik 18: 139-148, 1972.

Blumberg BD, Shulkin JD, Rotter JI, Thuluvancheri M, Kaback MM: Minor chromosomal variants and major chromosomal anomalies in couples with recurrent abortion. Am J Hum Genet 34: 948-960, 1982.

Byrd JR, Askew DE, McDonough PG: Cytogenetic findings in 55 couples with recurrent fetal wastage. Fertil Steril 28: 246-250, 1977.

Davis JR, Weinstein L, Veomett IC, Shenker L, Giles HR, Hauck L: Balanced translocation karyotypes in patients with repetitive abortion. Am J Obstet Gynecol 144: 229-233, 1982.

Fitzsimmons J, Wapner RJ, Jackson LG: Repeated pregnancy loss. Am J Med Genet 16: 7-13, 1983.

Fryns JP, Kleczkowska A, Kubien E, Petit P, Van den Berghe H: Cytogenetic survey in couples with recurrent fetal wastage. Hum Genet 65: 336-354, 1984.

Hansson A: Differences in the satellite association pattern in the human population. Hereditas 66: 21-30, 1970.

Hassold TJ: A cytogenetic study of repeated spontaneous abortions. Am J Hum Genet 32: 723-730, 1980.

Heritage DW, English SC, Young RB, Chen ATL: Cytogenetics of recurrent abortions. Fertil Steril 29: 414-417, 1978.

Husslein P, Huber J, Wagenbichler P, Schnedl W: Chromosome abnormalities in 150 couples with multiple spontaneous abortions. Fertil Steril 37: 379-383, 1982.

Jacobs PA, Mayer M, Morton NE: Acrocentric chromosome associations in man. Am J Hum Genet 28: 567-576, 1976.

Kardon NB, Davis JG, Berger AL, Brockman A: Incidence of chromosomal rearrangements in couples with reproductive loss. Hum Genet 53: 161-164, 1980.

Mattei JF, Ayme S, Mattei MG, Gouvernet J, Giraud F: Quantitative and qualitative study of acrocentric associations in 109 normal subjects. Hum Genet 34: 185-195, 1976.

Miller DA, Tantravahi R, Dev VG, Miller OJ: Frequency of satellite association of human chromosomes is correlated with amount of Ag-staining of the nucleolus organizer region. Am J Hum Genet 29: 490-502, 1977.

Neu RL, Entes K, Bannerman RM: Chromosome analysis in cases with repeated spontaneous abortions. Obstet Gynecol 53: 373-375, 1979.

Osztovics MK, Toth SP, Wessely JA: Cytogenetic investigations in 418 couples with recurrent fetal wastage. Ann Genet 25: 232-236, 1982.

Patil SR, Lubs HA: Non-random association of acrocentric chromosomes. Humangenetik 13: 157-159, 1971.

Schmid M, Krone W, Vogel W: On the relationship between the frequency of association and the nucleolar constriction of individual acrocentric chromosomes. Humangenetik 23: 267-277, 1974.

Stoll CG, Flori E, Beshara D: Interchromosomal effect in balanced translocations. Birth Defects 14: 393-397, 1978.

Stoll CG: Cytogenetic findings in 122 couples with recurrent abortions. Hum Genet 57-58: 101-103, 1981.

Tsenghi C, Metaxotou-Stavridaki C, Strataki-Benetou M, Kalpini-Mavrou A, Matsaniotis N: Chromosome studies in couples with repeated spontaneous abortions. Obstet Gynecol 47: 463-468, 1976.

Ward BE, Henry GP, Robinson A: Cytogenetic studies in 100 couples with recurrent spontaneous abortions. Am J Hum Genet 32: 549-554, 1980.

Zankl H, Zang KD: Quantitative studies on the arrangement of human metaphase chromosomes. Humangenetik 23: 267-277, 1974.