EFFECTS OF PARACENTRIC INVERSION ON MEIOSIS AND REPRODUCTION

MAYA THANGAVELU

EFFECTS OF PARACENTRIC INVERSION ON MEIOSIS AND REPRODUCTION

BY

© Maya Thangavelu, M.S.

A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Faculty of Medicine
Memorial University of Newfoundland
February 1987

St. John's Newfoundland

ISBN 0-315-36990-6

ABSTRACT

Paracentric inversions are intrachromosomal rearrangements that involve inversion of a segment on one arm of a chromosome. Unlike other rearrangements, the frequency of paracentric inversions and that of their recombinants is low in the normal human population as well as in clinically defined ones. The effect of heterozygosity for each of a variety of paracentric inversions (varying in the length of the inverted segment and location relative to the centromere), on some aspects of meiosis and reproduction, in male and female mice, were investigated to determine the existence of mechanisms which may explain this observation.

In male mice, heterozygosity for paracentric inversions involving large segments, was associated with meiotic disturbance, measured by a decrease in the proportion of haploid secondary spermatocytes and an increase in that of heteroploid secondary spermatocytes. This disturbance appears to be associated with dicentric recombinants. Studies suggest that loop formation during the pachytene stage of spermatogenesis has a role in preventing crossover and consequently recombinant formation. Association between size of the inverted segment and meiotic disturbance was not evident in female heterozygotes.

Despite meiotic disturbance in male heterozygotes, litter size, a measure of post-natal phenotypic expression of chromosomal rearrangement, is not affected, suggesting pre-gametic selection. Pre-gametic selection and consequent reduction or absence of phenotypic expression in male heterozygotes may result in underascertainment of paracentric inversion. On the contrary, despite lack of

significant meiotic disturbance, reduction in litter size is observed in females heterozygous for some inversions. These observations in females suggest post-gametic and possibly post fertilization selection. Differences between males and females in the stage at which selection operates may account for differences in the efficiency of selection in the two sexes. This may explain why the mother, rather than the father, is the carrier of paracentric inversion in most instances of recombinants reported in humans.

The observations made in this study, suggest that a variety of unique factors may be responsible for the low frequency of balanced paracentric inversions and their recombinants in humans. They are: cytogenetic limitations in the identification of the inversion, meiotic mechanisms that suppress recombinant formation, efficient pre-gametic selection in male heterozygotes and failure of the resulting gametic loss to affect reproductive fitness in male heterozygotes.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following:

Professor F. Clarke Fraser O.C., for his supervision, guidance and stimulating discussions.

Professor P.W. Allderdice for her supervision, encouragement, advice and criticism.

Dr. Thomas H. Roderick and Mr. Norman Hawes of the Jackson Laboratory, Bar Harbor, Maine, for generously providing the animals for this work and for the discussions.

Dr. Irene Uchida of McMaster University, Hamilton, for the help rendered in establishing the technique of in vitro culturing of mouse oocytes.

The Staff of the Animal Care, Health Sciences Centre, Memorial University for their help in maintaining the animals used in this study.

Dr. D. Bryant, Division of Community Medicine, Memorial University, for his advice and help in the statistical analysis of the data.

The Staff of the Health Sciences Library, Memorial University.

The Staff of the Computer Services Unit of Memorial University.

The Staff of the Medical Audiovisual Services, Health Sciences Centre, Memorial University.

The Staff of the Electron Microscopy Unit, Health Sciences Centre, Memorial University.

The School of Graduate Studies, Memorial University, and the School of Medicine for financial support in the form of a Graduate Fellowship and bursary respectively.

I also would like to express my appreciation to my parents, brothers and sister for their constant support and would like to dedicate this thesis to my parents, Mrs. & Dr. M. Thangavelu.

Table of Contents

List of Figures

List of Tables

LIST OF ABBREVIATIONS

°C	Degrees Celsius (Centigrade)
C57BL	C57BL/6J
C3H	C3HFeJ
DC	Oocytes dividing in culture
HetIIM	Heteroploid second meiotic metaphase
EDTA	Ethylenediaminetetraacetic acid
M	Molar
IM	First meiotic metaphase
IIM	Second meiotic metaphase
mcgm	Microgram
ml	Millilitre
mm	Millimeter
MO	Mature oocytes (oocytes in the germinal vesicle stage)
MV	Oocytes in meiosis in vivo
NIIM	Normal second meiotic metaphase
OC	Oocytes in culture
1RK	IN(1)1RK
12RK	IN(1)12RK
24RK	IN(1)24RK

11RK	IN(3)11RK
17RK	In(10)17RK
14RK	IN(8)14RK
20RK	IN(11)20RK
22RK	IN(14)22RK
W	Watts

Chapter 1

Introduction

Inversions are balanced chromosomal rearrangements resulting from two breaks on a chromosome followed by rotation of the intercalary segment by 180 degrees and incorporation in the same chromosome. The term paracentric inversion was proposed by Muller (Muller, 1952) to address inversions limited to one arm of the chromosome.

Paracentric inversions were initially identified as genetic variants (mutants) in *Drosophila melanogaster ampelophila*, which reduced the frequency of crossover in the heterozygous state (Sturtevant, 1919). The presence of inversions was later proved on the basis of extensive investigations on crossover frequencies in these mutants (Sturtevant, 1931). Since then they have been identified in a variety of plants and animals. (*Agave stricta* (Brandham, 1969), *Trillium erectum* (Smith, 1935), Aloineae (Brandham, 1977), *Zea mays* (McClintock, 1933), *Notophthalmus viridescens* (Hartley & Callan, 1977), *Drosophila virilis* (Komai & Takaku, 1942), Barley (Das, 1955, Ekberg, 1969), *Lolium perenne* (Simonsen, 1973), *Festuca pratensis* (Simonsen, 1975), *Tradescantiae* (Darlington, 1938), *Paeonia* (Stebbins & Ellerton, 1939) *Camnula pellucida* (Nur, 1968) and *Prosimulium multidentatum* (Rothfels & Nambiar, 1975).)

Paracentric inversions have not been reported in mice in the wild population. They have, however been induced in the laboratory by X-irradiation or administration of ethylmethane sulfonate or triethylene melamine (Roderick & Hawes, 1974; Evans & Phillips, 1975). Paracentric inversions have also been induced in mosquitoes by irradiation or chemicals (Kitzmiller, 1976).

1.1. Paracentric inversions in humans

Until recently the number of paracentric inversions identified in humans has been small compared to other balanced rearrangements such as translocations and pericentric inversions. Paracentric inversions were not identified in a population of 3835 liveborn infants whose chromosomes were studied with Giemsa banding, in which the frequency of reciprocal translocations and pericentric inversions was 0.10% and 0.05% respectively (Buckton, O'Riordan, Ratcliffe, Sligth, Mitchell & McBeath, 1980).

The apparent frequency of any chromosomal rearrangement depends upon the frequency of the rearrangement in the population and the ability to identify it. Prior to the introduction of chromosome banding techniques (Caspersson, Zech & Johansson, 1970), only those rearrangements which resulted in altered arm ratio (length of long arm divided by that of the short arm (q/p)) or centromere index (percentage of the short arm in terms of the total chromosome length [(p/(p+q)x100)] (Therman, 1986) could be identified. With the introduction of banding techniques, and more recently of techniques which facilitate the study of prophase chromosomes (Yunis, 1976) one can identify rearrangements that do not alter arm ratio.

As the centromere is not included in the inverted segment in paracentric inversions, the arm ratio is not altered. Therefore it was not until banding techniques were introduced that the first paracentric inversion was reported in humans (del Solar & Uchida, 1974). Since then there has been an increase in the number of cases reported every year. Some of the interesting characteristics of human paracentric inversions are;

1. Low frequency
2. Association of apparently balanced inversions with developmental abnormalities
3. Association with aneuploidy of other chromosomes and
4. Low frequency of recombinants.

1.2. Frequency of paracentric inversion in relation to that of other balanced rearrangements in various populations

The frequencies of paracentric inversions and that of other balanced rearrangements in different populations may be compared.

As paracentric inversions may be detected only on chromosomes that are banded, only those studies which used banding techniques will be reviewed. Few population studies have been carried out on healthy individuals after the introduction of banding techniques and using prophase analysis (Lin, Gedeon, Griffith, Smink, Newton, Wilkie & Sewell, 1976, Buckton, O'Riordan, Ratcliffe, Sligth, Mitchell & McBeath, 1980).

1.2.1. In amniocytes

Chromosome studies of amniocytes always use banding techniques and data from large studies are available. Data from three large collaborative studies (Ferguson-Smith & Yates, 1984, Hook, Schreinemachers, Wiley & Cross, 1984, Van Dyke, Weiss, Roberson & Babu, 1983) are summarized in Table [1-1].

The study by Van Dyke et al. (Van Dyke, Weiss, Roberson & Babu, 1983) was carried out to determine the differences in the frequencies of balanced rearrangements with and without the use of banding techniques. The higher frequency of translocations and paracentric inversions found in this study compared to others may have been because of the extra effort made to ensure that the number of rearrangements undetected was minimal.

1.2.2. In liveborn

Paracentric inversions were not identified in two studies of consecutive liveborns (Lin, Gedeon, Griffith, Smink, Newton, Wilkie & Sewell, 1976, Buckton, O'Riordan, Ratcliffe, Sligth, Mitchell & McBeath, 1980) involving a total of 4765 births. The frequency of paracentric inversions in the general population is not known. Fryns et al. have reported a frequency of 0.03% in a population of 44,000 individuals, investigated for various reasons (Fryns, Kleckowska & Van den Berghe, 1986). The difference in frequencies in amniocytes, 0.016 (weighted average), and liveborn, 0.03%, is not significant.

Table 1-1: Frequency of balanced structural rearrangements in amniocytes.

Investi-gator	Sample size	Trans-location	Peri-centric	Para-centric
Ferguson-Smith et al. [84]	52965	0.15%	0.03%	0.01%
Hook et al. [84]	24951	0.16%	0.08%	0.02%
Van Dyke et al. [83]	8158	0.28%	0.07%	0.05%

1.2.3. Mentally retarded individuals

In individuals with moderate and severe mental retardation, Fryns et al. observed that the frequency of paracentric inversion was 2/2000 (0.1%) (Fryns, Kleczkowska, Kubien & van den Berghe, 1984). The higher incidence of paracentric inversion in individuals with mental retardation than in the general population (0.03%) suggests an association between paracentric inversions and abnormal development, and implies that paracentric inversions may be of clinical significance. Another possible reason for the higher frequency among the mentally retarded individuals is that extra effort may have been taken in evaluating them cytogenetically.

1.3. Factors that may influence the true frequency of paracentric inversions

The low frequency of paracentric inversions identified despite the use of banding techniques may be due to a truly low frequency. As in the case of chromosome rearrangements in general, the prevalence of a paracentric inversion depends on;

1. The rate at which it is produced i.e., the mutation rate and
2. Its maintanence in the population, which depends on its reproductive fitness.

1.3.1. Mutation rate

Translocations involve breaks in two chromosomes followed by exchange of material between them. Inversions result from two breaks in the same chromosome followed by rotation of the intercalary segment by 180 degrees around a transverse axis and incorporation in the same chromosome. Incorporation at the DNA level requires that the polarity is maintained. (Figure 1-1).

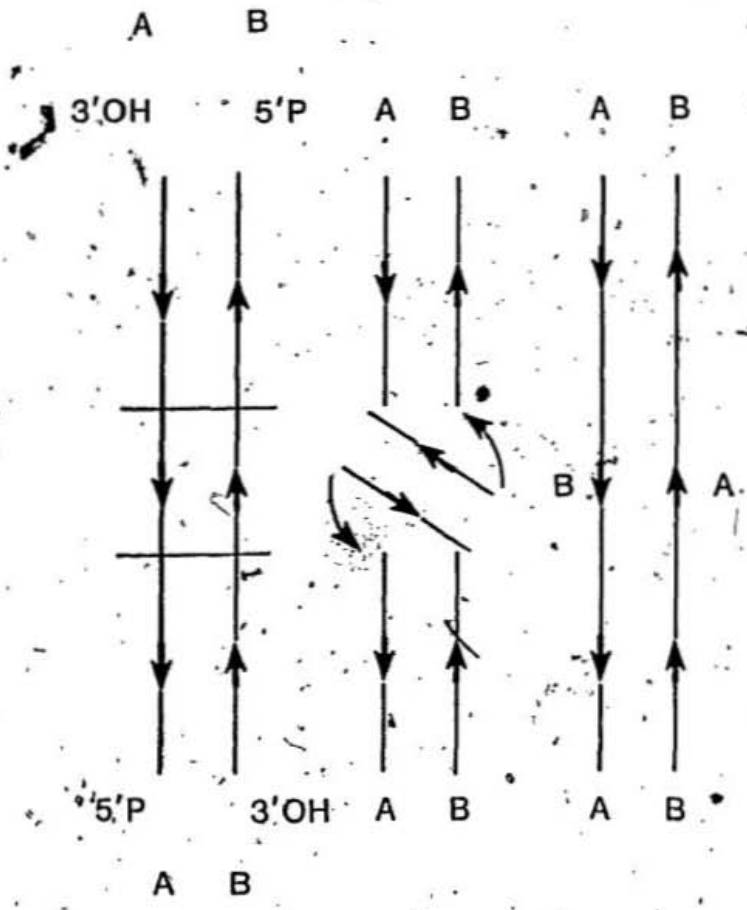
The location of two breaks and the resulting chromosome rearrangement (intra or inter) depend on the proximity of the breakpoints in the nucleus at the time of breakage and reunion.

Data from amniocytes may be analysed to compare mutation rates for the various intrachromosomal and interchromosomal rearrangements. The rate and 95% confidence intervals of mutant structural rearrangements in fetus of seven groups of women undergoing amniocentesis is as follows; Robertsonian translocations (2.0-2.8/10,000) reciprocal translocations (4.8-5.6/10,000) and inversions (0.8-1.2/10,000) (Hook, Schreinemachers, Wiley & Cross, 1984). The differences in the observed rates for translocations and inversions may be real or may result from difficulties in identifying inversions.

Involvement of two chromosomes in translocations and only one chromosome in inversions may be one explanation for the observed differences between the frequencies of mutant translocations and inversions. If the inversion is the result of three events, two breaks, rotation of the segment between the two breaks and

Figure 1-1

Dingrammatic representation of maintenance of polarity during formation of a paracentric inversion, essential for the maintenance of functional integrity of the DNA.


A B
3'OH 5'P A B A B
B A
5'P 3'OH A B A B
A B

incorporation of the inverted segment (classical model of chromosomal rearrangement), there ought not to be a difference in the frequencies of mutant pericentric and paracentric inversions. But if inversions result from breaks of crossed over regions of the arm, or arms, followed by incorporation of the alternate ends (exchange hypothesis of chromosomal rearrangement (White, 1973)) mutation rates of pericentric inversions would be higher than that of paracentric inversions. 120 of 390 (30.8%) chromosomal rearangements which occurred during primate evolution were pericentric inversions compared to only 22 (1>%) of paracentric inversions (Dutrillaux, Couturier, Sabatier, Muleris & Prieur, 1986), suggesting one or both of the following; (1) Pericentric inversions occur more frequently than paracentric inversions. This would be the case if it is assumed that overlapping of regions on either sides of the centromere is more likely compared to that of regions on the same arm. (2) Pericentric inversions are genetically less lethal or more pertinant to evolution than paracentric inversions. In humans, of 196 pericentric inversions investigated, the frequency of de novo mutants was 18 (9.2%) (Kaiser, 1984). In 83 cases of paracentric inversions reported in the literature, the frequency was 8 (9.6%). This data does not however represent the rate of mutation in the gametes.

The observed similarity in the proportion of *de novo* paracentric and pericentric inversions suggests that they have the same reproductive fitness. However, the absolute numbers of new cases of paracentric inversions is much less.

1.3.2. Reproduction in human carriers of balanced rearrangements

Although balanced chromosomal rearrangements, in most cases, will not have much effect on the development of individuals, they may have an effect on their reproductive fitness. Relative reproductive fitness may be estimated as a function of fertility, survival and generation time (Morton, Jacobs, Frackiewicz, Law & Hilditch, 1975). Pairing of homologous chromosomes is a prerequisite for recombination and normal disjunction. Chromosomal rearrangements that disrupt the homology of chromosome pairs can disrupt the process of meiosis and impair reproduction. Chromosomal rearrangements can interfere with (1) gametogenesis (2) viability or function of the gametes formed and (3) development of the products of conception. In humans detrimental effect on gametogenesis and viability of gametes manifest as lowered gamete count. Interference with development of the products of conception is clinically recognised as spontaneous abortion of a chromosomally unbalanced fetus or an offspring with chromosomal imbalance and congenital abnormalities.

Insight into the relative effects of paracentric inversions and other balanced rearrangements on reproductive fitness may help in evaluating the role of selection in determining the observed frequency of the rearrangement, as well as in providing estimates of risk for involved couples.

1.3.2.1. Chromosome abnormalities and gamete count

For reasons of easy availability evaluation of gamete count in humans is limited to males. Chromosome abnormalities of both sex chromosomes and autosomes are in general more frequent in individuals with low sperm count (2%) than in the general population (Chandley, Maclean, Edmond, Fletcher & Watson, 1976).

1.3.2.1.1. Translocations in subfertile men

There are numerous reports of probands with chromosome rearrangements and azoospermia or oligospermia. Both reciprocal translocation (Moreau & Teyssier, 1984, Chandley, Seuánez & Fletcher, 1976, Chandley, Christie, Fletcher, Frackiewicz & Jacobs, 1972, Gonzales, Losourd & Duttrillaux, 1981, Laurent, Chandley, Dutrillaux & Speed, 1982, Yamada, Nanko, Hattori & Isurugi, 1982, Cantú, Díaz, Möller, Jiménez-Sáinz, Sandoval, Vaca & Rivera, 1985, Román, Sordo & García-Sagredo, 1979, Micic & Micic , 1981, Callen, Woolatt & Sutherland, 1985, Faed, Lamont & Baxby, 1982, Blattner, Kistenmacher, Tsai, Punnett & Giblett, 1980, Laurent, Biemont, Cognat & Dutrillaux, 1977, Viguié, Romani & Dadoune, 1982, Chandley, 1981, Léonard, Bisson & David, 1979, Retief, Van Zyl, Menkveld, Fox, Kotzè& Brusnicky, 1984) and Robertsonian translocation (Chandley, Hargreave & Fletcher, 1982, Chandley, Christie, Fletcher, Frackiewicz & Jacobs, 1972, Román, Sordo & García-Sagredo, 1979, Plymate, Bremner & Paulsen, 1976, Faed, Lamont & Baxby, 1982, Menkveld, van Zyl & Retief, 1983) have been reported in subfertile men.

The incidence of translocations in men with oligozoospermia and azoospermia

from six studies (McIlree, Price, Brown, Tulloch, Newsam & Maclean, 1966, Retief, Van Zyl, Menkveld, Fox, Kotzè& Brusnicky, 1984, van Zyl, Menkveld, van Kotzè, Retief & van Niekerk, 1975, Chandley, Edmond, Christie, Gowans, Fletcher, Frackiewicz & Newton, 1975, Tiepolo, Zuffardi, Fraccaro & Giarola, 1981, Hendry, Polani, Pugh, Sommerville & Wallace, 1976) is reviewed in Table [1-2].

The range in frequencies (0%-4%) observed in these studies could be because;

1. Individuals classified as subfertile and included in the various studies range from those ascertained due to azoospermia to those due to a pregnancy resulting in stillbirth. (Chandley, Edmond, Christie, Gowans, Fletcher, Frackiewicz & Newton, 1975, Marmor, Taillemite, van den Akker, Portnoi, Joyce, Delafontaine & Roux, 1980); the samples may therefore be heterogeneous.

2. The techniques used in the various studies may be different. For example only 3 of the 8 translocations identified by Hendry et al. were identified in somatic chromosomes. The remaining 5 were identified in meiotic preparations (Hendry, Polani, Pugh, Sommerville & Wallace, 1976).

3. Small sample size and use of staining techniques that do not have the ability to identify certain rearrangements may be the reason why McIlree et al. (McIlree, Price, Brown, Tulloch, Newsam & Maclean, 1966) did not detect rearrangements in their study.

1.3.2.1.2. Prospective studies on carriers of translocations

There are at least two studies on sperm count in carriers of balanced translocations. In two cases out of three, Abramsson et al. found a sperm count less than 20 million/ml (Abramsson, Beckman, Duchek & Nordenson, 1982). The value of 20 million considered as the appropriate value to distinguish between normal and abnormal based on reports, has since been contested (Eliasson, 1981).

Table 1-2: Frequency of translocations among subfertile men.

Investigator	Frequency of translocations
McIlree et al. [66]	0/50 (0%)
Retief et al. [84]	3/496 (0.60%)
Van Zyl et al. [75]	3/130 (2.30%)
Chandley et al. [76]	9/1599 (0.56%)
Tiepolo et al. [81]	27/2247 (1.20%)
Hendry et al. [76]	8/200 (4.0%)

Marmor et al. analysed the spermiograms of nineteen subfertile translocation carriers. Sperm count in the eleven who were carrriers of reciprocal translocations was between 0-105 million/ml. The count in the 8 carriers of Robertsonian translocations was between 1.5-47 million/ml. Sperm count among individuals who presented with primary infertility (i.e., no recognisable pregnancy) was 0-47 million/ml (Marmor, Taillemite, van den Akker, Portnoi, Joyce, Delafontaine & Roux, 1980). Both reports are based on few subjects. Therefore it is difficult to draw any conclusion on the correlation between the rearrangement and sperm count. The observed range of sperm count (0-105 million/ml) may be due to the inclusion of heterogeneous populations. (The sperm count in those cases presenting with primary infertility (ie., no recognisable pregnancy) is 0-18 million/ml, whereas that in those who have had at least 2 pregnancies and ascertained due to spontaneous abortions or stillbirths is 23-105 million/ml).

1.3.2.1.3. Inversions and reduced sperm count

Reduced sperm count has been reported in carriers of both pericentric inversions (Tóth, Gaál, Sára & László, 1982, Giraldo, Silva, Martínez, Campos & Guzmán, 1981, Faed, Lamont & Baxby, 1982, Rivera, Alvarez-Arratia, Moller, Díaz & Cantú, 1984) and paracentric inversions (Faed, Robertson, Lamont, MacIntosh, Grieve, Baxby, James & Crowder, 1979, Madan, Seabright, Lindenbaum & Bobrows, 1984) (Refer Appendix A). Both the probands with paracentric inversions were investigated for subfertility. In the French Collaborative Study 12% (36/304) pericentric inversions (A French Collaborative Study, 1986a) and 6% (2/32) paracentric inversions (A French Collaborative Study, 1986b) were ascertained due to male sterility.

The wide range of sperm count reported by Marmor et al. (Marmor, Taillemite, van den Akker, Portnoi, Joyce, Delafontaine & Roux, 1980) suggests that retrospective cytogenetic studies on individuals with low sperm counts could result in the exclusion of those individuals who are carriers of balanced rearrangements but whose sperm counts are not affected to any significant level. There is evidence that fertility need not be affected even when the sperm count is below 10 million/ml (Eliasson, 1981).

The lack of paracentric inversions in the various studies could be because:

1. Since the frequency of paracentric inversions is low they may not be identified in small samples.
2. The cytogenetic techniques used may not have been appropriate to identify paracentric inversions.
3. The minimal effects on spermatogenesis may not result in apparent subfertility.
4. Spermatogenesis may not be affected.

As in the case of other rearrangements, retrospective studies attempting to correlate low sperm count with paracentric inversions are subject to ascertainment bias. In addition the observed range of sperm count in prospective studies and the observation that low sperm counts need not necessarily affect reproduction suggests that at least in humans, low sperm count cannot be used as a reliable estimate of reproductive fitness. Aberrations in sperm count, however may be an indication of a selection mechanism in operation.

1.3.2.2. Balanced rearrangements and reproductive impairment evident during the post-fertilization stage

At the post-fertilization stage of reproduction, balanced rearrangements in an individual are ascertained following:

1. The spontaneous abortion of a chromosomally unbalanced product of conception.
2. The association of stillbirth or neonatal death with chromosome imbalance.
3. The birth of an offspring with developmental abnormalities and chromosome imbalance.
4. Population screening.

1.3.2.2.1. Balanced rearrangements and fetal wastage

Balanced rearrangements can give rise to genetically unbalanced recombinants which can affect embryonic or fetal development. One factor that may affect fetal survival is the nature of the fetus (Stein, Susser, Warburton, Wittes & Kline, 1975). The more severely affected embryos and fetuses are eliminated prenatally in the form of spontaneous abortions. Therefore balanced rearrangements would be represented more in couples with repeated abortions than in the general population. The observation from four studies (Fitzsimmons, Wapner & Jackson, 1983, Lippman-Hand & Vekemans, 1983, Pantzar, Allanson, Kalousek & Poland, 1984, Fryns, Kleckowska, Kubién, Petit, van den Bergh, 1984) on couples who experienced spontaneous abortions are summarized in Table [1-3]. The differences in frequencies in these studies may be due to variation in ascertainment. The number of spontaneous abortions experienced before the couple was identified is

Table 1-3: Frequency of chromosomal rearrangements in couples who have experienced spontaneous abortions.

Investigator	Frequency of Translocations	Frequency of Inversions
Fitzsimmons		
et al. [83]	3/175 (1.71%)[a]	2/175 (1.143%)[a]
	5/161 (3.11%)[b]	1/161 (0.62%)[b]
	0/39 (0%)[c]	1/39 (2.56%)[c]
Lippman-Hand		
et al. [83]	10/177 (5.65%)[d]	0/177 (0%)[d]
Pantzar		
et al. [84]	4/318 (1.26%)[d]	0/318 (0%)[d]
Fryns		
et al. [84]	53/1068 (4.96%)[e]	2/1068 (0.19%)[*e]

[*] Paracentric inversion

[a] 2 consecutive spontaneous abortions

[b] 3 or more consecutive spontaneous abortions

[c] 50% fetal loss

[d] 2 or more spontaneous abortions

[e] 2 spontaneous abortions and 1 spontaneous abortion and 1 fetal death particularly with congenital malformations.

one source of variation. Differences in the frequency of chromosomal rearrangements have been observed in couples with differences in the number of spontaneous abortions (Pantzar, Allanson, Kalousek & Poland, 1984). Another source of variation is inclusion of cases with a history of late fetal death with congenital abnormalities. The study by Fryns et al. (Fryns, Kleckowska, Kubién, Petit, van den Bergh, 1984) included couples with such a history. Yet another reason for the high frequency in some studies may be attributed to the fact that the women are investigated for other probable reasons for spontaneous abortions before inclusion in the study.

1.3.2.2.2. Prospective studies on carriers of balanced rearrangements

There are at least 2 studies investigating the rate of spontaneous abortions among carriers of balanced rearrangements. In 16 cases of reciprocal translocation ascertained following birth of a malformed child, recurrent abortions or hypogonadism, the incidence of spontaneous abortion was 50% after correction for ascertainment bias (Neri, Serra, Campana & Tedeschi, 1983). The genetic risk for reciprocal translocations have been found to depend on the probability of disjunction/segregation and the resulting degree of imbalance. The probability of disjunction/segregation and degree of imbalance depends on the breakpoints and sex of the carrier (Stene & Stengel-Rutkowski, 1982). In the study by Neri et al., the incidence among carriers of Robertsonian translocations was found to be 20% for t(13q14q) and 25% for t(14q21q) after correction for ascertainment bias (Neri, Serra, Campana & Tedeschi, 1983). Differences in segregation ratio have also been

observed in male and female carriers of Robertsonian (D/G) translocation (Stene, 1970).

The rate of abortion among 349 carriers of pericentric inversions is reported as 11.2% (Kaiser, 1984). If those cases that were ascertained on the basis of an abortion are disregarded, the frequency of abortion is 9.1%, which is lower than that observed in carriers of translocations (20%-50%) as well as in the general population (15%) (Warburton & Fraser, 1964).

1.3.2.2.3. Paracentric inversion and fetal wastage

Paracentric inversions have been ascertained on the basis of fetal wastage (Stetten & Rock, 1983, Finley, Scarbrough, Carroll & Feng, 1985, Fryns & Van den Berg, 1980, A French Collaborative Study, 1986b, Yang-Feng, Finley, Finley & Franke, 1985) (Refer Appendix B). Trunca and Finley et al. have attributed the fetal wastage to the inversion. Until the products of conception are proved to be chromosomally abnormal the relationship between the miscarriages and inversion cannot be established with certainty. Thirty-four percent (11/32) of the paracentric inversions identified by the French Collaborative Study was ascertained following investigations for recurrent abortions. This is higher than that reported in literature (15%) (A French Collaborative Study, 1986b). Use of high resolution cytogenetic evaluation in the French Study and/or under reporting in literature may be the reason for the difference.

Miscarriages have also been reported in some familial paracentric inversions not

ascertained on the basis of spontaneous abortions. (Valárcel, Benítez, Martínez, Rey & Sánchez - Casos, 1983, Speevak, Hunter, Hughes & Cox, 1985, Madan, Seabright, Lindenbaum & Bobrows, 1984, Ridler & Sutton, 1981, del Porto, d'Alessandro, de Matteis, d'Innocenzo, Baldi, Pachi & Cappa, 1984, Venter, Dawson, Du Toit, Smith, Kritzinger, Landman, Cronje & Hof, 1984, Stetten & Rock, 1983, Peters-Slough, Planteydt, Timmerman & Vooren, 1982, Djalali, Steinbach & Barbi, 1984, Fryns & Van den Berg, 1980, Fryns, Kleckowska, Kubién, Petit, van den Bergh, 1984). (Refer Appendix C). In 26 carriers of various paracentric inversions in these families, of 100 pregnancies, 23 resulted in spontaneous abortions (23%), which unlike pericentric inversions is higher than the proportion of pregnancies that result in spontaneous abortions in the normal population. In one family ascertained on the basis of miscarriages, the frequency of miscarriages was not different between that in nine carriers and five non-carrier family members (Johnson, Dobyns, Dewald & Gordon, 1985). In those instances of fetal wastage in which a paracentric inversion heterozygote has been identified, there is no cytogenetic evidence suggesting a causal relationship between the fetal wastage and inversion.

1.3.2.2.4. Stillbirths and infants with congenital malformations in carriers of balanced rearrangements

Chromosomal imbalance may also result in late fetal death (stillbirth) or birth of an infant with congenital abnormalities. The average risk of a malformed infant for reciprocal translocation carriers, and the Robertsonian translocation t(14q21q) mothers are 6% and 23% respectively (Neri, Serra, Campana & Tedeschi, 1983).

Chromosomes 5, 9, 13 and 15 were most frequently involved in reciprocal translocations and adjacent type of disjunction-segregation was the most frequent type capable of producing a viable unbalanced zygote. For pericentric inversions the rate of stillbirths has been estimated to be 2.37% (23/972) of births, a significant increase over the normal rate is 0.77% (Kaiser, 1984). One familial paracentric inversion of chromosome 15 (q15q24) has been ascertained on the basis of 2 neonatal deaths with congenital abnormalities (del Porto, d'Alessandro, de Matteis, d'Innocenzo, Baldi, Pachi & Cappa, 1984). In the French Collaborative Study, 4% (11/301) pericentric inversions (A French Collaborative Study, 1986a) and 6% (2/32) paracentric inversions were identified subsequent to stillbirths (A French Collaborative Study, 1986b).

In all the above mentioned situations, i.e., those identified due to reduced gamete count, fetal wastage or perinatal mortality, it appears that the contribution of paracentric inversions is minimal compared to that of other rearrangements. Most studies on subfertility, fetal wastage and perinatal mortality used cytogenetic techniques which could identify only gross structural rearrangements and were not efficient in identifying subtle rearrangements and paracentric inversions. The high frequency of spontaneous abortions among carriers of paracentric inversions (Refer section 1.3.2.2.3) however, suggests that paracentric inversions may be of clinical significance.

1.3.2.2.5. Abnormal recombinants in liveborn offspring of carriers of balanced rearrangements

The extent of genetic imbalance in reciprocal translocations and pericentric inversions has been suggested as one factor which influences the survival of a genetically abnormal fetus to term. Percentage haploid autosomal length has been suggested as a measure of the potential chromosomal imbalance. The absence of large chromosomal imbalance in full term offspring has been explained on this basis (Daniel, 1981, Daniel, 1979).

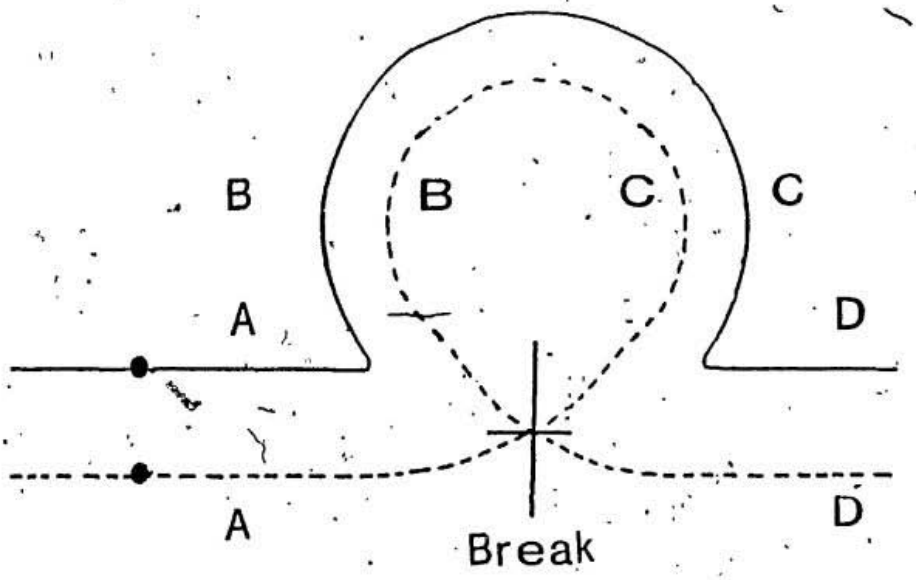
1.3.2.2.6. Abnormal recombinants in liveborn offspring of carriers of paracentric inversion

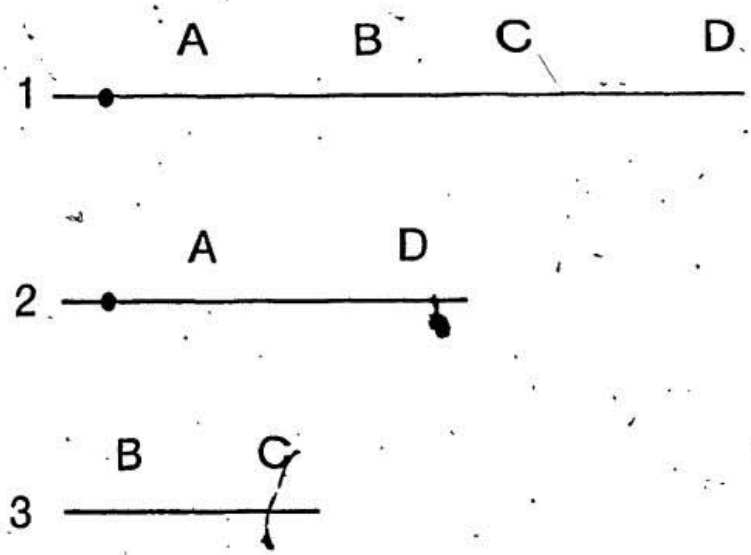
Individuals with congenital abnormalities and possible abnormal recombinants of paracentric inversion have been reported (Valárcel, Benitez, Martinez, Rey & Sánchez - Casos, 1983, Speevak, Hunter, Hughes & Cox, 1985, Hoo, Lorenz, Fischer & Furhmann, 1982, Sparkes, Muller & Klisak, 1979, Mules & Stanberg, 1984, Kelly, Wyandt, Kasprzak, Ennis, Wilson, Koch & Schnatterly, 1979) (Refer Appendix D)

Whether the recombinant is due to crossing over in the rearranged chromosome is questionable in at least three of these reports. Various mechanisms, as suggested by Sparkes et al. (Sparkes, Muller & Klisak, 1979) could result in the observed interstitial deletion in chromosome number 13. Crossover between the inverted and the normal chromosome in the inverted region and deletion of the resulting dicentric chromosome is one mechanism. Others include (1) breaks in both chromosomes in the inverted segment followed by rejoining with the segment on

Figure 1-2

Diagrammatic representation of the mechanism by which the inverted segment may be deleted during meiosis in a paracentric inversion heterozygote.


B
B
C
C
A
D
A
D
Break


A
B
C
D
1
A
D
2
B
C
3

the alternate chromosome on the same side of the break or (rejoining with the segment on the alternate chromosome on the other side of the break would result in a crossover) and (2) two breaks on the same chromosome arm and loss of the interstitial material. A break at the point where regions on the normal chromosome crosses to form the loop will also have the same result (Figure 1-2) (Sparkes, Muller & Klisak, 1979). The recombinant described by Kelly et al. is reported to involve deletion of the region inverted in one chromosome 3 of the father (Kelly, Wyandt, Kasprzak, Ehnis, Wilson, Koch & Schnatterly, 1979). In this case also, excision of the inversion loop by exchange within the chromosome is thought to result in the interstitial deletion.

Neither genetic nor clinical information is available to substantiate the assumption made by Hoo et al. that the additional band on the inverted chromosome 7 is an interstitial duplication (Hoo, Lorenz, Fischer & Furhmann, 1082).

To result in the recombinants reported by Valcarel et al., breaks have to occur at the same point in the dicentric bridge in all the three cases (Valárcel, Benitez, Martinez, Rey & Sánchez - Casos, 1983). An alternative is that the rearrangement is an insertion as discussed by Callen et al. (Callen, Woolatt & Sutherland, 1985).

Until these possible mechanisms are excluded, one can accept only those recombinants reported by Speevak et al. (Speevak, Hunter, Hughes & Cox, 1985) and Mules and Stanberg (Mules & Stanberg, 1984) as resulting from

crossover in the inverted segment, formation of dicentric chromosome and the observed recombinants resulting from a break in the dicentric chromosome. Attempts to explain the low frequency of paracentric inversions compared to that of translocations on the basis of differences in reproductive fitness assessed at various stages of reproduction is restricted by the small numbers of paracentric inversions available for analysis.

1.4. Balanced rearrangements and developmental abnormalities

The chromosomal basis of developmental abnormalities depends on duplication or deficiency of a segment of the chromosome. Therefore balanced rearrangements ought not to result in developmental abnormalities. They have, however been reported in a variety of populations and are reviewed below. Position effect has been suggested as one reason for the association of balanced rearrangements and developmental abnormalities (Refer section 1.4.4).

1.4.1. Frequency of balanced rearrangement in abortuses

Warburton et al. (Warburton, Grady & Jagiello, 1981) studied twelve abortuses from nine carriers of balanced rearrangements. Three were normal, three were unbalanced derivatives of the parental rearrangements and two had anomalies unrelated to the parental karyotype. Interestingly, the remaining four had the balanced rearrangement. Observations from four studies (Boué, Boué, Lazar & Gueguen, 1973, Kajii & Ferrier, 1978, Boué& Boué, 1973, Jacobs, 1981) are summarized in Table [1-4]. Two abortuses with inversions were observed in one study (Jacobs, 1981).

Table 1-4: Frequency of balanced translocation in spontaneous abortuses.

Investigator	Frequency of translocation
Boué & Boué [73]	0/86 (0%)
Boué et al. [73]	1/60 (1.67%)
Kajii et al. [78]	0/310 (0%)
Jacobs [81]	14/5726 (0.24%)
Total	15/5452 (0.26

1.4.2. Balanced rearrangement and perinatal mortality

Three studies (Machin & Crolla, 1974, Sutherland, Carter, Bauld, Smith & Bain, 1978, Kuleshov, 1976) are summarized in Table [1-5].

There are no significant differences between the observations in the various studies. The relatively low incidence of balanced rearrangements in abortuses and cases of perinatal mortality suggests that they have a small, if any, deleterious effect on development.

1.4.3. Frequency of balanced rearrangements associated with developmental abnormalities

There are a number of reports of developmental abnormalities associated with balanced rearrangements (Tharapel, Summitt, Wilroy & Martens, 1977, Nielsen & Krag-Olsen, 1981, Funderburk, Spence & Sparkes, 1977, Jacobs, 1974, Breg, Miller, Allderdice & Miller, 1972). Most of the rearrangements were balanced translocations. Funderburk et al. identified one pericentric inversion among 455 patients (0.22%) with mental retardation and four cases of pericentric inversion among 1679 (0.24%)in patients with psychiatric disorders (Funderburk, Spence & Sparkes, 1977). These frequencies are significant compared to the frequency of 0.05% reported in liveborns (Lin, Gedeon, Griffith, Smink, Newton, Wilkie & Sewell, 1976, Buckton, O'Riordan, Ratcliffe, Sligth, Mitchell & McBeath, 1980).

Table 1-5: Frequency of balanced rearrangements in perinatal deaths.

Investigator	Frequency of Translocations	Frequency of Peri. Inv.
Machin et al. [74][a]	3/500 (0.6%)	0/500 (0%)
Kuleshov [76][b]	0/175 (0%)	0/175 (0%)
Sutherland et al. [78][c]	4/1193 (0.34%)	2/1193 (0.17%)

[a] Infants dying in perinatal period
[b] Infants dying during perinatal period and premature infants
[c] Late abortions (spontaneous and therapeutic) included

1.4.3.1. Balanced paracentric inversions and developmental abnormalities

Many probands with paracentric inversions present developmental abnormalities or features suggestive of chromosomal rearrangement or imbalance, such as mental retardation, family history of neural dysraphia and spontaneous abortions (Fryns & Van den Berg, 1980), cerbral palsy, undescended testis, Klinefelter syndrome, infertility, azoospermia or Down syndrome (Madan, Seabright, Lindenbaum & Bobrows, 1984), neonatal hypotonia (del Solar & Uchida, 1974), multiple abnormalities (del Porto, d'Alessandro, de Matteis, d'Innocenzo, Baldi, Pachi & Cappa, 1984, A French Collaborative Study, 1986b), features suggestive of Langer Giedion syndrome (Shabtai, Sandowski, Nissimov, Klar & Halbrecht, 1985), growth retardation (Peters-Slough, Planteydt, Timmerman & Vooren, 1982), psychomotor retardation (Deroover, Fryns, Haegaman, Van Den Berghe, 1979), unusual facies and other abnormalities (Callen, Woolatt & Sutherland, 1985),and minor physical anomalies (Allderdice, Sreenivasan & Eales, 1980). The balanced nature of the rearrangement and in many cases its occurrence in normal members of the family suggest that the rearrangement is coincidental and not of clinical significance (Refer Appendix E).

1.4.4. Balanced paracentric inversions and translocations associated with developmental abnormalities - comparative analysis

Association between *de novo* rearrangements and developmental abnormalities has been explained on the basis of (1) interruption of the genetic locus rendering it inactive, (2) position effect or (3) deletion of very small magnitude (Tharapel, Summitt, Wilroy & Martens, 1977).

Inactivation of the gene by interruption of the gene locus and position effect as possible mechanisms resulting in developmental abnormalities are applicable only to *de novo* rearrangements. Abnormal recombinants are formed only in familial situations. Ascertainment bias and deletion of a small segment are possible in both *de novo* and familial cases. Reduced penetrance may be another reason for variable expression and difficulties in ascertainment.

Apparently balanced paracentric inversions that are familial (Fryns & Van den Berg, 1980, Madan, Seabright, Lindenbaum & Bobrows, 1984, del Solar & Uchida, 1974, Orye & Van Bever, 1983, Peters-Slough, Planteydt, Timmerman & Vooren, 1982, Deroover, Fryns, Haegaman, Van Den Berghe, 1979, Callen, Woolatt & Sutherland, 1985) as well as those that are *de novo* in origin (Ho[illegible], Horton & Scott, 1985, Jacken, Fryns, Standaert, Cock & Van den Berghe, 1980, Riccardi & Holmquist, 1979) have been ascertained on the basis of phenotypic abnormalities.

Possible reasons for the observed association of developmental abnormalities and presence of an apparently balanced paracentric inversion may be:

1. Ascertainment bias: Cytogenetic investigations on probands with paracentric inversions are often carried out because of a phenotype suggestive of chromosomal abnormalities. If these probands are not excluded a falsely high estimate will be derived.

2. Reporting bias: Because paracentric inversions are balanced rearrangements, when detected in phenotypically abnormal individuals they may be reported.

3. Abnormal recombinants present only during the early stages of development: Uneven number of crosssovers (recombination) in the inverted segment of a paracentric inversion results in the formation of a dicentric anaphase bridge and an acentric fragment (McClintock, 1933). If the fragment is functional as in maize (Rhoades & Dempsey, 1953) it may have some effect on the development of the embryo. However, during subsequent cell divisions, it may be lost and may not be present in the tissue examined postnatally.

4. Abnormal recombinants due to crossover in the uninverted segment: Unequal crossing over in regions adjacent to the breakpoints in an inversion could result in very small duplications or deletions which might not be easily identifiable. Therefore what appears like a balanced paracentric inversion may in fact not be balanced.

De novo translocations have been found to be more frequent among individuals with developmental abnormalities than familial translocations (Warburton, 1982).

Among translocations it is observed that non-Robertsonian translocations that are *de novo* in origin are more detrimental than familial and Robertsonian translocations (Tharapel, Summitt, Wilroy & Martens, 1977, Funderburk, Spence & Sparkes, 1977, Warburton, 1984, Jacobs, 1974). Warburton estimated that the incidence of apparently balanced *de novo* rearrangements in mentally retarded individuals was almost seven times as high as that in newborn infants (Warburton, 1984). No conclusion can be drawn from the only prospective study on five *de*

novo translocation cases as they did not show any significant developmental abnormalities compared to those who had inherited translocations (Nielsen & Krag-Olsen, 1981).

The number of cases of paracentric inversions associated with developmental abnormalities is too small to determine the relationship between the origin of the rearrangement and clinical significance as in the case of translocations.

1.5. Paracentric inversions and interchromosomal effect

Some of the paracentric inversions in humans were found in association with aneuploidy of the sex chromosomes (Madan, Seabright, Lindenbaum & Bobrows, 1984, Singh, 1981, Canki & Duttrillaux, 1979) or autosomes (Madan, Seabright, Lindenbaum & Bobrows, 1984). Disturbances in meiotic behaviour of chromosomes due to structural rearrangements of other chromosomes is termed "interchromosomal effect" (Grell, 1962, Grell, 1970). In humans, the distributive-pairing hypothesis has been evoked to explain this form of interchromosomal effect. The co-existence of aneuploidy and structural rearrangements of unrelated chromosomes is thought to be one form of interchromosomal effect (Grell & Valencia, 1964). Reciprocal translocations were found in 1.06% of parents of Down syndrome, compared to 0.16% in newborn infants (Lindenbaum, Hultén, McDermott & Seabright, 1985). A 3.2 fold increase in risk for aneuploidy is observed in offspring of carriers of pericentric inversions (A French Collaborative Study, 1986a). In the case of the autosome (Madan, Seabright, Lindenbaum & Bobrows, 1984) the chromosome with the inversion as well as that involved in

aneuploidy was chromosome 21. However it is not reported if the chromosome involved in aneuploidy was the one with the inversion. There is one report of a paracentric inversion (7)(q22q35) in the mother of an individual with a structural abnormality of the short arm of the X chromosome (Watt & Couzin, 1984) (Refer Appendix F).

The association between paracentric inversion and non-disjunction of other chromosomes may be the result of ascertainment and/or reporting bias, as the individuals were investigated because of abnormalities suggestive of aneuploidy. Non-disjunction in the inversion carrier parent has to be proven before interchromosomal effect is accepted as an established phenomenon in the case of paracentric inversions as is associated with other rearrangements.

1.6. Paracentric inversions and recombinants

Unlike other rearrangements, very few recombinants have been identified in humans heterozygous for paracentric inversion (Sparkes, Müller & Klisak, 1979, Mules & Stanberg, 1984; Hoo, Lorenz, Fischer & Furhmann, 1982, Valárcel, Benítez, Martínez, Rey & Sánchez - Casos, 1983, Speevak, Hunter, Hughes & Cox, 1985, Kelly, Wyandt, Kasprzak, Ennis, Wilson, Koch & Schnatterly, 1979). Heterozygous paracentric inversions appear to function as suppressors of recombination. A number of possible mechanisms have been invoked for the observed suppression (Schulz-Schaeffer, 1980, Greenbaum & Reed, 1984):

1. Probability of recombination is higher in the case of large inversions. In very short inversions pairing of the inverted segment is eliminated altogether.

2. Both incomplete and heterosynaptic pairing prevents crossing over and results in crossover suppression within the loop and around it.

3. Elimination of unbalanced recombinants may be incorrectly interpreted as suppression of crossover.

1.7. Meiosis in inversion paracentric heterozygotes.

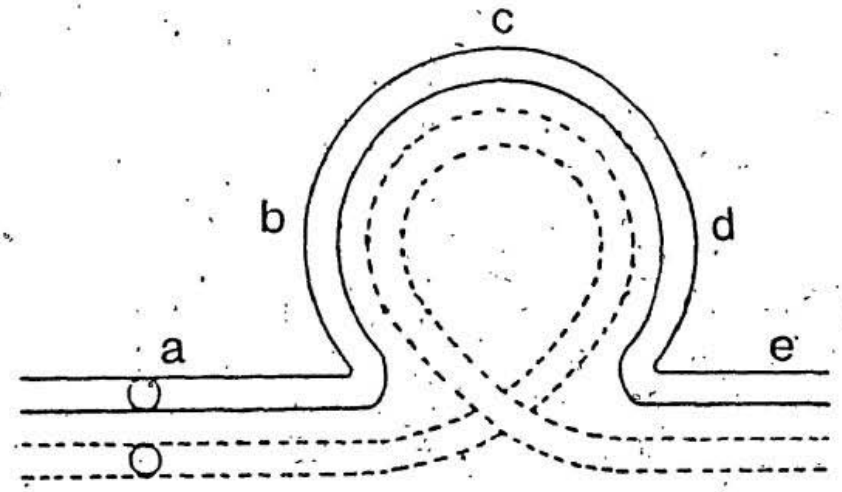
Homologous pairing of the inverted segment is achieved by the formation of a loop or by reverse pairing during zygotene. (Reverse pairing is brought about by homologous pairing of the inverted segment and non-homologous pairing or asynapsis of the flanking segments.) Chiasmata formation at specific sites is a prerequisite for the formation of abnormal recombinants in paracentric inversion.

If no chiasma is formed, meiosis results in balanced normal and inverted chromosomes as in the carrier (Figure 1-3). If chiasmata are formed in the region distal to the inversion, or in the interstitial segment balanced normal and inverted chromosomes are formed (Figures 1-4 & 1-5). If, however uneven numbers of chiasmata involving the same two chromatids, occur in the inverted segment, a dicentric chromosome that forms a bridge during first anaphase and an acentric fragment result (Figure 1-6).

If one chiasma is formed in the interstitial segment and the other in the inverted segment, the nature of the recombinants depends on the chromatids involved in the 2 chiasmata. If the same two chromatids or different ones are involved in the crossover in the inverted segment and the interstitial segment, a dicentric chromosome that forms an anaphase bridge during first anaphase and an acentric

Figure 1-3

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation and the chromosomes formed when chiasmata are not formed. (1) and (2) are normal. (3) and (4) are balanced inverted chromosomes.


c
b
d
a
e

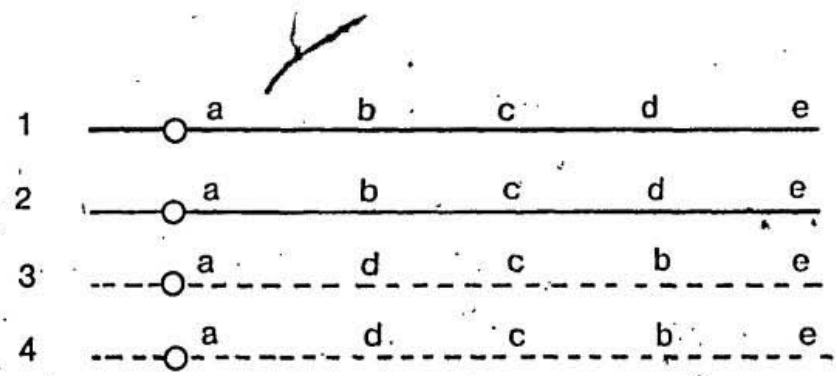
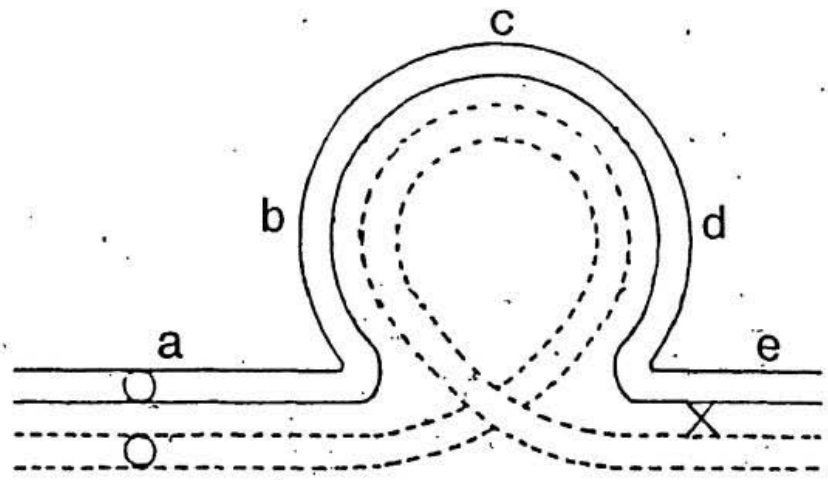

1
a b c d e
2
a b c d e
3
a d c b e
4
a d c b e

Figure 1-4

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, chiasma formation in the segment distal to the inverted region and the recombinants formed. (1) and (2) are normal. (3) and (4) are balanced inverted chromosomes.


c
b
d
a
e

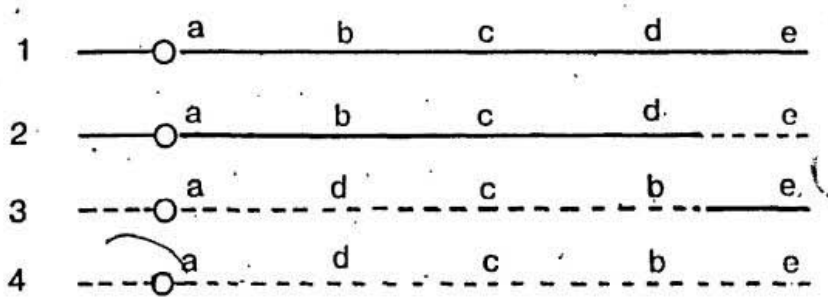
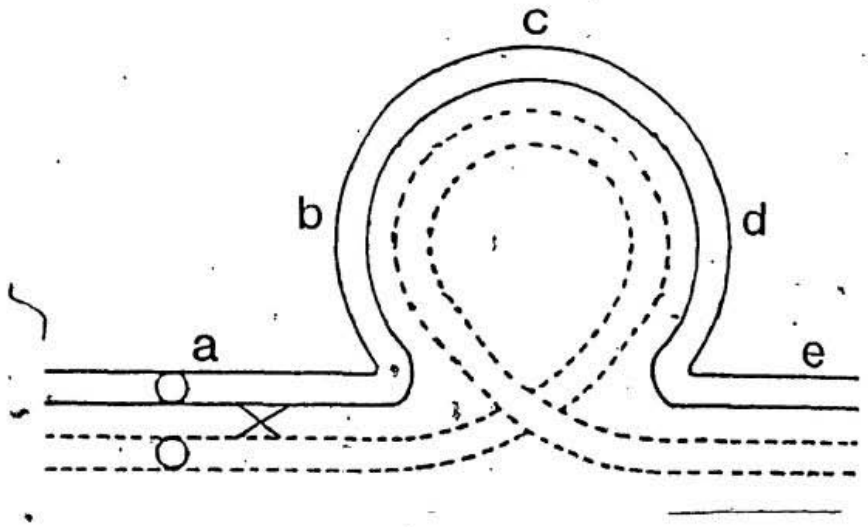

1
a b c d e
2
a b c d e
3
a d c b e
4
a d c b e

Figure 1-5.

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, chiasma formation in the interstitial segment and recombinants formed. (1) and (3) are normal. (2) and (4) are balanced inverted chromosomes.


c
b
d
a
e

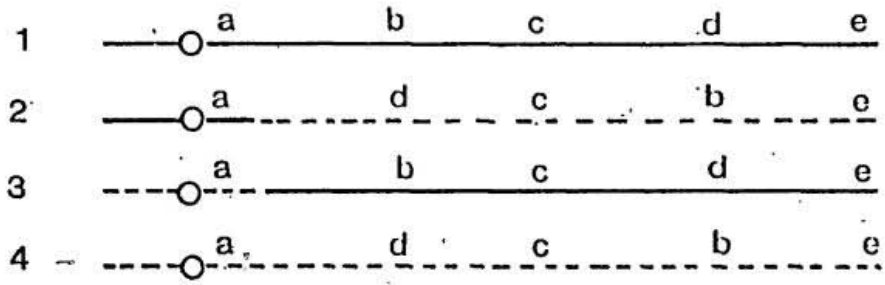
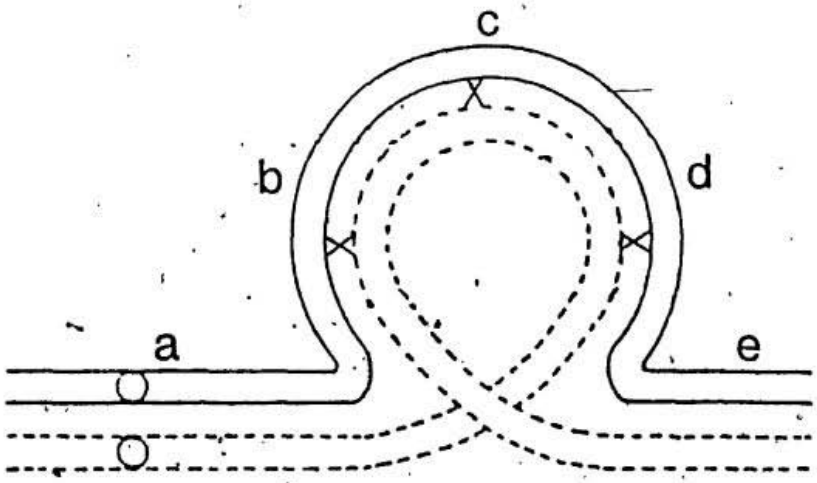

1
a b c d e
2
a d c b e
3
a b c d e
4
a d c b e

Figure 1-6

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, uneven number of chiasmata formation between non-sister chromatids in the inverted segment and the recombinants formed. (1) is a normal chromosome, (2) is a dicentric recombinant with duplication of the locus 'a' and deficiency of locus 'e', (3) is a balanced inverted chromosome and (4) an acentric fragment with deficiency of the 'a' locus and duplication of the 'e' locus. The dicentric recombinant forms a bridge during first anaphase.


c
b
d
a
e

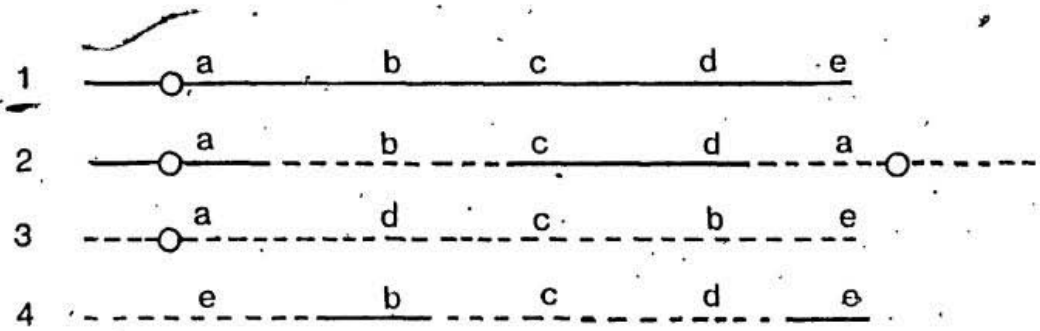
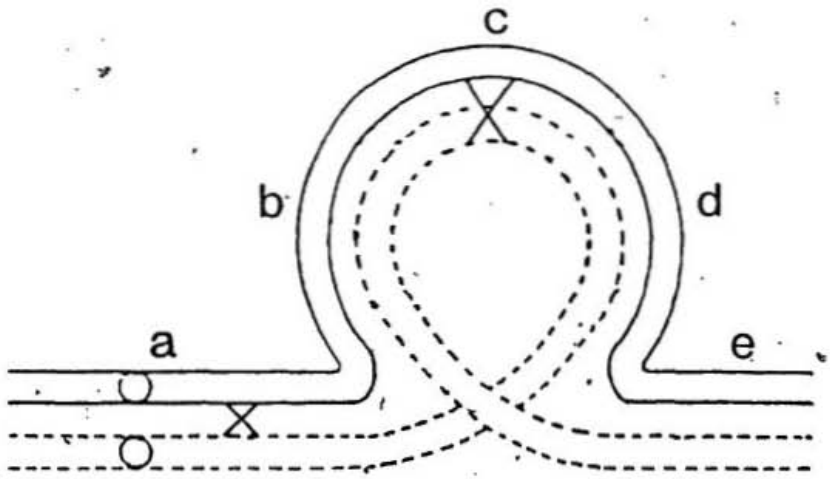

1
a b c d e
2
a b c d a
3
a d c b e
4
e b c d e

Figure 1-7

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, chiasmata formation involving the same non-sister chromatids, one in the interstitial segment and the other in the inverted segment and the recombinants formed. (1) is a normal chromosome, (2) is a dicentric recombinant with duplication of the 'a' locus and deficiency of the 'e' locus, (3) is an acentric fragment with deficiency of the 'a' locus and duplication of the 'e' locus and (4) is a balanced inverted chromosome. The dicentric recombinant forms a bridge during first anaphase.


c
b
d
a
e

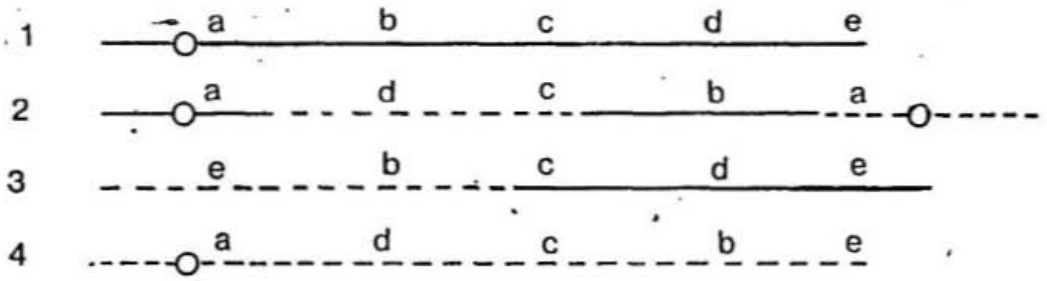
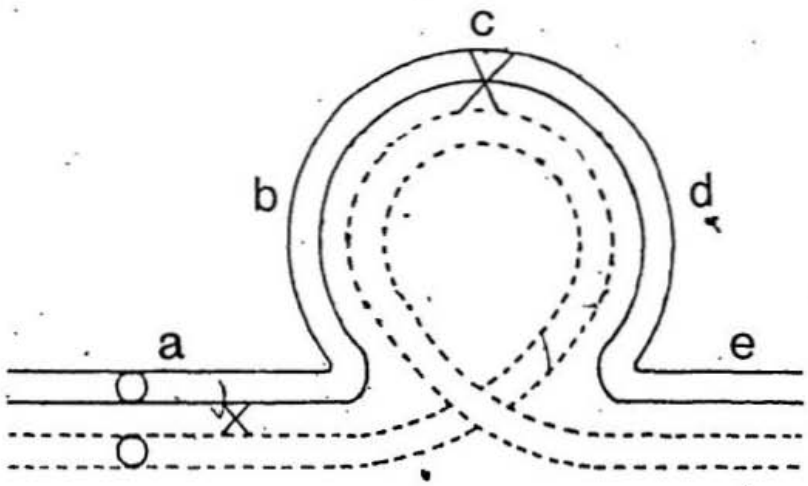
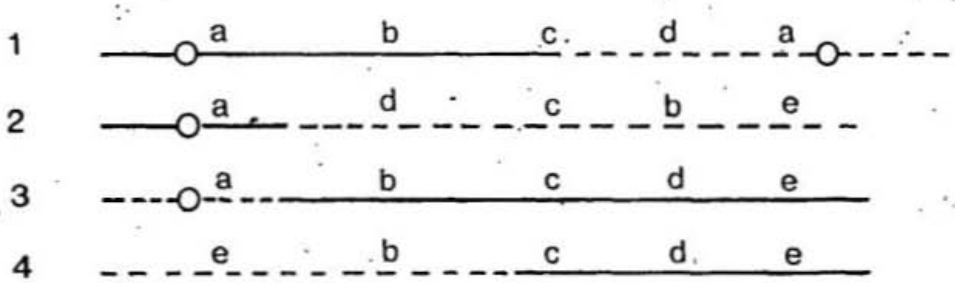

1
a b c d e
2
a d c b a
3
e b c d e
4
a d c b e

Figure 1-8

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, chiasmata formation involving different non-sister chromatids, one in the interstitial segment and the other in the inverted segment and the recombinants formed. (1) is a dicentric recombinant with duplication of locus 'a' and deficiency of 'e', (2) is a balanced inverted chromosome, (3) is a normal chromosome and (4) is an acentric fragment with deficiency of the 'a' locus and duplication of 'e'. The dicentric recombinant forms a bridge during first anaphase.


c
b
d
a
e


1
a
b
c
d
a
2
a
d
c
b
e
3
a
b
c
d
e
4
e
b
c
d
e

fragment are formed (Figure 1-7 & 1-8). If, however only one of the two chromatids involved in the crossover in the inverted segment is also involved in the interstitial segment, a dicentric chromosome that forms a loop during first meiosis and a dicentric bridge during second anaphase and an acentric fragment result (Figure 1-9).

The dicentric chromosome and the acentric fragment are duplicated for some loci and deficient in others. The size of the acentric fragment is the sum of the length of the inverted segment and twice the length of the uninverted segment from the distal breakpoint to the telomere. The size of the dicentric bridge is the sum of the length of the inverted segment and twice the length of the interstitial segment.

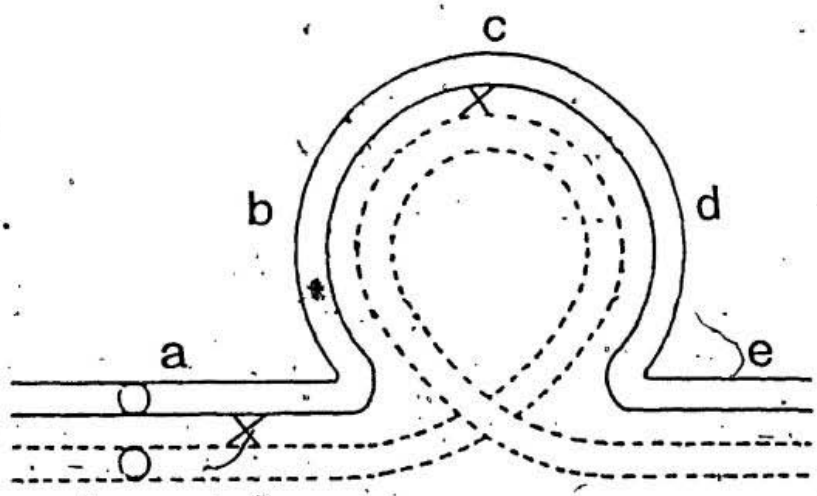
1.7.1. Frequency and location of chiasmata in the normal state

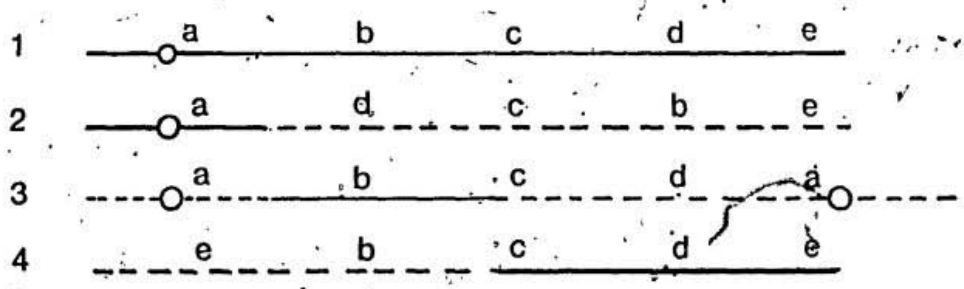
Crossovers are points of physical exchange between homologous non-sister chromatids. Use of staining techniques that enable differentiation of sister chromatid suggests that crossovers occur at positions identified as chiasmata cytologically (Tease, 1978, Polani, Crolla, Seller & Moir, 1979). Observations in *Lilium formosanum* heterozygous for paracentric inversions suggested the relationship between chiasmata and crossing over (Brown & Zohary, 1955).

Data on the frequency and location of chiasmata in a variety of species has been used to estimate the frequency and location of crossovers. For example, in the Locust *Schistocera gregaria* when only one chiasma is present on one arm of a

Figure 1-9

Diagrammatic representation of synapsis of the inverted segment during pachytene by loop formation, with two chiasmata formed between non-sister chomatids, one in the interstitial segment and the other in the inverted segment. One chromatid, represented by the solid line, is involved in both chiasmata and the other chromatids represented by the dotted lines differ in each chiasmata. The recombinants formed are (1) a normal chromosome, (2) a balanced inverted chromosome, (3) a dicentric recombinant with duplication of the locus 'a' and deficiency of 'e' and (4) an acentric fragment with deficiency of 'a' locus and duplication of the 'e' locus. The dicentric recombinant forms a bridge during second anaphase.


c
b
d
a
e


1
a
b
c
d
e
2
a
d
c
b
e
3
a
b
c
d
a
4
e
b
c
d
e

chromosome, it is normally formed near the telomere. Based on this observation it has been suggested that the sequence of chiasma formation is from the telomere to centromere (Fox, 1973). This is also the sequence of synapsis (Moses, 1977). Similar observations have been made in the mouse (Maudlin & Evans, 1980).

The possible movement of chiasmata towards the telomere (terminalization) could interfere with the use of chiasmata to study crossing over. Some investigators have observed evidence suggestive of terminalization (Hultén, 1974), whereas others have evidence to the contrary (Tease & Jones, 1978, Fox, 1973, Tease, 1978) or evidence that is inconclusive (Polani, Crolla, Seller & Moir, 1979). Maudlin and Evans have reviewed the literature on chiasma terminalization and discussed possible mechanisms for the apparent process of terminalization (Maudlin & Evans, 1980). Terminalization, therefore must be considered when interpreting these reports on the location of chiasmata.

It is suggested that following formation of the first chiasma, the next one is located beyond a certain distance. The interference distance represents the minimum distance within which chiasma formation is precluded. In the mouse it may have a role in determining the distribution of chiasmata (Maudlin & Evans, 1980). Studies of cells in diakinesis in a human male suggests interference (Hultén, 1974). As it is technically easy to study male cells in meiosis, information available on interference in the much more difficult females is limited.

1.7.2. Factors which appear to influence location of chiasma

Differences in the number and location of chiasmata in the two sexes in a variety of species has been reviewed by White (White, 1973). Differences in the location of chiasmata between sexes have been reported in newts (Watson & Callan, 1963), plants (Fogwill, 1958, Ved Brat, 1966) and in at least two strains of mice (Polani, 1972). The frequency of distal chiasmata is significantly greater in males than in the females. It is not known whether this difference is due to technical, cytological or unknown biological differences in the two sexes (Polani, 1972).

Under normal circumstances chiasma frequency on one arm of the chromosome increases with the length of the arm (Fox, 1973, Maudlin & Evans, 1980). Differences in the frequencies of chiasmata between different sexes in Lilium and Fritillaria has been reported by Foqwill (Fogwill, 1958). Differences in the frequencies of chiasmata between different sexes in a number of plant and animal species has been reviewed by Callan & Perry (Callan & Perry, 1977).

1.7.3. Frequency and location of chiasmata in inversion heterozygotes

The rules applicable to structurally normal chromosomes in determining the frequency and location of chiasma may not be valid in the presence of an inverted segment. Frequency of crossing over within the inverted segment depends on the length of the inverted segment, its relative location on the chromosome and the crossover characteristics of the individual (Swanson, Merz & Young, 1981).

Paracentric inversions appear to suppress crossover in the inverted segment (Refer section 1.6). To maintain a crossover frequency close to an optimal value, the frequency of crossover is increased in the uninverted region of the chromosome as well as in other chromosomes. Although this phenomenon, known as the Schultz-Redfield effect, has been studied and reported extensively only in *Drosophila*, it may occur in other species as well (Schultz-Schaeffer, 1980).

1.8. Reproductive outcome in carriers of paracentric inversion

The abnormal recombinants formed in paracentric inversion heterozygotes are dicentric chromosomes and acentric fragments. The acentric fragment may be lost. The dicentric chromosome is duplicated for some loci and deficient in others and may have an effect on the fertility of the heterozygote. Prospective studies are essential for an unbiased assessment of the effects of paracentric inversion on reproduction. Meiotic behaviour of paracentric inversions and their reproductive consequences has been studied in some plants and animals.

In female carriers of paracentric inversions in *Drosophila*, preferential

elimination of the dicentric chromosome has been observed. By selective orientation, the dicentric chromosome is included in the two inner nuclei of the linear quartet formed following meiosis. These cells form polar bodies and are not fertilized. The fertility of female *Drosophila* heterozygous for a paracentric inversion is therefore not affected (Sturtevant & Beadle, 1936). This phenomenon has also been observed in *Sciara* (Carson, 1946). In mammals linear quartets are not formed.

In maize, plants heterozygous for a paracentric inversion on chromosome 4 (4a) showed an ovule abortion rate of 4% and pollen abortion rate of 25%. Crossover characteristics in male and female flowers are not different. The observed difference in abortion rate has therefore been attributed to preferential exclusion of dicentric chromatids with duplications and deficiencies from the nucleus of the egg. (Morgan, 1950).

The observed degree of pollen abortion in maize heterozygous for paracentric inversions is found to be lower than expected on the basis of cytological observations. It has been suggested that the degree of pollen abortion is a function of various mechanisms capable of restoring a genetic constitution in the spores compatible with viability. The dicentric bridge, if formed, ruptures during anaphase or telophase. If the genetic deficiency is sufficiently small, the pollen develops normally. It is also suggested that spores may develop normally if the acentric fragment complements the deficiency resulting from rupture of the dicentric bridge (Rhoades & Dempsey, 1953).

Heterozygosity for paracentric inversions in the grass, *Festuca pratensis* (Simonsen, 1975), does not appear to effect its fertility. As in *Drosophila*, selective orientation is thought to be responsible.

Fertility has been found to be affected in barley heterozygous for paracentric inversions (Ekberg, 1969). Both ovule and pollen abortions were found to range between 37.0%-49.2% for pollens and 32.8%-44.9% for ovules (in different plants heterozygous for the same inversion) (Das, 1955). Absence of selective orientation or presence of a gene similar to the lethal ovule gene is suggested as possible explanations for similar rates of ovule and pollen abortions.

In mice, investigation of two inversions suggest that fertility is impaired in heterozygotes of one paracentric inversion in both sexes, In(5)0RK and in females alone in the other (In(2)5RK) due to pre- and post-implantation losses (Ford, Evans & Burtenshaw, 1976).

It is evident that even if recombinants are formed, their effect on fertility may differ between the two sexes in some species.

1.9. Characteristics of pericentric and paracentric inversions - an analysis

Pericentric inversions differ from paracentric inversions in their reproductive implications and population frequencies.

Paracentric inversions result from two breaks on the same arm of a chromosome, whereas pericentric inversions result from two breaks on the two arms. Therefore in a paracentric inversion the inverted segment does not include the centromere, whereas in pericentric inversion it does. Based on inversions reported in literature, the proportion of *de novo* pericentric and paracentric inversions does not appear to be different (Refer section 1.3.1).

Although loops are formed during meiosis to enable homologous pairing in heterozygotes of both pericentric and paracentric inversions, recombinants with duplications and deficiencies have been identified predominantly in the case of pericentric inversions. Differences in the structure and behaviour of recombinants in the two types of inversions may account for this observation. Unless one centromere is inactivated the dicentric recombinants formed in paracentric inversion heterozygotes, may be eliminated unlike monocentric recombinants in pericentric inversion heterozygotes.

The frequency of pericentric inversions is significantly higher than the frequency of paracentric inversions. In the French Collaborative Study 304 pericentric inversions were identified compared to 34 paracentric inversions (A French Collaborative Study, 1986b). If it is assumed that most breaks occur at the

junction of R- and G- bands, at the 802 band level, 7650 pericentric inversions and 8607 paracentric inversions are expected and at the 273 band level the expected numbers are 917 pericentric and 862 paracentric inversions (Dutrillaux, Prieur & Aurias, 1986). The apparent difference in frequencies may be due to the technical inadequacies that existed until recently in identifying paracentric inversions. Because the frequency of recombinants is higher for pericentric than in paracentric inversion carrier, ascertainment on the basis of recombinants is lower for paracentric inversions.

On analysis of observations related to paracentric inversions and other balanced rearrangements, it is apparent that features such as increased incidence among individuals who have developmental abnormalities and presence of interchromosomal effects are common to both groups. However features such as a low frequency of recombinants and low frequency in the general population and in some clinically definable populations compared to those of other balanced rearrangements (subfertile males and couples who have experienced repeated spontaneous abortions) are unique to paracentric inversions.

1.10. Phenotypic effects of chromosomal rearrangements and ascertainment

Minimal phenotypic effects of heterozygosity for a paracentric inversion is one possible explanation for its low frequency, since overt phenotypic deviations aid in ascertainment of individuals with chromosomal rearrangements. Elimination of recombinants prior to a stage when they may be identified may be one effect of

the rearrangement with apparently minimal implications. Some of the effects, although insignificant, may have a role in preventing production of gametes with recombinants. Prospective studies on the effects of heterozygosity for chromosomal rearrangements on reproduction may enable one to identify the effects and determine the extent of these effects. Various aspects of reproduction need consideration in this context.

1.10.1. Effects of chromosomal rearrangement evident at various stages of reproduction

Chromosome rearrangements may or may not have major reproductive implications. When present, the reproductive effects of chromosomal rearrangements may be evident at different stages of the reproductive process. Some rearrangements affect gametogenesis resulting in reduced gamete count. This effect may go unnoticed if the reduction in gamete count is small enough not to affect fertility.

In carriers of some rearrangements, abnormal recombinants are included in gametes that have the ability to fertilize successfully. Some of the resulting individuals are identified as prenatally eliminated unbalanced zygotes and/or as offspring with congenital abnormalities. If heterozygosity for paracentric inversions results in selection at the pre-gametic and/or pre-fertilization stages of reproduction, and if fertility is not affected, the chances of its ascertainment due to effects apparent at the post-fertilization stage is considerably reduced. In some cases the probability of ascertainment may even be eliminated.

An ideal assessment of the reproductive implications of any rearrangement under consideration ought to involve prospective investigations which would enable determining the effects of the rearrangement at various stages of reproduction. They also ought to take into consideration the various factors that could influence the effects of the rearrangement. Sex of the carrier of the rearrangement is one factor.

1.10.2. Possible differences in the effect of a rearrangement on reproduction in the two sexes

Differences between male and female gametogenesis in mammals need to be considered when evaluating the effects of chromosomal rearrangements. A major difference between male and female gametogenesis involves the products of meiosis in the two sexes. All four cells that result from spermatogenesis of a single spermatogonium have the potential to develop into sperm. Following oogenesis, however, only one cell matures into the oocyte which is fertilized. The remaining three (polar bodies) degenerate.

There are at least two consequences of this difference that are important in determining the reproductive implications of chromosome rearrangements. One is that in females the abnormal recombinants may be excluded (selectively or not) from the reproductive path by being included in the polar bodies. The other is related to the production of large numbers of sperm which is a characteristic of males in a variety of species (Bedford, Rodger & Breed, 1984). The maintenance of this characteristic through evolution suggests its importance in efficient

reproduction. Because of this characteristic, reduction in number of sperms may occur without significant reduction in male fertility.

Rearrangements which affect products of meiosis may therefore have differnt effects in the two sexes. As only one out of 4 cells resulting from meiosis is functional in females, preferential inclusion of the recombinant in one of the cells destined to degenerate, will not seriously affect female fertility. This has been well demonstrated in Drosophila (Sturtevant & Beadle, 1936). If however, preferential exclusion is not a feature, female fertility may be affected.

As the number of sperm produced is normally in excess of what is required, reduction in sperm count due to heterozygosity may be tolerated to a certain extent before fertility is affected. An effect on sperm production may be identified by a reduced sperm count. If however sperm with abnormal recombinants are functionally normal, fertility at the post-fertilization stage may be affected as a result of genomic imbalance. Therefore, differences in the effects of a chromosomal rearrangement in the two sexes, and differences in the stage at which selection occurs in male and female carriers may provide information on the biological mechanisms behind the observed effects.

Rearrangements have been reported in humans and mice that affect fertility in male and not female carriers, resulting in male specific sterility. On the basis of cytological observations, interaction of the XY bivalent and the rearranged chromosome has been postulated as a possible mechanism and is extensively documented (Searle, Beechey & Evans, 1978).

Additional aspects of reproduction to be considered in the context of male and female gametogenesis is the difference in developmental stages at which meiosis commences and the time required for completion of gametogenesis in males and females. In females the meiotic process begins in the fetal stage. The process is arrested at the diplotene stage so that at the time of birth the oocytes are all at diplotene. At puberty, the process is resumed. In males the meiotic process starts at puberty and is completed with the formation of sperm within a few days. Duration of spermatogenesis is species specific (Searle, Ford & Beechey, 1971). Male mice are reported to become sexually mature by about 6 weeks of age (Engle & Rosasco, 1927) with spermatogenesis taking 34.5 days (Roosen-Runge, 1962). The effect of chromosomal rearrangements on reproduction in the two sexes may be different because: (1) gametogenesis occur at different stages of development and (2) because the time required for the process is different.

Differences in the meiotic process and observed differences in the fertility of male and female carriers of various rearrangements in humans and mice, necessitate investigations in both sexes when determining the effects of chromosome rearrangements on reproduction. These investigations will provide information on the biology of the observed effects, and differences in reproductive risk.

1.10.3. Relationship between physical characteristics of paracentric inversions and recombinant formation

In translocation carriers abnormal recombinants result from segregation. In heterozygotes for inversions, crossover (genetic recombination) is a prerequisite for the formation of abnormal recombinants ("aneusomie du recombinasion"). Probability of crossover in the inverted segment is positively correlated with the size of the inverted segment. Location of the inverted segment may be significant in recombinant formation if a relationship exists between location of the inversion on the chromosome, relative to the centromere or telomere, and the probabiliy of crossover in the inverted segment. Investigations of paracentric inversions of varying lengths and location may provide information on the physical characteristics of the inversion required for the production of gametes with recombinants (dicentric chromatid and/or acentric fragment).

1.10.4. Effects of chromosomal rearrangement evident at various stages of meiosis

When a chromosomal rearrangement affects gametogenesis, meiotic disturbance may be anticipated. More than one meiotic stage must be investigated in order to determine the nature and etiology of the disturbance. One reason for the evolution of the diploid state of higher organisms appears to be to facilitate synapsis of chromosomes in germ cells during meiosis prior to recombination. Pairing of homologous chromosomes occur during the pachytene stage of meiosis. Abnormalities in the pairing process have been observed to be associated with

meiotic disturbances and abnormal gametogenesis (Egozcue, Templado, Vidal, Navarro, Morer-Fargas & Marina, 1983, Vidal, Templado, Navarro, Brusadin, Marina & Egozcue, 1982, Koulischer, Schoysman, Gillerot & Debry, 1982, Westergaard & Wettstein, 1972, Chaganthi & German, 1979).

Heterozygosity for a chromosomal rearrangement may affect synapsis. In heterozygotes for chromosomal rearrangements synapsis of a rearranged chromosome with its normal homolog during pachytene may be identified as an abnormal configuration. In inversion heterozygotes a loop may be formed during pachytene. Study of germ cells from pachytene in these heterozygotes will provide insight into loop formation and its effects on meiosis and reproduction.

Maturation arrest has been reported in mice and humans heterozygous for chromosomal rearrangements (Forejt, 1982, Rodriguez, Martin & Abrisqueta, 1985, Egozcue, Templado, Vidal, Navarro, Morer-Fargas & Marina, 1983, Vidal, Templado, Navarro, Brusadin, Marina & Egozcue, 1982, Koulischer, Schoysman, Gillerot & Debry, 1982, Faed, Lamont & Baxby, 1982, Giraldo, Silva, Martínez, Campos & Guzmán, 1981, Plymate, Bremner & Paulsen, 1976, Chandley, Christie, Fletcher, Frackiewicz & Jacobs, 1972, Vidal, Templado, Navarro, Marina & Egozcue, 1982). It is also observed in individuals without apparent chromosome rearrangement (Sung, Komatsu & Jagiello, 1983, Westergaard & Wettstein, 1972, Chaganthi & German, 1979). The arrest may be at any stage of meiosis, but the outcome of the arrest is oligospermia or azoospermia in males (Laurent, Biemont,

Cognat & Dutrillaux, 1977, Chandley, Christie, Fletcher, Frackiewicz & Jacobs, 1972, Vidal, Templado, Navarro, Marina & Egozcue, 1982). The relative frequency of cells in various stages of meiosis has been used as a determinant of meiotic disturbance associated with impaired fertility (Micic & Micic, 1984, Micic & Micic , 1981, Román, Sordo & García-Sagredo, 1979).

Recombinants in heterozygotes for paracentric inversion are the dicentric chromatid and acentric fragment formed as a result of uneven number of crossovers in the inverted segment. Meiotic metaphases may be analysed to identify recombinants and aneuploidy. There is some evidence suggesting increased frequency of individuals with aneuploidy among offspring of heterozygotes for chromosomal rearrangement (Lindenbaum, Hultén, McDermott & Seabright, 1985).

1.10.5. Effect of chromsomal rearrangement and litter size

Some effects of chromosomal rearrangements on reproduction are apparent during the post-fertilization stages. In certain species, reduced litter size indicates existence of a chromosomal rearrangement, which affects fertility. It does not provide information on the nature of the defect (or stage at which reproduction is affected). Observation of reduced litter size may be followed up by studies of the meiotic process, of gametes and of the post-fertilization stages of reproduction. Counts of corpora lutea, implantation sites and resorption sites may provide a reliable estimate of possible loss in the post-fertilization stage due to unbalanced recombinants and subsequent genomic imbalance in the zygote.

The aim of the thesis is to determine if some of the observations on human carriers of paracentric inversions may be explained in terms of the effect of paracentric inversion on meiosis and gametogenesis in male and female carriers.

As prospective studies on humans is not feasible, the available mouse model was used to investigate some aspects of meiosis and reproduction in male and female mice heterozygous for paracentric inversions. Paracentric inversions involving segments of varying lengths and location on the chromosome were investigated. Reduced phenotypic expression is one obstacle in the ascertainment of chromosome rearrangements. Therefore the effects of the various paracentric inversions on litter size has been used as a basis for interpretation of observations at selected stages of the meiotic process.

Chapter II provides a detailed description of the inversions investigated, the materials used and methodology employed. Chapter III comprises an introduction to effects of chromosomal rearrangement on male fertility, observations and discussions on male mice heterozygous for the various paracentric inversions investigated. Introduction to effects of chromosomal rearrangement on female fertility, observations and related discussions on female mice heterozygous for the paracentric inversions investigated constitute Chapter IV. Ramifications of the observations on the apparent effect of heterozygosity for paracentric inversions in the two sexes are presented in Chapter V. An attempt is made to explain observations in human carriers of paracentric inversions in Chapter V.

Chapter 2

Materials and Methods

2.1. Animals

Mice homozygous for most of the paracentric inversions investigated were generously provided by Dr. T.H. Roderick (The Jackson Laboratory, Bar Harbor, Maine 04609, USA.). Inversions obtained in the homozygous state were: IN(1)1RK, IN(1)12RK, IN(1)24RK, IN(3)11RK, IN(8)14RK, IN(11)20RK and IN(14)22RK. Male mice heterozygous for IN(10)17RK were obtained from the same source. [Homozygotes for IN10(17)RK are sterile and are therefore maintained in the heterozygous state.]

2.1.1. Production and nomenclature of the inversions

Structural rearrangements of chromosomes in spermatocytes were induced by X-irradiation of the lower half of the body of male mice (Roderick, 1971), oral or intraperitoneal administration of mutagens like ethylmethane sulfonate (EMS) or triethylene melamine (Roderick & Hawes, 1974). Male off-spring of these males were screened for paracentric inversions.

Of the inversions investigated, only 1N(1)1RK was induced by X-irradiation. The others were induced by triethylene melamine. All the inversions investigated

were induced in DBA/2J males, except IN3(11)RK which was induced in a C57BL/6J male (Roderick, 1983).

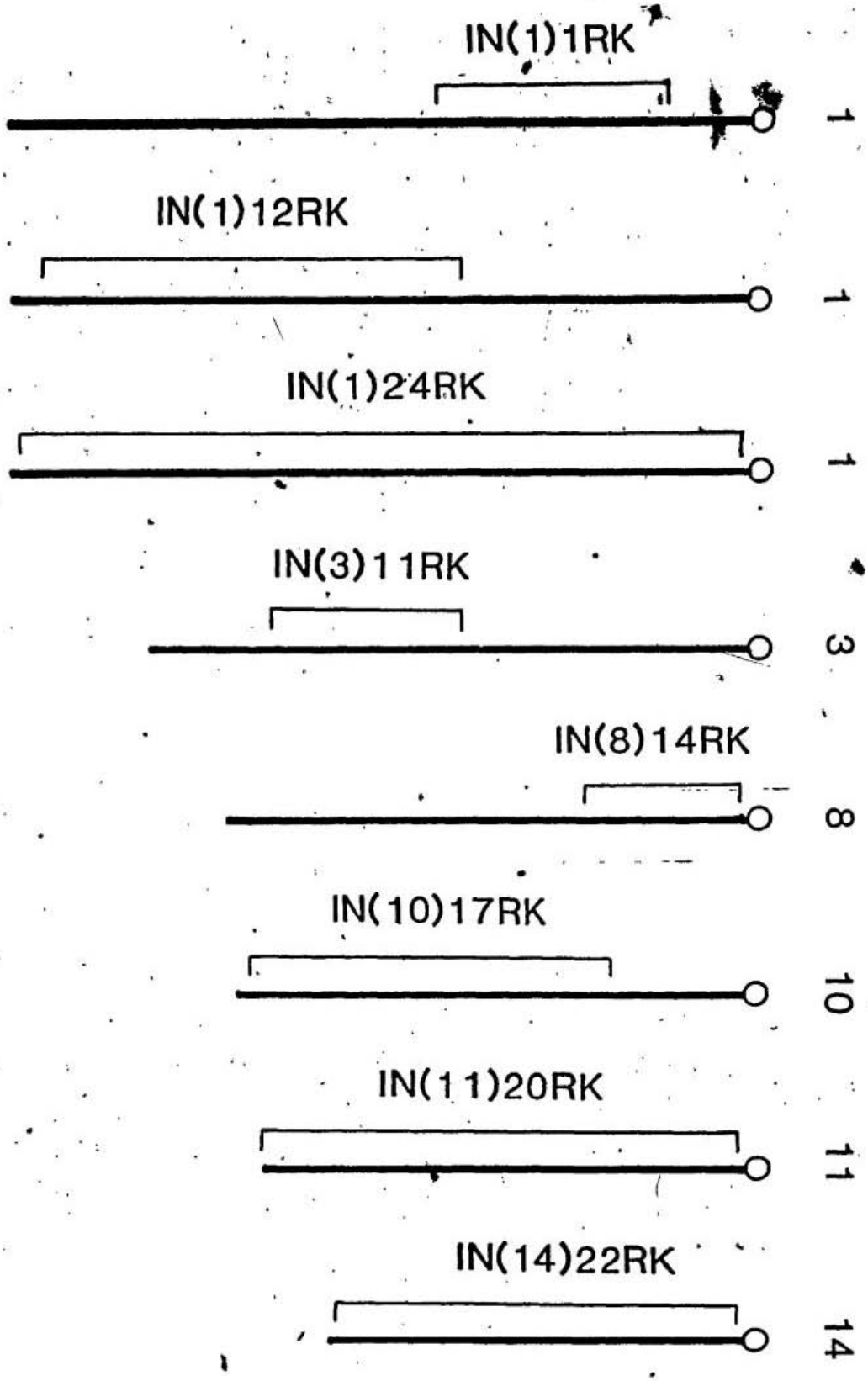
The number in parenthesis refers to the chromosome carrying the inversion. The number outside the parenthesis refers to the order in which it was identified. Existence of an inversion was initially established by the presence of high frequencies of anaphase bridges in histological preparation of testes. Dominant inheritance of the increased frequency of anaphase bridges, absence of recombination of genes in the inverted region, no significant effect on fertility and cytogenetic evidence were used in confirming the presence of an inversion (Roderick, 1979). (Anaphase bridges may be observed in animals heterozygous for inversions associated with translocations. Unlike simple inversions, however, the fertility of these heterozygotes is reduced). Anaphase bridge frequency appears to correlate with the length of the inversion and is consistent for the specific inversion across generations (Roderick, 1979).

Selection of inversions for the study was based on the length of the inverted segment relative to the length of the chromosome on which it is located i.e., relative physical length (Davisson & Roderick, 1973) and its location relative to the centromere (Figure 2-1). The initial rationale for employing this wide range of physical characteristics was to provide the prerequisite for crossover in the inverted segment and formation of dicentric chromatid and acentric fragment.

Heterozygotes for the inversions were produced by mating male or female mice

Figure 2-1

Diagrammatic representation of paracentric inversions in mice that were investigated. The number in paranthesis refers to the chromosome carrying the inversion. The number outside refers to the order in which it was identified.


IN(1)1RK
1
IN(1)12RK
1
IN(1)24RK
1
IN(3)11RK
3
IN(8)14RK
8
IN(10)17RK
10
IN(11)20RK
11
IN(14)22RK
14

homozygous for the inversion with C57BL/6J or C3HFeJ mice. The C57BL/6J mice were obtained from the Health Sciences Centre Animal Care Facility and the C3HFeJ mice were obtained from the Jackson Laboratory, Bar Harbor, Maine 04609, USA. Compound heterozygotes[1] and double heterozygotes[2] were produced by mating homozygotes for single inversions.

2.1.2. Maintenance of animals

Animals were housed at the "minimal disease" quarters of the Animal Care Facility of the Health Sciences Centre, 4 animals to a cage (polycarbonate mouse cage). Purina Mouse Chow and filtered water was available 24 hours a day. The temperature in the quarters was maintained at 21°C-24°C and humidity at 45%. The animals were kept on a 10-hours dark to 14 hours-light cycle.

2.1.3. Breeding

Breeding cages held 1 male and 1-3 females. Pregnant mice were maintained one animal per cage. Cages of pregnant mice were examined daily to determine the date of birth of the litter. Pups were weaned on day 21-25.

---

[1]Compound heterozygote: Two different inversions on homologous chromosomes.

[2]Double heterozygote: Inversions on non-homologous chromosomes.

2.1.4. Fibroblast culturing and mitotic metaphase preparation

Randomly selected mice were karyotyped. Fibroblast cultures were established in 25 cm^2 Falcon flasks (Appendix N) from finely chopped tissue from the abdominal wall of the mouse. Cells were cultured in 5ml of RPMI 1640 with fetal calf serum and pencillin and streptomycin (Appendix M) in 5% CO_2 at 37°C. Once sufficent outgrowth of cells from the explant was observed (after approximately 7-10 days), the culture was trypsinized (Appendix M) and subcultured. When sufficient number of cells in mitosis was observed, the culture was treated with 0.5 ml of Colcemid (10 mcgm/ml solution) (Appendix N) for 90 minutes. The culture was then trypsinised (Appendix M) and transferred into a centrifuge tube. After centrifugation at 200 g for 5 minutes, the supernatant was removed by suction. The cells were then exposed to 0.54% KCl for 10 minutes at room temperature. The suspension was again centrifuged for 5 minutes at 200 g. The supernatant was removed and the cells fixed in freshly prepared fixative (Appendix M) for 30 minutes. The fixative was changed twice. Two drops of cell suspension after the last change of fixative were dropped on a clean slide held at an angle of 30 degrees. The slide was then placed on a hotplate at 60°C for 2 minutes.

2.1.4.1. Giemsa staining of mitotic metaphase

The slides were aged for 48 hours at room temperature. They were then treated for 5-10 seconds with freshly prepared trypsin (Appendix N & M), washed in 0.9% NaCl and stained with freshly prepared Wright stain (Appendix M) in borate buffer (Appendix M) for 40 seconds. Excess stain was washed away with distilled water. The slides were dried and mounted in di-n-butyl phthalate.

2.1.4.2. Analysis of mitotic metaphase

Slides were analysed on a Carl Ziess light microscope. When possible, 20 well spread and well banded metaphases were photographed at a magnification of 1250X (Objective 100X Eyepeice 12.5X). The inverted chromosome could be identified with certainty only in heterozygotes for 1RK [Figure 2-2], 12RK [Figure 2-3], 24RK [Figure 2-4] and 20RK [Figure 2-7] because of distinct rearrangement of the banding pattern. Metaphases from heterozygotes for 11RK, 14RK, and 22RK are shown in Figures 2-5, 2-6, and 2-8.

2.2. Preparation of spermatocytes for metaphase analysis

The method described by Evans et al. for meiotic preparation from mammalian testes (Evans, Breckon & Ford, 1964) was used with modifcations.

2-7 month old male mice (Appendix II) were sacrificed by cervical dislocation. Testes were removed and placed in isotonic sodium citrate (Appendix M) at room temperature. The tubuli were released by removing the tunica albuginea. They were chopped finely with a pair of scissors and teased with a pair of 26G needles. The suspension was repeatedly pipetted approximately 50 times to release the sperinatocytes. The large fragments were allowed to sediment and the supernatant with spermatocytes was centrifuged for 5 minutes at 80 g. The sedimented spermatocytes were resuspended in 1% sodium citrate. After 12 minutes at room temperature the suspension was centrifuged at 80 g for 5 minutes. Spermatocytes were fixed by resuspending the sediment in freshly prepared fixative (Appendix M). After 30 minutes at 4°C, the suspension was centrifuged for 5 minutes at 80 g.

Figure 2-2

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion IRK. The structurally normal (N) and the inverted (I) chromosomes are readily identifiable.


N
I

Figure 2-3

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 12RK. The structurally normal (N) and the inverted (I) chromosomes are readily identifiable.


N

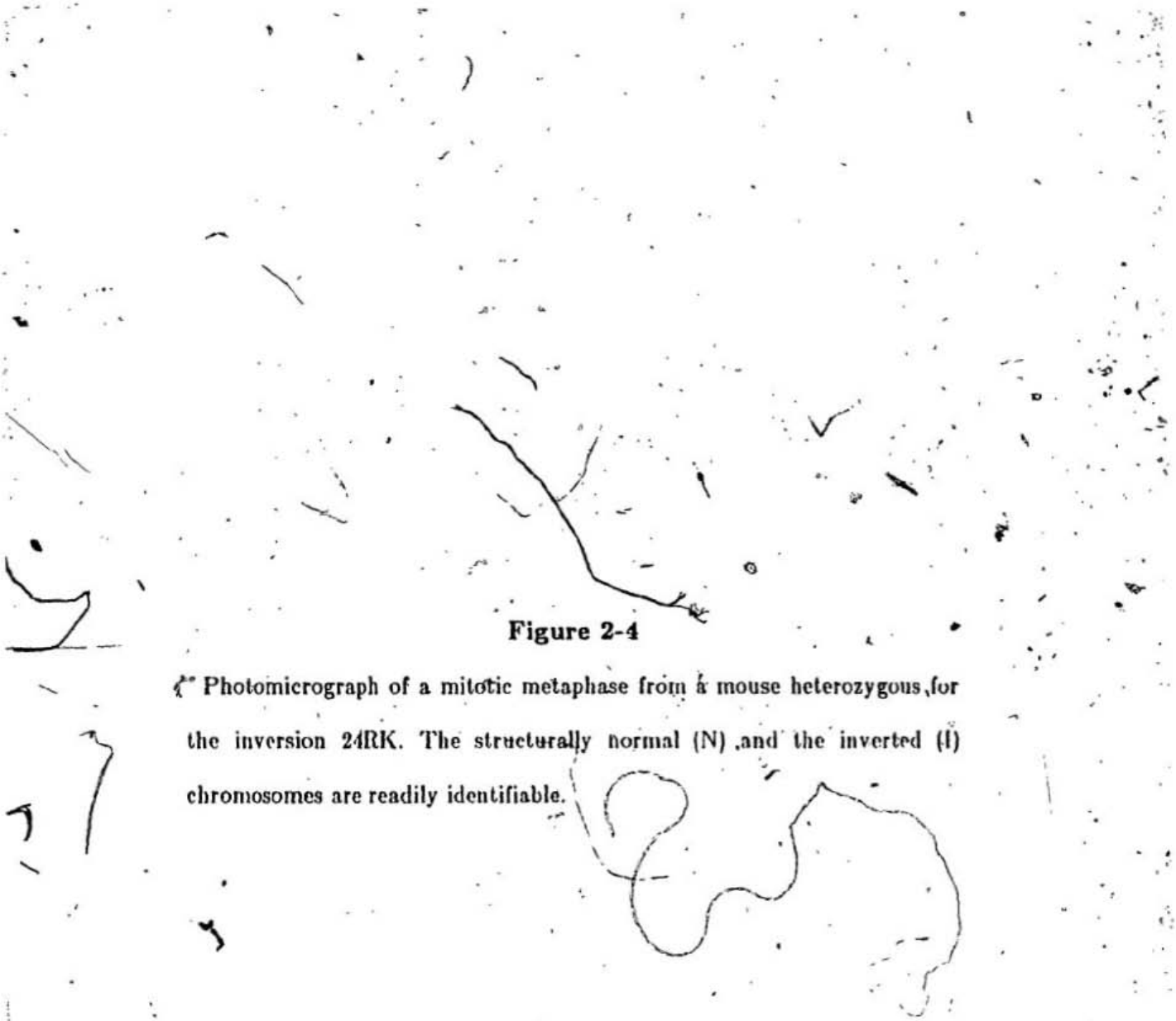


Figure 2-4

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 24RK. The structurally normal (N) and the inverted (I) chromosomes are readily identifiable.


N

Figure 2-5

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 11RK. The structurally normal and inverted chromosomes (arrows) are not readily identifiable.

Figure 2-6

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 14RK. The structurally normal and inverted chromosomes (arrows) are not readily identifiable.

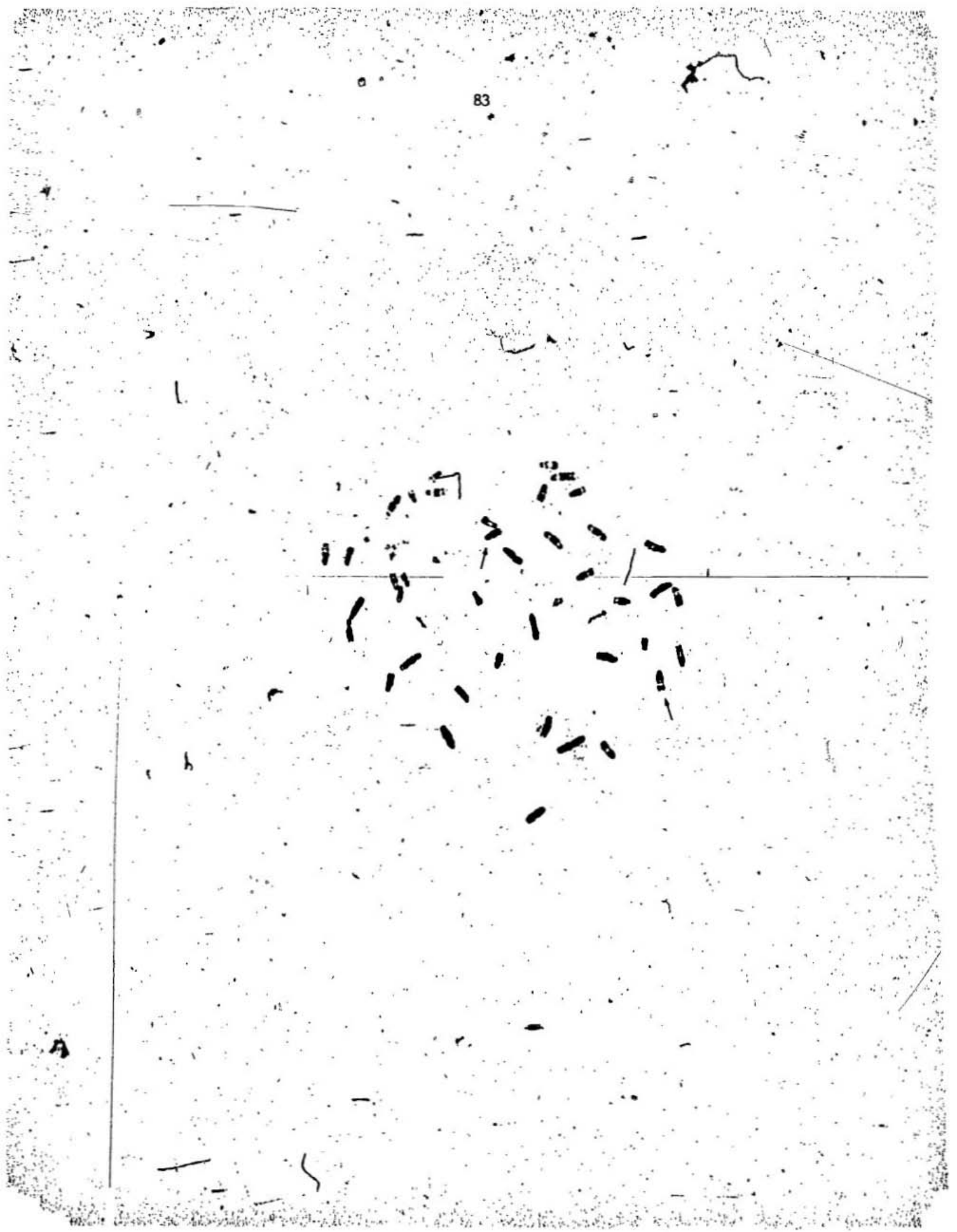


Figure 2-7

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 20RK. The structurally normal (N) and inverted chromosomes (I) are readily identifiable.


N

Figure 2-8

Photomicrograph of a mitotic metaphase from a mouse heterozygous for the inversion 22RK. The structurally normal and inverted chromosomes (arrows) are not readily identifiable.

The fixative was changed 3 times by resuspending the sediment in fresh fixative and centrifuging it for 5 minutes at 80 g. Slides were prepared by dropping 3 drops of the cell suspension on a clean slide held at an angle of 30 degrees. The slide with fixed spermatocytes was dried by placing it on a hot plate at 60°C for 2 minutes.

2.2.1. G-staining of spermatocytes

Slides with fixed spermatocytes were stained for 5 minutes with 2% Giemsa in phosphate buffer at pH 6.8 (Appendix M). Slides were rinsed in deionised water to remove excess stain and mounted in di-n-butyl phthalate. (Appendix N).

2.2.1.1. Analysis of metaphases

Slides were coded prior to microscopic analysis. Metaphases were analysed on a Leitz Wetzlar microscope at a magnification of 1250X (Objective 100X Eyepiece 12.5X). Whenever possible first meiotic metaphases (Figure 2-9) and haploid (apparent) second meiotic metaphases (Figure 2-10) totalling 100 were counted for each animal. The number of heteroploid second meiotic metaphases (Figure 2-11) observed while counting the 100 metaphases was also noted.

The proportions of haploid [(NIIM/IM+NIIM)x100] (an index of the number of spermatocytes that would form normal sperm), heteroploid [(HetIIM/NIIM+HetIIM)x100] (an index of the number of spermatocytes in which meiosis is disturbed) and total second meiotic metaphase [(NIIM+HetIIM/IM+NIIM+HetIIM)x100] (an index of the number of spermatocytes that proceeded to second meiosis) were calculated for each mouse.

Figure 2-9

Photomicrographs of first meiotic metaphase in spermatocytes. Giemsa stained (above) and centromere stained (below).

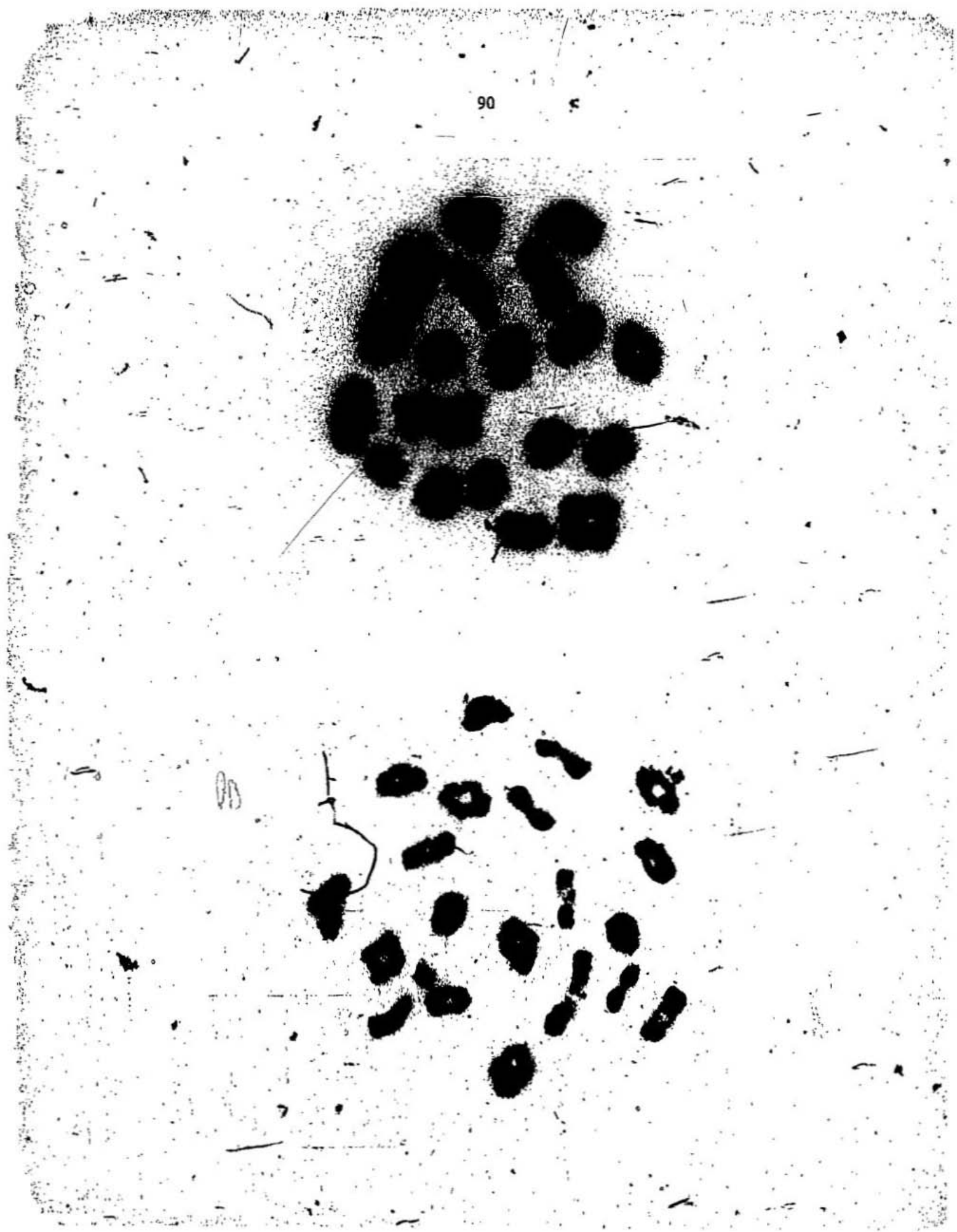


Figure 2-10

Photomicrographs of haploid second meiotic metaphase in spermatocytes. Giemsa stained (above) and centromere stained (below).

Figure 2-11

Photomicrographs of heteroploid second meiotic metaphase in spermatocytes. Giemsa stained (above) and centromere stained (below).

2.2.2. Light microscopic analysis of spermatocytes in pachytene

The method used to prepare the specimen and slides for light microscopic analysis of synaptonemal complex in male mice is a modified version of the method described by Dietrich and Mudler (Dietrich & Mudler, 1981).

3-5 month old male mice were sacrificed by cervical dislocation. Testes were collected in RPMI 1640 (with L-Glutamine) (Appendix N). Tubuli were released into the medium by removing the tunica albuginea. They were chopped with a pair of scissors and the spermatocytes released by teasing the fragments with a pair of 26G needles and repeated pipetting. The large fragments were allowed to sediment and the supernatant centrifuged at 500 rpm for 10 minutes. The supernatant was discarded and sedimented cells resuspended in 1 ml of 0.1M sucrose in water and placed on ice. After 7 minutes 1 ml of freshly prepared fixative (Appendix M) was added to the suspension and the final suspension was returned to the ice. After 2 minutes another ml of the fixative was added and the suspension once again was returned to the ice. After 5 minutes the suspension was centrifuged for 5 minutes at 80 g. The supernatant was discarded and spermatocytes resuspended in 3 ml to 5 ml of fixative.

About 0.5 ml of the suspension was placed on a slide and dried for 3 hours on a hotplate at 40°C. Excess of sucrose was removed by rinsing in deionised water for 5 minutes. Five drops of freshly dissolved silver nitrate solution (50% in deionised water) were placed on the slide and a coverslip placed on it. The slide was stained

for 15-17 hours at 37°C in the dark in a humid incubator. The cover slip was then removed and excess silver nitrate removed by rinsing with deionised water. Slides were dried and mounted with di-n-butyl phthalate. Cells in early pachytene were analysed on a Leitz Wetzlar light microscope. Cells in early pachytene were identified by the absence of the heterochromatic knobs (Figures 2-12 & 2-13), one of the criteria used by Moses in identifying mid-late stages of pachytene (Moses, 1980).

2.2.3. Electron microscopic analysis of spermatocytes in pachytene

The method used to prepare the specimen for electron microscopic analysis of pachytene chromosomes was a modification of those described by Mahadeviah et al. (Mahadeviah, Mittwoch & Moses, 1984) and that described by Navarro et al. (Navarro, Vidal, Guitart & Egozcue, 1981).

3 to 5 month old male mice were sacrificied by cervical dislocation and testes collected in RPMI 1640 (with L-Glutamine) (Appendix N). Tubuli were released into the medium by removing the tunica albuginea. They were chopped with a pair of scissors and spermatocytes released by teasing the fragments with a pair of 26G needles and repeated pipetting of the suspension. Large segments were allowed to sediment. The supernatant was centrifuged at 80 g for 5 minutes. The supernatant was discarded and the sedimented spermatocytes resuspended in RPMI 1640. The suspension was placed on ice.

One drop of the suspension was placed on a slide coated with 1% Formvar and

Figure 2-12

Photomicrograph of early pachytene preparation. The heterochromatic knobs are absent.

Figure 2-13

Photomicrograph of late pachytene preparation. The heterochromatic knobs are present.

0.01% Cytochrome C (Appendix M) and 2 drops of 0.5% sodium chloride were added to the drop of cell suspension. They were mixed and kept in a humid chamber. After 5 minutes 4 drops of freshly prepared fixative (Appendix M) was added to the mixture of cell suspension and sodium chloride.

After 5 minutes, the liquid on the slide was drained and 4 drops of fixative was placed on the slide where the cells adhered. After 5 minutes the fixative was drained and slides washed twice with 0.4% Photoflo (Appendix M).

Slides were stained with silver nitrate (Section 2.2.2) and analysed on a light microscope to locate cells in late zygotene or early pachytene. The selected areas were marked on the Formvar film with a water proof marker. The Formvar film was released from the slide by marking the film with a scalpel and introducing water between the film and the slide. The released film was floated off on deionised water. 75 mesh copper grids were placed on the marked area. The grids were picked up on paper, allowed to dry for 24 hours,and analysed on a Philips 300 EM.

2.3. In vitro culturing of oocytes

The method used for *in vitro* culturing of oocytes is a modified version of those used by Tarkowski (Tarkowski, 1966), by Henderson and Edwards (Henderson & Edwards, 1968) and by Tsuchida and Uchida (Tschuida and Uchida, 1974).

2-7 month old female mice (Appendix I) were sacrificed by cervical dislocation.

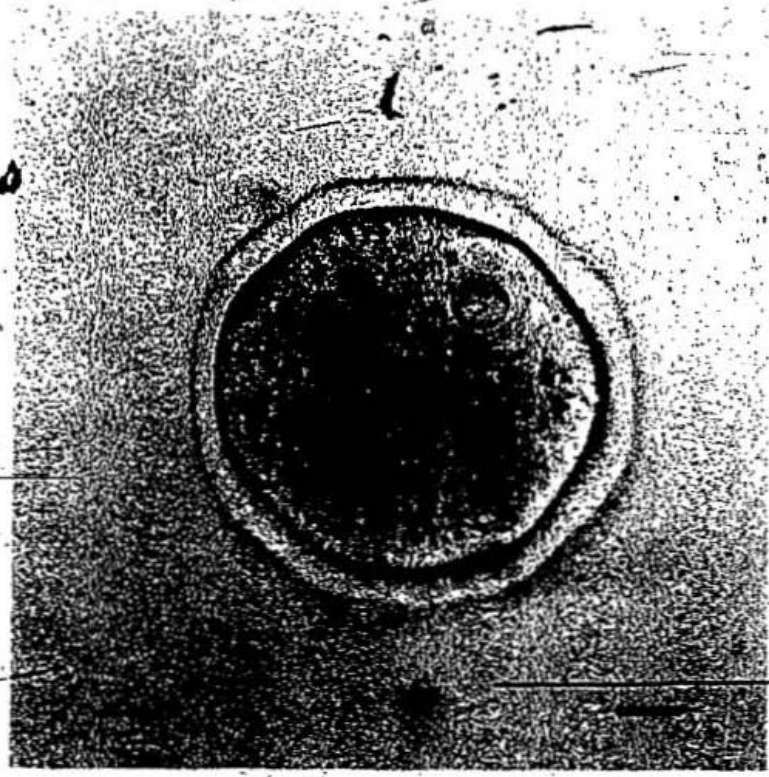
The abdominal region was cleaned with an alcohol swab prior and the abdominal wall dissected. The ovaries were removed and placed in TC Hanks Solution (Appendix N) in a sterile petri dish. They were transferred to another petri dish with TC Hanks solution to remove the lipid molecules on their surface. The clean ovaries were then transferred into TC Hanks solution in a sterile micro concavity slide (Appendix N). Dimensions of the concavity are 36mm. (diameter) and 5mm. (deep).

Ovaries were shredded with a pair of 26G sterile needles under a dissecting microscope. Released ooeytes with a visible germinal vesicle and nucleolus (Figure 2-14) were transferred into another sterile micro concavity slide with TC Hanks solution by mouth pipetting with a finely drawn out sterile pipette. Cells of the corona radiata surrounding the oocytes were removed by vigorously pipetting the oocytes repeatedly through a finely drawn sterile pipette.

The cleaned oocytes were transferred into fetal bovine serum (Appendix N) in a sterile micro concavity slide. They were once again transferred into another sterile micro concavity slide, containing 1.5 ml fetal bovine serum (Appendix M). 10 - 20 oocytes were placed in each slide. The micro concavity slide with the fetal bovine serum and oocytes was covered with a sterile glass slide. Maturation of oocytes *in vitro* may be affected by factors in the serum, which could interfere with interpretation of the observation. To detect any such effect, serum from the same aliquot was used to set up oocytes from 2 mice with different karyotypes.

Figure 2-14

Oocyte with a visible germinal vesicle and nucleolus. [Bar = 10 μm]



Slides with the oocytes were incubated at 37°C for 20 hours. The humidity inside the incubator was maintained by placing a large tray with water at the bottom of the incubator. The level of carbon dioxide inside the incubator was maintained at 5% with a Carbondioxide Control Master (Appendix N).

At the end of the incubation period the oocytes were transferred by mouth pipetting into 1.1% sodium citrate in a micro concavity slide. After 15 minutes at room temperature the oocytes were transferred on to a slide precleaned with 70% ethyl alcohol, 5 oocytes per slide. The area with the oocytes was marked on the lower side of the slide with a glass marker and excess hypotonic solution was pipetted off. The oocytes were fixed by placing a tiny drop of freshly prepared fixative on the oocytes (Appendix M1) and the slide was quickly blown dry under the heat of a 25 W incandescent bulb.

Additional information obtained from each female mouse was, the number of oocytes in division *in vivo*, and the number of oocytes with a visible germinal vesicle. Oocytes in division were identified by the absence of the nucleolus (Figure 2-15) and in some cases by the presence of the polar body (Figure 2-16).

Some oocytes with the germinal vesicle were identified after the culture had been set up, while screening the shredded ovaries for oocytes in division. In these instances, the number of oocytes set up in culture is lower than the number of oocytes in the germinal vesicle stage. When cultures were harvested the number of oocytes that had resumed meiotic division was recorded. They were identified by

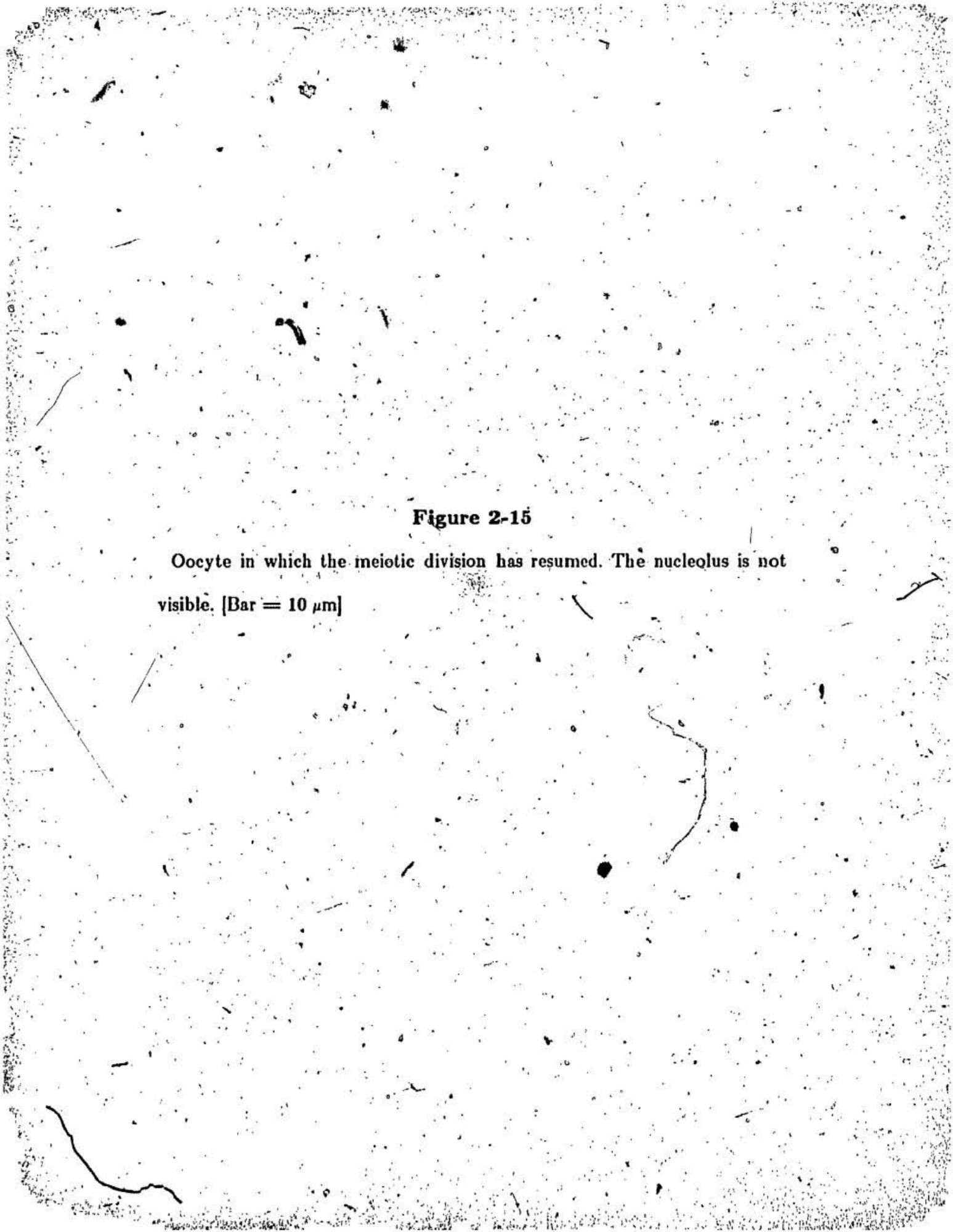


Figure 2-15

Oocyte in which the meiotic division has resumed. The nucleolus is not visible. [Bar = 10 μm]

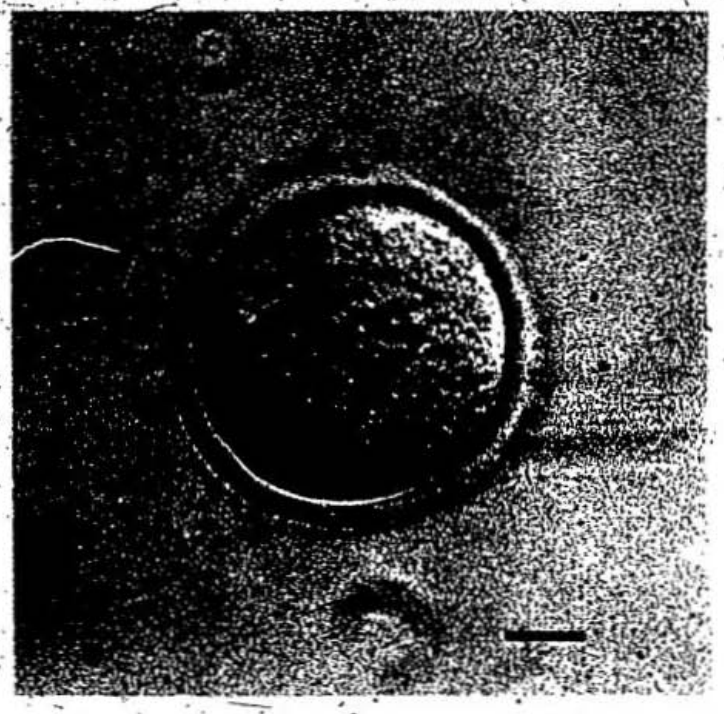
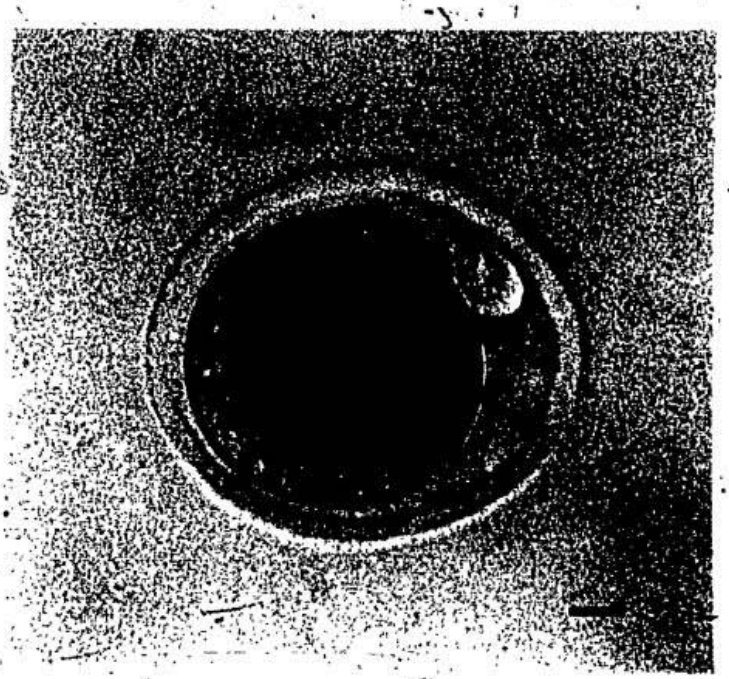


Figure 2-16

Oocyte in second meiosis. Completion of first meiotic division is evident by the presence of polar body. [Bar = 10 μm]



the absence of the nucleolus (Figure 2-15) and presence of the polar body (Figure 2-16).

2.3.1. C-staining of female meiotic metaphases

The method described by Chandley and Fletcher to stain centromeres of meiotic chromosomes in humans (Chandley & Fletcher, 1973) was used with modifications.

Slides with the oocytes in the meiotic metaphase stage were aged for 5 days at room tempertaure. They were treated for 1 hour with 0.2M hydrochloric acid at room temperature. After rinsing in deionised water they were treated with 4% barium hydroxide for 35 seconds at 37°C. After rinsing in deionised water, they were incubated for 1 hour in 2 X SSC (Appendix M) at 60°C. They were then rinsed in deionised water and stained in 2% Giemsa (GURR, improved R66) in phosphate buffer at pH 6.8 for 1 hour (Appendix M). The slides were then rinsed in deionised water, dried and mounted with di-n-butyl phthalate.

2.3.1.1. Analysis of meiotic metaphase in oocytes

To determine meiotic disturbance, the number of oocytes in first and second metaphase (Figures 2-17 & 2-18) were recorded for each mouse. From the number of oocytes in first and second meiotic metaphase, the frequency of second meiotic metaphase [(IIM/IM+IIM)x100] was calculated. Well spread metaphases were photographed on a Leitz Wetzlar microscope at a magnification of 1250X (Objective 100X Eyepiece 12.5X). Photographs were analysed in detail for dicentric and acentric fragments and for aneuploidy.

Figure 2-17

Photomicrograph of first meiotic metaphase (centromere stained) obtained by in vitro culturing of mouse oocyte.

Figure 2-18

Photomicrograph of second meiotic metaphase (centromere stained) obtained by in vitro culturing of mouse oocyte.

2.4. Microphotography

Microphotographs were taken on 35 mm Kodak technical pan Film 2415, Estar_AH base (Appendix N). Films were developed for 5 minutes in HC 110 (Appendix N and Appendix M). 320 ml of the working solution of HC 110 was used to develop a film of 20 exposures. They were rinsed twice in water at 68°C and fixed in Kodak rapid fix (Appendix N and Appendix M) for 5 minutes. The fixed film was rinsed in running water at 68° C for 20 minutes. The film was then rinsed in water with photoflo (Appendix N and Appendix M).

2.4.1. Printing of photographs

Microphotographs were printed on Kodak Ektamatic SC paper (Appendix N), developed in a Kodak Ektamatic Processor (Appendix N) using Kodak S II Activator (Appendix N) as developer and Kodak Ektamatic S80 Stabilizer (Appendix N) as stabilizer.

2.5. Statistical analysis

The statistical analysis package (SPSSX) was used in the analysis of data. Differences were considered significant at the $P<0.05$ level. Proportions were transformed (arcsine transformation) before they were analysed by analysis of variance.

2.5.1. Litter size

When mates of different genotypes were used to determine the litter size, data was pooled when there was no difference between the groups. In both sexes, differences in litter size of homozygotes and heterozygotes were tested by oneway analysis of variance (litter as the unit).

2.5.2. Relationship between age and proportion of second meiotic metaphases

Linear relationship between age and proportion of second meiotic metaphase was determined in male and female mice. In male mice linear relationship between age and proportion of haploid secondary spermatocytes was determined.

2.5.3. Relationship between proportion of haploid and heteroploid secondary spermatocytes

In male mice, linear relationship between the proportion of haploid and that of heteroploid second meiotic metaphase was determined.

2.5.4. Meiosis in males

The mean proportion of haploid second meiotic metaphase and that of heteroploid second meiotic metaphase for the various groups were compared by one way analysis of variance after arcsine transformation of the proportions. Each group consisted of data from C57Bl/6J mice, that from mice homozygous and from those heterozygous for an inversion. For inversions 1RK, 12RK and 24RK, data from C3HFeJ mice and heterozygotes from C3H crosses were included. Data

from compound inversions 1RK/24RK and 12RK/24RK and that from the double inversion 22RK/24RK, were compared to data from homozygotes and heterozygotes from C57Bl crosses. For inversions 1RK, 12RK and 24RK data from heterozygotes from C3HFeJ crosses were also included in the analysis. (Heterozygotes for 17RK were not included in this analysis).

2.5.5. Meiosis in females

The proportion of oocytes that resumed meiosis *in vitro* and the mean proportion of second meiotic metaphase for the various groups was compared by one way analysis variance after arcsine transformation of the proportions. Each group consisted of data from C57BL, that from homozygotes and heterozygotes for an inversion. For inversions 1RK, 12RK and 24RK data from C3H and heterozygotes from C3H crosses were included. Data from compound inversions 1RK/12RK, 12RK/24RK and 1RK/24RK were compared with data from heterozygotes from C57Bl and C3HFeJ crosses for the single inversions.

Chapter 3

Male fertility

Fertility may be impaired by two mechanisms in male carriers of chromosomal rearrangements: segregational impairment of fertility and chromosomally derived sterility (or male dependent infertility).

3.1. Segregational impairment of fertility

Prenatal elimination of zygotes due to chromosomal imbalance results in secondary segregational impairment of fertility (Gropp, Winking & Redi, 1982). Origin of imbalance in these cases may be traced to meiotic segregation in male or female carriers of a balanced rearrangement. The prerequisite for secondary segregational impairment of fertility in males heterozygous for a paracentric inversion is fertilization of an oocyte by a sperm carrying a recombinant. Ford et al. (Ford, Evans & Burtenshaw, 1976) have reported increased post-implantation deaths (21.5%) in zygotes of males heterozygous for In(5)9RK (an inversion involving approximately 90% of chromosome 5) compared to normal controls (5.1% - 5.6%) in 2 sets of experiments, suggesting segregational impairment of fertility.

3.2. Male dependent infertility

In mice and humans, the effects of heterozygosity for some chromosomal rearrangements on gametogenesis has been observed to be different in males and females. Sterility due to sex chromosome and autosomal aberrations that result in impairment of male germ cell maturation (with no apparent effect on female fertility) is known as 'chromosomally derived' sterility (Searle, 1974) or male dependent infertility (Searle, Beechey & Evans, 1978). Rearrangements of this kind were first described by Lyon & Meredith (Lyon & Meredith, 1966). Since then there have been numerous reports of male dependent infertility in humans (Rivera, Alvarez-Arratia, Moller, Díaz & Cantú, 1984, Blattner, Kistenmacher, Tsai, Punnett & Giblett, 1980, Cantú, Díaz, Möller, Jiménez-Sáinz, Sandoval, Vaca & Rivera, 1985, Yamada, Nanko, Hattori & Isurugi, 1982, Chandley, Seuánez & Fletcher, 1976, Tóth, Gaál, Sára & László, 1982) and mice (Searle, 1982, Forejt, 1982, de Boer & Searle, 1980, Searle, Beechey & Evans, 1978).

Fertility is not impaired in homozygotes for these rearrangements (Forejt, 1982). This suggests that heterozygosity for the chromosomal rearrangement and the associated structural non-homology is responsible for the infertility. This excludes possible effect at the gene level due to the rearrangement e.g., position effect. Male sterility has been reported in mice heterozygous for translocations involving sex chromosomes, reciprocal translocations involving autosomes and one insertion involving autosomes (Searle, 1981). Male sterility has been reported in mice heterozygous for a double overlapping inversion on chromosome 1 (Chandley, 1982a).

'Variegated' phenotype is observed in male dependent infertility (Forejt, 1982). That is, breakdown of spermatogenesis is not observed in all the cells, suggesting that the etiology for breakdown is at the cellular level and not at the level of the organ. 'Variegated' phenotype can result in variable expressivity. Variation in the sperm count may range from oligospermia to azoospermia. Fertility in male mice heterozygous for most paracentric inversions is not significantly impaired. This characteristic was used by Roderick in distinguishing paracentric inversions from translocations (Roderick, 1079). Heterozygosity for rearrangements like paracentric inversions may have the potential to express male dependent infertility which may be be minimal and therefore not easily demonstrated.

3.3. Rationale for the investigations on male mice heterozygous for paracentric inversions.

Meiosis in male mice heterozygous for paracentric inversions of varying lengths and location relative to the centromere and/or telomere were studied to determine if heterozygosity for paracentric inversions can result in impairment of fertility due to secondary segregation and/or male dependent infertility. Impairment of fertility due to secondary segregation was determined by estimating litter sizes of male mice heterozygous for 1RK, 12RK and 24RK. Proportions of spermatocytes in first and second meiotic metaphases were estimated to determine the effect of heterozygosity for paracentric inversion on the normal meiotic process. Male mice heterozygous for paracentric inversions were investigated for meiotic arrest as an indicator of male dependent infertility. Homozygotes and heterozygotes for

inversions 1RK, 12RK, 24RK, 11RK, 14RK, 20RK and 22RK were studied. In the case of 17RK only heterozygotes were investigated. In addition, compound heterozygotes 1RK/24RK and 12RK/24RK and the double heterozygote 22RK/24RK were studied.

During meiosis, homologous pairing of the inverted segment in inversion heterozygotes is achieved by the formation of a loop during zygotene (prophase I). Observations on spermatocytes at meiotic metaphase prompted investigations of spermatocytes in the pachytene (prophase I) stage. Cells in late zygotene and early pachytene from the above groups of males were investigated for loop formation and its effects on spermatogenesis.

Refer to sections 2.2, 2.2.1 and 2.2.1.1 for materials and methodology for metaphase analysis, section 2.2.2 for materials and methodology for light microscopic analysis of spermatocytes in pachytene and section 2.2.3 for materials and methodology for electron microscopic analysis of spermatocytes in pachytene.

3.4. Results

3.4.1. Litter size

There is no significant difference between the mean litter sizes in offspring of male mice homozygous for and those heterozygous (from a inversion homozygote male and C3HFeJ female cross) for inversions 1RK, 12RK and 24RK [Table 3-1]. [Appendix J].

3.4.2. Correlation between age and haploid secondary spermatocytes

Regression coefficient for the 2 variables, age of mice (in days) and proportion of haploid secondary spermatocytes is -0.056 (± 0.179).

3.4.3. Meiosis

The control value for the proportion of haploid secondary spermatocytes is between 38.3% (C3HFeJ) and 41.3% (C57Bl) [Table 3-2]. For inversion homozygotes the proportion of haploid secondary spermatocytes was not significantly different from that of controls with the exception of 22RK (31%). Compared to homozygotes, the proportion of haploid secondary spermatocytes is decreased in mice heterozygous for all inversions except 11RK and 14RK. The decrease is significant in heterozygotes for the two large inversions, 22RK and 24RK. In the compound heterozygote 12RK/24RK with two different inversions on homologous chromosomes, the frequency of haploid secondary spermatocytes was significantly higher than that in 24RK/C57 and 24RK/C3H. In the double heterozygote 22RK/24RK (inversions on non-homologous chromosomes), the

Table 3-1: Litter size of 1RK, 12RK and 24RK homozygotes and heterozygotes

Genotype of males	No. of litters	No. of males	Mean litter size
1RK/1RK	24	8	5.5 ± 0.67
1RK/C3H	15	5	7.0 ± 0.46
12RK/12RK	11	6	8.0 ± 0.74
12RK/C3H	16	4	6.6 ± 0.42
24RK/24RK	28	11	5.2 ± 0.35
24RK/C3H	13	4	5.0 ± 0.62

The values are the mean litter size (± S.E.M.). Heterozygotes were compared with homozygotes by analysis of variance. Differences were considered significant (Tukey's w-procedure) at the $P<0.05$ level.

Table 3-2: Proportion of haploid (NIIM) and heteroploid (HETIIM) secondary spermatocytes

GENOTYPE (no. of animals)	NIIM (%)	HETIIM (%)	NIIM+HETIIM (%)
C57BL/6J (4)	41.3 ± 2.6	17.0 ± 3.8	46.0 ± 2.1
C3HFeJ (6)	38.3 ± 4.3	25.2 ± 3.3	45.1 ± 3.8
DBA (4)	42.5 ± 4.6	25.8 ± 1.9	47.1 ± 3.8
1RK/1RK (4)	49.6 ± 3.8	22.9 ± 3.4	56.1 ± 3.5
1RK/C57 (10)	33.7 ± 3.1	40.1 ± 2.9[a]	45.6 ± 2.7
1RK/C3H (3)	36.7 ± 4.4	32.5 ± 2.7	46.0 ± 3.6
12RK/12RK (4)	43.3 ± 4.4	15.7 ± 2.3	47.4 ± 3.9
12RK/C57 (6)	38.4 ± 4.7	31.8 ± 4.5[a]	47.4 ± 3.4
12RK/C3H (8)	37.5 ± 3.0	24.5 ± 1.6	44.1 ± 3.3
24RK/24RK (7)	43.7 ± 2.9	21.6 ± 1.7	49.7 ± 3.4
24RK/C57 (7)	18.7 ± 1.9[a]	62.5 ± 5.2[a]	39.2 ± 2.7[a]
24RK/C3H (4)	16.0 ± 3.1[a]	64.5 ± 3.4[a]	34.3 ± 3.6[a]
11RK/11RK (4)	47.0 ± 3.6	16.8 ± 3.0	51.6 ± 2.8
11RK/C57 (4)	50.3 ± 3.1	24.5 ± 3.1	57.3 ± 2.8
14RK/14RK (4)	42.4 ± 2.3	21.4 ± 2.4	48.4 ± 1.8
14RK/C57 (6)	45.5 ± 2.9	39.8 ± 3.8[a]	58.2 ± 3.0[a]
17RK/+ (6)	17.0 ± 2.8	60.1 ± 5.0	33.6 ± 2.1
20RK/20RK (6)	37.5 ± 2.5	21.7 ± 4.3	43.5 ± 2.7
20RK/C57 (4)	34.7 ± 3.9	36.8 ± 2.3[a]	45.4 ± 3.7
22RK/22RK (6)	31.0 ± 2.7[d]	27.3 ± 3.0	38.1 ± 2.7
22RK/C57 (6)	16.2 ± 1.8[a]	67.2 ± 3.6[a]	37.5 ± 2.7[a]
1RK/24RK (7)	32.3 ± 2.6	35.8 ± 2.7[b]	42.4 ± 2.5
12RK/24RK (6)	32.5 ± 2.8[b]	33.3 ± 3.0[b]	41.8 ± 3.0[b]
22RK/24RK (5)	6.3 ± 2.1[c]	88.0 ± 4.2[c]	36.8 ± 2.6[c]

NIIM=NIIM/IM+NIIM HETIIM=HetIIM/NIIM+HetIIM
NIIM+HETIIM= NIIM+HetIIM/IM+NIIM+HETIIM

The values are the mean proportions (± S.E.M.). Heterozygotes were compared with the respective homozygotes and normal controls by analysis of variance. Differences were considered significant (Tukey's w-procedure) at $P<0.05$.

[a] Significantly different from homozygotes at $P<0.05$
[b] Significantly different from 24RK/C57 and 24RK/C3H at $P<0.05$
[c] Significantly different from 24RK/C57, 24RK/C3H and 22RK/C57 at $P<0.05$
[d] Significantly different from C57BL/6J

frequency of haploid secondary spermatocytes was significantly lower than for 24RK/C57, 24RK/C3H and 22RK/C57, and in fact, the lowest value of any cross. [Table3-2] (Appendix II).

It was also observed that the proportion of heteroploid secondary spermatocytes is significantly higher in heterozygotes for all inversions from C57BL/6J crosses except 11RK, and in 24RK/C3H than in the respective homozygote [Table 3-2]. The proportion of heteroploid secondary spermatocytes is negatively correlated with that of haploid secondary spermatocytes (Figure 3-1), with a regression coefficient of -0.916 (± 0.499).

No difference is observed between 1RK/C57Bl and 1RK/C3HFeJ crosses in the proportion of haploid or heteroploid secondary spermatocytes. This was also the case for the heterozygotes for each of the single inversions 12RK and 24RK [Table3-2].

In heterozygotes for inversions 1RK, 11RK and 14RK, in which the inverted segment is small, the proportion of cells in late zygotene/early pachytene with an inversion loop is high (70%-84%). In heterozygotes for inversions 17RK, 20RK 22RK and 24RK in which a large segment of the chromosome is inverted, the loop was observed in few (0%-21%) cells in late zygotene (early pachytene). In heterozygotes for 12RK, in which a medium sized segment of the chromosome is inverted, loops were present in 50% of cells in late zygotene/early pachytene [Table 3-3].

Figure 3-1

Linear relationship between the proportion of haploid secondary spermatocytes [(NIIM/IM+NIIM)100] and that of heteroploid secondary spermatocytes [Het IIM/NIIM+Het IIM)100] with a regression co-efficient of -0.016 ± 0.499.

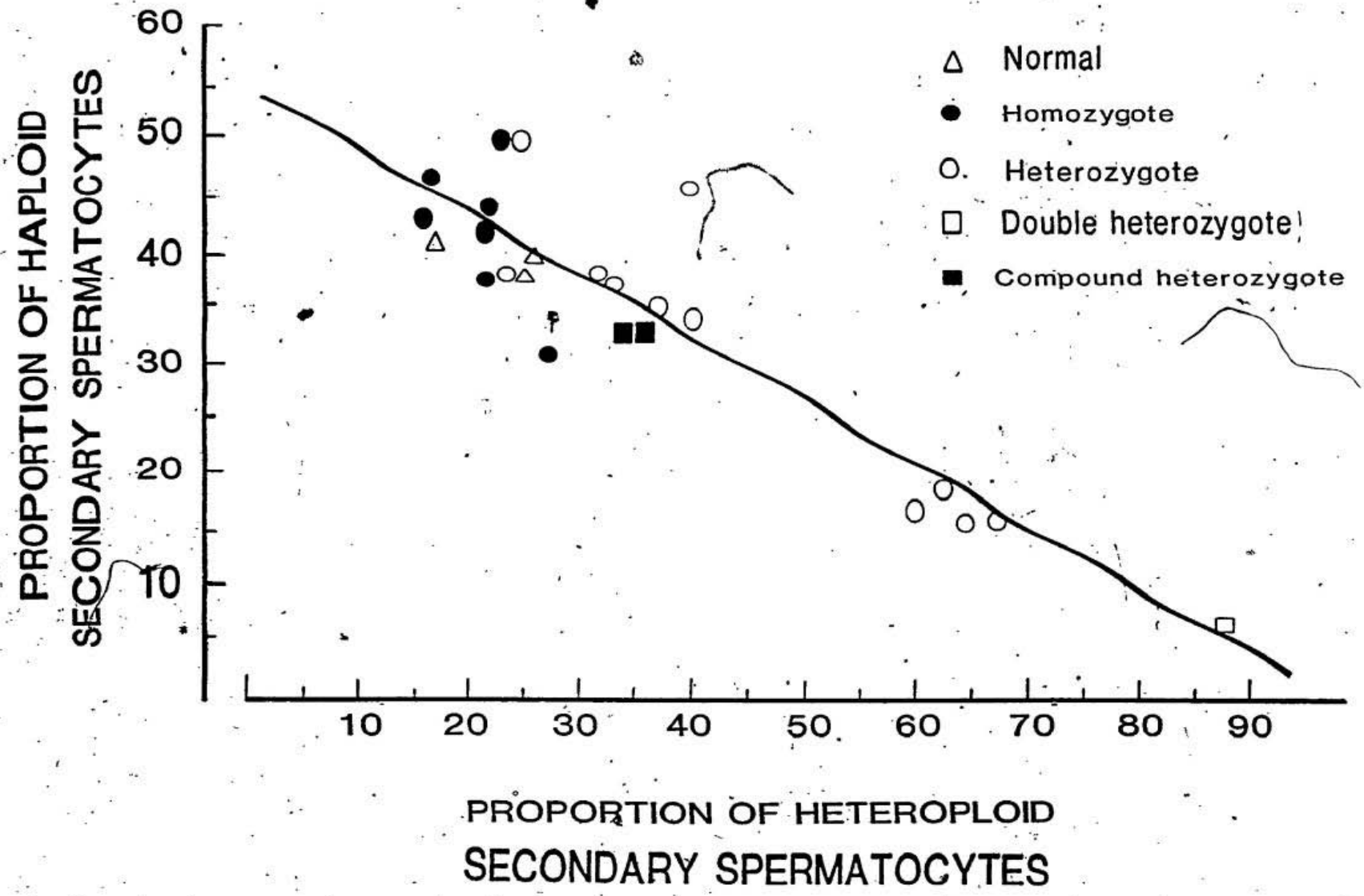

PROPORTION OF HAPLOID SECONDARY SPERMATOCYTES
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
PROPORTION OF HETEROPLOID SECONDARY SPERMATOCYTES
Normal
Homozygote
Heterozygote
Double heterozygote
Compound heterozygote

Table 3-3: Proportion of cells in pachytene with the loop in males heterozygous for each of the various inversions and in compound and double heterozygotes

Genotype	Frequency of cells with loop
1RK/C57	70% (35/50)
14RK/C57	84% (42/50)
11RK/C57	82.46% (47/57)
12RK/C57	50% (50/100)
17RK/+	21% (12/56)
20RK/C57	0% (0/30)
22RK/C57	2% (2/100)
24RK/C57	8% (8/100)
1RK/12RK	89% (89/100)
12RK/24RK	63% (63/100)
22RK/24RK	7% (7/100)

3.5. Discussion

3.5.1. Litter size of some male inversion heterozygotes

The mean litter size of male mice heterozygous for the two inversions, 1RK, 12RK and 24RK, on chromosome 1, indicates that heterozygosity for these inversions does not affect fertility [Table 3-1]. Meiotic studies indicate that heterozygosity for the large inversion 24RK shows significant meiotic disturbance [Table 3-2]. In the absence of reduced litter size, it appears that the extent of meiotic disturbance observed in inversion 24RK heterozygotes is not significant enough to impair fertility, either because it does not lead to abnormal gametes, or it does not reduce the sperm count below the threshold level associated with reduced fertility. If so, the minor disturbances associated with other inversions would not be expected to have any apparent effect on fertility. The meiotic disturbance however, may be accompanied by selection against production of gametes with recombinants. There is no reduction in the litter size despite the heterozygosity of the female mice [Appendix J].

Normal fertility in heterozygotes for 24RK associated with meiotic disturbance emphasizes the difficulty of ascertaining heterozygosity for paracentric inversions at least in males by their phenotype.

The observation in this study is in contrast to the significantly reduced fertility reported in male mice heterozygous for 9RK (Ford, Evans & Burtenshaw, 1976), compared to normal control, evaluated on the basis of post-implantation death

(resorption). Estimating fertility by post-implantation deaths and use of normals as controls, rather than homozygotes, may account for the observed difference. The percent death during the post-implantation period in the study by Ford et al. is 21.5% for male heterozygotes which appears to be significantly less than the 43.8% observed in female heterozygotes.

3.5.2. Meiotic disturbance - nature, etiology and role in selection

The nature of the observed meiotic disturbance, its etiology and role in selection will be discussed in the light of the various observations.

3.5.2.1. Nature of observed meiotic disturbance

If haploid secondary spermatocytes are considered (as a requirement for normal spermatogenesis and reproduction), it is readily apparent that their frequency is consistently, and in some cases significantly lower in heterozygotes compared to homozygotes for all the inversions. [Table 3-2]. If the total frequency of normal and heteroploid second meiotic metaphase is considered, most differences do not persist (all except 24RK), whereas others appear (14RK) [Table 3-2]. This observation suggests that the disturbance is not an arrest but a delay at the metaphase I stage.

3.5.2.2. Age of animal and frequency of haploid secondary spermatocytes

Frequencies of haploid secondary spermatocytes do not appear to correlate with the age of the animal (Refer section 3.4.2), suggesting that the age of the animal does not contribute significantly to the observed differences.

3.5.2.3. Meiotic disturbance and genetic background

The effect of some Robertsonian translocations on male fertility is reported to be altered by the genetic background (Winking, 1980, Gropp & Winking, 1981). Similar observations have been made in heterozygotes for the translocation T6Ca [T(14;15)6Ca translocation] (Forejt, 1976). In the present study the genetic background does not appear to make a difference in the case of the one inversion (24RK). The proportion of haploid and heteroploid secondary spermatocytes in heterozygotes for this inversion, obtained from 2 different crosses (C57Bl and C3HFeJ) were not different. Some of the reported differences in translocation heterozygotes may be due to different criteria employed in defining and identifying fertility. If litter size rather than sperm count is used as a criterion differences may be expected, since litter size is not a true index of sperm count. Gropp and Winking used data from breeding in some cases and in others elaborate fertility tests were used (Gropp & Winking, 1981). In the former, only litter sizes may have been used for evaluating fertility. Alternatively the absence of any difference between the two heterozygotes in this study may be because sufficient backcrosses were not carried out to ensure differences in the background. Nevertheless, it seems clear that the differences observed in the kinetics of meiosis

are due to heterozygosity for the inversion and not due to genes contributed by the C57Bl or C3HFeJ mice.

It may be speculated that the influence of genetic background on the effects of chromosomal rearrangement is specific for the type of rearrangement. In heterozygotes for translocations more than one pair of chromosomes is involved and atypical pairing and segregation at the chromosomal level may be affected by the genetic background. In rearrangements such as paracentric inversions, which involve only one pair of chromosomes, recombination prior to chromosomal segregation and the structurally abnormal products of recombination may affect chromosomal segregation. Chromosomal segregation may not be influenced by the genetic background. Use of littermates as controls in the F_2 or subsequent generations are essential to support this suggestion.

3.5.2.4. Correlation between proportions of haploid and heteroploid secondary spermatocytes

The negative correlation between the proportion of haploid secondary spermatocytes and that of heteroploid secondary spermatocytes (Refer section 3.4.3) suggests that the decreased proportion of haploid secondary spermatocytes and increased proportion of heteroploid secondary spermatocytes probably have a common etiology. A significant increase in the proportion of heteroploidy even in those heterozygotes in which the decrease in proportion of haploid secondary spermatocytes is not significant [1RK/C57, 12RK/C57, 14RK/C57, and 20RK/C57] [Table3-2], suggests that the mechanism responsible for minimal

changes in the proportion of haploid spermatocytes can result in significant changes in the proportion of heteroploid spermatocytes. An alternative interpretation is that spermatogenesis is arrested at the second meiotic metaphase stage in those cells which are heteroploid. A negative correlation between sperm count and proportion of heteroploid secondary spermatocytes would suggest the existence of such an arrest.

3.5.2.5. Relationship between physical characteristics of the inversion and observed meiotic disturbance

In an attempt to formulate a hypothesis for the etiology for the meiotic disturbance, information on meiotic disturbance from heterozygotes of the various inversions was analysed, primarily on the basis of the physical characteristics of the inverted segment. The location of one breakpoint in the heterochromatic region proximal to the centromere is a feature observed in a number of autosomal translocations that result in male specific sterility (Forejt, 1982). The relationship between this physical characteristic and maturation arrest is thought to be due to: translocation of small segments (resulting in chain configurations during first meiosis), rare chiasma formation in the translocated segments and non-disjunction (Searle, 1982, Cacheiro, Russell & Swartout, 1974). Position effect has also been suggested as a possible cause for sterility (Ford, Searle, Evans & Jean West, 1969).

The proximal breakpoint in a number of the paracentric inversions investigated in this study is close to the centromere. These inversions are 1RK, 24RK, 14RK, 20RK and 22RK (Figure 2-1). Of these inversions only heterozygosity for 22RK

and 24RK result in meiotic disturbance, suggesting that a breakpoint close to the centromere alone does not significantly affect meiosis in the paracentric inversion heterozygotes investigated.

The location of a breakpoint close to the telomere, also does not appear to be of importance. Although 12RK has a breakpoint close to the telomere, it is not associated with meiotic disturbance, in contrast to 22RK and 24RK where one breakpoint is close to the telomere and there is an effect.

The observed meiotic disturbance appears to be consistent in its association with the physical length of the inversion relative to the length of the respective chromosome. Meiotic disturbance is observed in the case of heterozygotes for inversions which extend practically the entire chromosome length with the exception of heterozygotes for 20RK (Refer section 3.5.2.8).

3.5.2.5.1. Possible consequences of meiosis in heterozygotes for paracentric inversions involving a large segment

An increased proportion of diploid secondary spermatocytes has been reported in males heterozygous for the inversion 5RK and those for 9RK. Dicentric chromatids that are intact, stretched or broken have been observed in these diploid restitution nuclei (Evans & Ford, 1976). There are at least three possible consequences for meiosis in heterozygotes of paracentric inversions involving a large segment. They may be those related to (1) probability of crossover, (2) length of the dicentric bridge formed, and (3) size of the loop and synaptic adjustment.

3.5.2.5.1.1. Size of inverted segment and probability of crossover in the inverted segment

Dicentric anaphase bridges in first anaphase resulting from uneven number of crossovers in the inverted segment could interfere with the normal meiotic process, and might be responsible for delay at first meiotic metaphase. The apparent positive correlation between the anaphase bridge frequency reported by Roderick (Roderick, 1983) and length of the inverted segment [Table 3-4] suggests a positive relationship between length of the inverted segment and probability of crossover. (20RK is somewhat of an exception (Refer section 3.5.2.8).) Thus, since crossover and anaphase bridge formation occur more frequently in heterozygotes for large inversions, meiotic disturbance may be expected to be more prevalent in heterozygotes for large than small inversions.

Those inversions whose heterozygotes are reported to have a high frequency of anaphase bridges, 17RK, 22RK and 24RK, are found to have significant meiotic disturbance. This suggests a causal relationship between size of the inverted segment, probability of crossover in the inverted segment, frequency of anaphase bridges and meiotic disturbance. Heterozygotes for 20RK are an exception (Refer section 3.5.2.8). The relationship between the (high) frequency of anaphase bridges and extent of meiotic disturbance is maintained in heterozygotes for 20RK. Despite involvement of a large segment, unlike heterozygotes for other large inversions, the frequency of anaphase bridges is small. Nevertheless, the low frequency of anaphase bridges is associated with a normal proportion of haploid secondary spermatocytes emphasizing the importance of the role of anaphase bridges in meiotic disturbance.

Table 3-4: Frequency of anaphase bridges in inversion heterozygotes (Roderick, 1983)

Genotype	Frequency of anaphase bridges (%)
1RK/+	34
11RK/+	29
14RK/+	35
12RK/+	22
17RK/+	65
20RK/+	47
22RK/+	66
24RK/+	73

3.5.2.5.1.2. Length of dicentric bridge and meiotic disturbance

The length of the dicentric bridge is the sum of the length of the inverted segment and twice the length of the interstitial segment. The high frequency of dicentric bridges observed in heterozygotes for paracentric inversions involving a large segment, may be due to the relative inability of the bridge to break compared to that of the short bridges.

In this regard the observations in heterozygotes of 12RK, 22RK and 24RK are interesting. Inversions 22RK and 24RK occupy practically the entire length of the chromosomes involved, show high frequency of anaphase bridges and similar meiotic disturbances. If the absolute length of the inverted segment is considered, 12RK and 22RK may be of the same range. This would imply that the probability of crossover ought to be similar. Therefore, if meiotic disturbance in 22RK is the consequence of crossover in the inverted segment, similar observations ought to be made in the case of 12RK. Nevertheless, significant meiotic disturbance is not observed in heterozygotes for 12RK.

Size of the bridge formed may be considered as the cause of the meiotic arrest. The distal break in 12RK is located at the telomeric region of the chromosome 1. Therefore, a crossover in the inverted segment would result in a dicentric bridge which would be the sum of the length of the inverted segment and twice the length of the interstitial segment. This bridge ought to be longer than the bridge formed from a crossover in the inverted segment in a 24RK heterozygote.

However, the frequency of bridges in heterozygotes of 12RK, 24RK and 22RK are 22%, 73% and 66% respectively. These observations suggest that the frequency of bridges and the resulting meiotic disturbance is related to the physical length of the inverted segment relative to the length of the chromosome on which the inversion is located and not genetic length or physical length of the inverted segment relative to the sum of the length of all the chromosomes in the nucleus.

3.5.2.5.1.3. Size of inverted segment and synaptic adjustment

Synaptic adjustment is a phenomenon by which the loop formed during pachytene in heterozygotes of inversions is resolved. It is proposed that synapsis in inversion heterozygotes may consist of two phases. During the initial phase synapsis and synaptonemal complex formation is limited by synapsis between homologous regions, giving rise to the loop (homosynaptic phase). This is followed by a phase when desynapsis and resynapsis result in elimination of the loop. This is the heterosynaptic phase, when the inverted segment is non-homologously paired. With progression of the pachytene stage, synaptic adjustment is observed with decrease in the size of the loop (Moses, Poorman, Roderick & Davisson, 1982). Large loops may take longer to resolve, disrupting the synchrony between chromosomal disjunction and nuclear division. The products of meiosis in such a cell may be heteroploid.

3.5.2.6. Loop formation and its effects on meiosis

With differences in time required for synaptic adjustment in mind, the effects of loop formation during pachytene on meiosis and reproduction were analysed. (Figure 3-2). Synaptic adjustment is a potential source of error when determining the frequency of spermatocytes with the loop. To avoid this, spermatocytes in late zygotene/early pachytene were analysed, since synaptic adjustment occurs as the cells proceed through pachytene and the loop is resolved by the end of pachytene (Moses, Poorman, Roderick & Davisson, 1982). Large loops may be expected to take a longer time to resolve than the small ones. Therefore one would expect loops to be readily identifiable in preparations of heterozygotes for inversions involving large segments. Contrary to expectation, however, fewer spermatocytes with the loop were observed in heterozygotes for 22RK and 24RK than in heterozygotes for short inversions (Refer section 3.4.3) [Table 3-3].

In the absence of the loop in most spermatocytes and with increased frequency of bridges in heterozygotes of large inversions (suggesting crossover in the inverted segment), it may be assumed that homologous pairing of the inverted segments in these heterozygotes is accompanied by non-homologous pairing or asynapsis of the flanking segment and centromeres, rather than loop formation (Figure 3-3).

Electron microscopic studies on a heterozygote for 24RK (Figure 3-4) did not show asynapsis of the inverted segment or thickened axis indicating asynapsis (Moses, Poorman, Roderick & Davisson, 1982). This observation supports the

Above: Figure 3-2a

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 1RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment.

Below: Figure 3-2b

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 12RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment. Asynapsis of the region distal to the inversion is observed.

IN(1)1RK

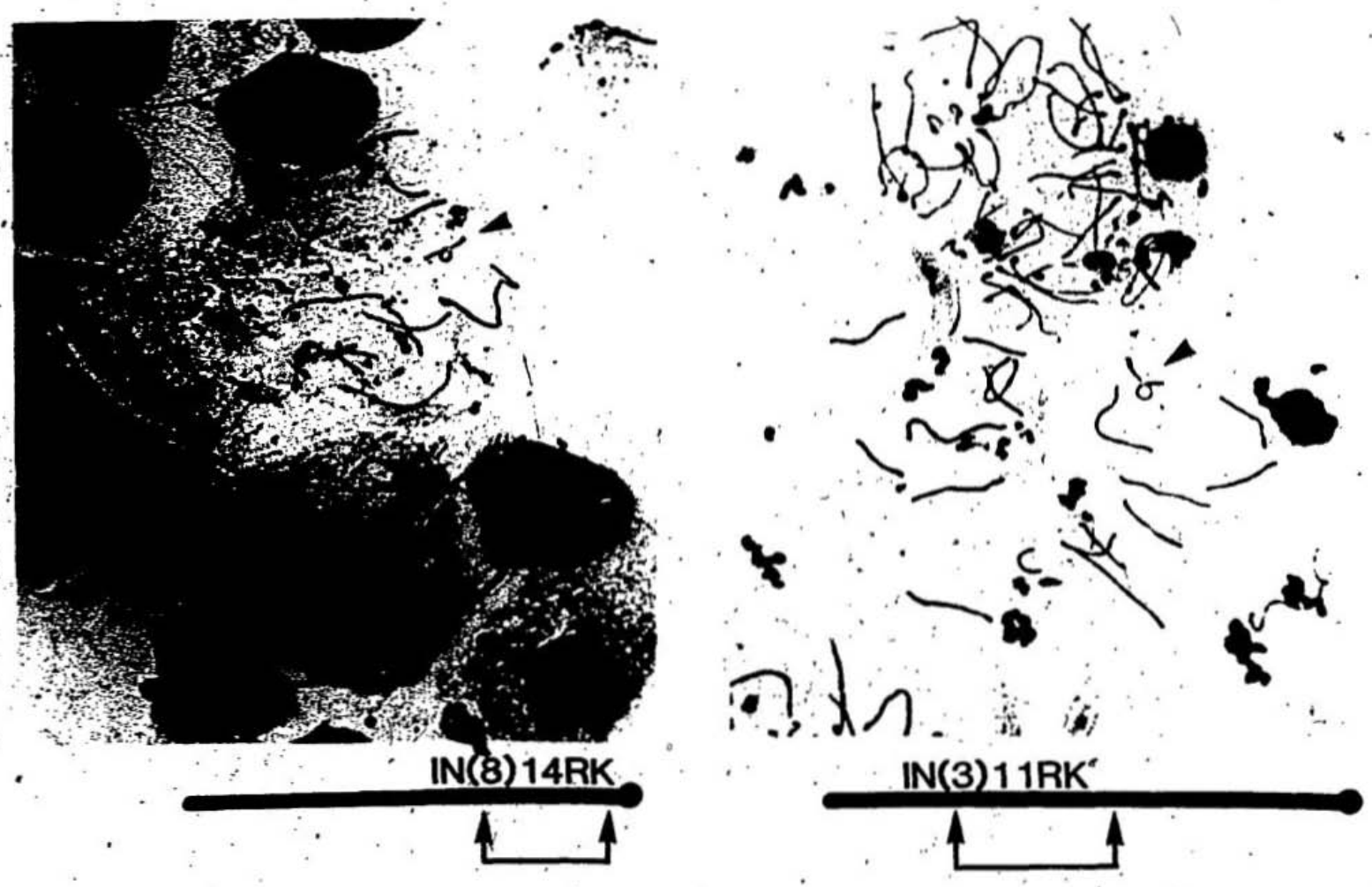
IN(1)12RK

Above: Figure 3-2c

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 11RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment.

Below: Figure 3-2d

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 14RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment.


IN(8)14RK
IN(3)11RK

Above: Figure 3-2e

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 24RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment.

Below: Figure 3-2f

Photomicrograph of pachytene preparation from a male mouse heterozygous for inversion 22RK. A loop (arrowhead) is formed to enable synapsis of the inverted segment.


IN(14)22RK


IN(1)24RK

Figure 3-3

Diagrammatic representation of reverse pairing. Homologous pairing of the inverted segment is associated with asynapsis of the centromere and the flanking segments.

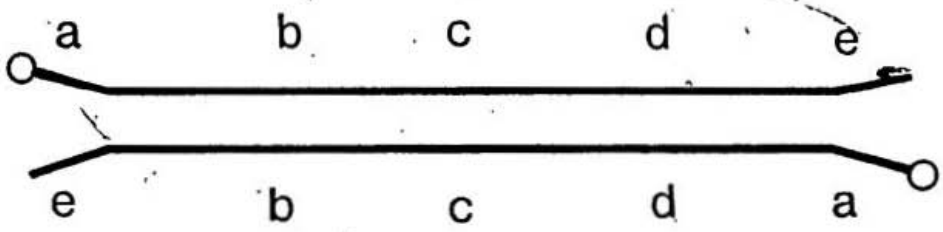

a
b
c
d
e
e
b
c
d
a

Figure 3-4

Electron micrograph of pachytene preparation from a male mouse heterozygous for inversion 24RK. Bar= 1μm

mode of pairing illustrated in Figure 3-3. Asynapsis of the flanking regions was not observed in the electron micrographs (Figure 3-4), perhaps because the flanking regions are very small. The centromeres were not identifiable in electron micrographs of specimens stained using conventional techniques. This mode of pairing has been demonstrated by C-staining pachytene oocytes from a female mouse heterozygous for a paracentric inversion on the X chromosome (Tease & Fisher, 1980). Reverse pairing has also been reported in mice heterozygous for inversion 9RK (an inversion involving approximately 90% of chromosome 5) by C-staining of spermatocytes in pachytene (Evans, 1979).

Absence of loop formation has been observed in heterozygotes for inversions in domestic fowl (Kaelbing & Fechheimer, 1985). In the sand rat (Ashley, Moses & Solari, 1981) absence of loop formation is associated with non-homologous pairing. Absence of loop formation and heterosynaptic pairing in a species of the deer mouse heterozygous for a terminally located pericentric inversion prevents recombinantant formation and gametic loss and maintanence of the inversion polymorphism (Greenbaum & Reed, 1984). Synapsis of the inverted segment and asynapsis of the flanking segments has been suggested in a human male heterozygous for a pericentric inversion, inv (7) (p22q32) (Winsor, Palmer, Ellis, Hunter & Ferguson-Smith, 1978).

3.5.2.6.1. Possible factors determining mode of pairing in paracentric inversion heterozygotes

Factors which determine loop formation in paracentric inversion heterozygotes may be postulated on the basis of the various mechanisms proposed for initiation

and progression of synapsis. One mechanism suggests initiation of synapsis by attachment of telomeres and/or centromeres to specalized sites on the nuclear membrane. Synapsis is then thought to proceed from these paired regions, accompanied by formation of the synaptonemal complex (Maguire, 1977). An alternative mechanism is based on observations in heterozygotes for rearranged chromosomes. It has been suggested that synapsis is these rearranged regions is mediated by the existence of numerous sites on the chromosome capable of attaching to the nuclear membrane and initiating synapsis (Maguire, 1977). In at least 1 paracentric inversion, 5RK, Moses et al. suggested the existence of three initiation sites for synapsis. Differences in the length of the synaptonemal complex at the different sites in different cells, suggests that there is no priority with regard to the site of initiation (Moses, Poorman, Roderick & Davisson, 1982).

The suggested non-homologous pairing of centromere and telomere in the case of 17RK, 22RK and 24RK heterozygotes suggests homologous synapsis can occur even when centromeres and telomeres are not synapsed. This observation supports the suggestions that homologous synapsis does not depend on homologous centromere associations (Burnham, Stout, Weinheimer, Kowles & Phillips, 1972) and that synapsis need not necessarily be initiated at the telomere or centromere (Maguire, 1977).

Physical factors to be considered in determining whether initiation of synapsis is at the centromere, telomere or in the inverted segment are (1) size of the inverted

segment, (2) location of the inverted segment in relation to the centromere, (3) location of the inverted segment in relation to the telomere and (4) size of the segments flanking the inverted segment. The three short inversions 1RK, 11RK and 14RK and the medium sized inversion 12RK show a high frequency of cells with the loop. Frequency of cells with the loop in heterozygotes for 12RK is lower than that in heterozygotes for 1RK, 11RK and 14RK. This suggests a negative relationship between length of the inverted segment and loop formation.

All these chromosomes have large segments of unaltered sequences. In 1RK and 14RK, the unaltered segment is predominantly telomeric. In 12RK, the unaltered sequence is proximal to the centromere and in 11RK unaltered segments of appreciable lengths flank the inverted segment. Thus the location of unaltered segments relative to the centromere or telomere do not appear to affect formation of the loop. The effect of location of the inverted segment relative to the centromere or telomere cannot be determined from these investigations, since 12RK, which is located close to the telomere also involves inversion of a larger segment of the chromosome than 1RK, 11RK and 14RK.

The four large inversions, 17RK, 20RK, 22RK and 24RK involving segments which include nearly the entire length of the chromosome, show a low frequency of cells with the loop. Based on this observation, the following speculation is made. In heterozygotes for paracentric inversions, synapsis is more likely to be initiated in the inverted segment if the inversion is large. If synapsis is initiated in the

unaltered segment, the loop is formed in the inverted segment, since asynapsis of the inverted region was not observed. But if synapsis is initiated in the inverted segment, the loop may or may not be formed. When the loop is not formed homologous pairing of the inverted segment and non-homologous pairing of the flanking segments take place.

3.5.2.7. Effect of loop formation on cross over frequency, in the inverted segment

As mentioned earlier, the physical lengths of the inverted segment in 12RK and 22RK are similar though 12RK is relatively shorter in terms of fraction of the length of the chromosome. The proportion of cells with the loop in heterozygotes for these inversions, however, is 50% and 2% respectively. And the frequency of anaphase bridges in heterozygotes for these inversions is 22% and 66% respectively. That is, loops are formed infrequently in heterozygotes for inversions involving a large segment of the chromosome compared to those for inversions involving short segments as predicted by the hypothesis. But anaphase bridges are formed more frequently in the former compared to latter. It is suggested that loop formation may interfere more with crossing over in the inverted segment than does pairing of the inversion with non-homologous pairing of the flanking segments.

Observations in compound heterozygotes also suggests a relationship between loop formation and crossover. Bridge frequencies in these heterozygotes are not available. The frequency of haploid secondary spermatocytes can be used as an

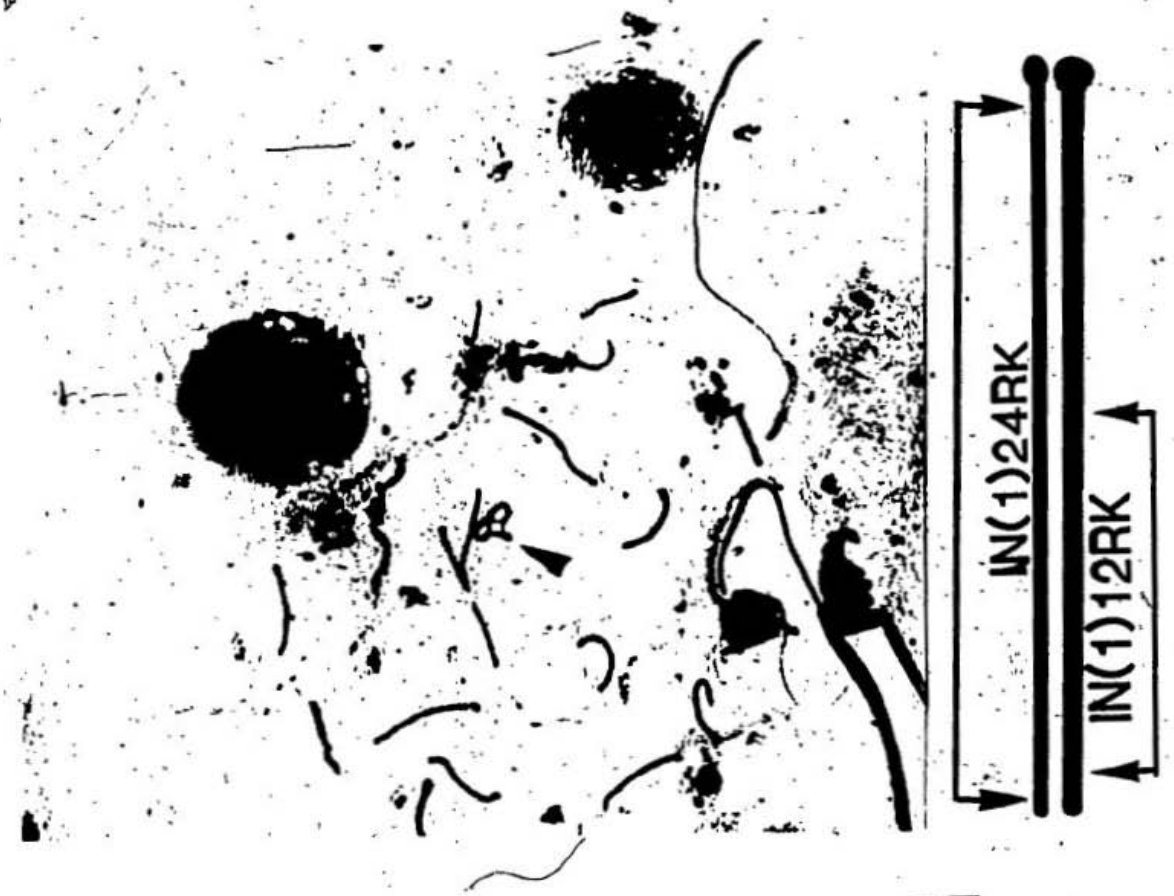
index of crossover frequency. In heterozygotes 12RK/24RK, the proportion of cells with the loop is 63% and that of haploid secondary spermatocytes is 32.5% i.e., somewhat increased proportion of haploid secondary spermatocytes and spermatocytes with loop are found in association [Table 3-2]. In compound heterozygotes, however, the loops formed are so complicated (Figure 3-5), that if the loop is formed it may not be resolved and the cells may breakdown at the pachytene stage thereby preventing the formation of heteroploid secondary spermatocytes. Meiotic arrest at pachytene may be determined by investigating cytogenetic or histological preparations.

3.5.2.8. Observations in heterozygotes for 20RK

Inversion 20RK extends practically the entire length of the respective chromosome as in inversions 22RK and 24RK. Unlike heterozygotes for 22RK and 24RK, male heterozygotes for 20RK, do not show significant meiotic arrest [Table 3-2]. Also, compared to heterozygotes for 22RK and 24RK which show significantly few cells with the loop, 2% and 8% respectively, heterozygotes for 20RK did not show loops in any of the cells investigated [Table 3-3]. If low frequency of loops (2% and 8%) and high frequency of anaphase bridges (66% and 73%) are correlated, absence of loops in 20RK ought to be be associated with a high frequency of anaphase bridge (even higher than that observed in heterozygotes for 22RK and 24RK). The low frequency of anaphase bridges, in heterozygotes for 20RK, suggests that crossover may be prevented to a certain extent due to some other factor in these heterozygotes. One possiblity is that the rearrangement is not a simple inversion. This could explain the absence of meiotic disturbance in heterozygotes for 20RK.

Figure 3-5

Photomicrograph of pachytene preparations from a male mouse heterozygous for inversions 1IRK and 12RK. A complex loop (arrowhead) is formed to enable synapsis of the inverted segments.


IN(1)24RK
IN(1)12RK

3.5.2.9. Observations in double heterozygotes

The meiotic process is arrested at the pachytene stage in the compound heterozygote 1RK/12RK. Similar observations were made by Chandley (Chandley, 1982a). Spermatocytes in first and second meiotic metaphase are observed in compound heterozygotes 1RK/24RK and 12RK/24RK. One difference between the 1RK/12RK and the other two compound heterozygotes is that, in 1RK/12RK the inverted segments overlap. In 1RK/24RK and 12RK/24RK, the smaller inversions, 1RK and 12RK are included within the region inverted in 24RK. The nature of the loops formed is therefore different in 1RK/12RK and 1RK/24RK and 12RK/24RK. This may be one reason for the observed differences in meiotic disturbance.

The extremely low frequency of haploid secondary spermatocytes and very high frequency of heteroploid secondary spermatocytes suggests that the etiology for meiotic disturbance contributed by heterozygotes for inversions 24RK and 22RK are additive, or at least act independently of one another.

3.5.2.10. Possible etiology for meiotic disturbance in male mice heterozygous for paracentric inversions

Based on the various observations the following is proposed. The observed meiotic disturbance is a function of dicentric anaphase bridge. Crossover required for the formation of the dicentric bridge may, however, be influenced by the absence of loop formation as in heterozygotes for 22RK and 24RK in addition to other unknown factors (as in heterozygotes for 20RK). A bridge may be formed

in heterozygotes for 12RK just as easily as in heterozygotes for 22RK since the size of the inverted segments are similar, except that loops are not formed in 22RK. Also a bridge formed in heterozygotes for 12RK would be as long as that formed in heterozygotes for 24RK, and may be expected to have similar effects. The minimal effects observed in heterozygotes for inversion 12RK suggest that the loop formed in these heterozygotes prevent crossover in the inverted segment. Therefore it may be suggested that absence of loop formation in heterozygotes for 22RK or 24RK is accompanied by crossover whereas loop formation interferes with crossover in heterozygotes for 12RK.

The decreased frequency of loop formation in heterozygotes for 22RK and 24RK unlike those for 12RK may be because of the non-availability of sufficient lengths of flanking segments for initiation of synapsis. Another aspect to be considered is the possibility of increased probability of crossover in the region close to the centromere rather than the telomere. If so crossover would be expected to be more frequent in heterozygotes for 22RK and 24RK compared to heterozygotes for 12RK.

As mentioned earlier, homologous pairing in the absence of loop formation has been suggested in heterozygotes for pericentric inversions (Refer section 3.5.2.6). It would be interesting to determine if this effect of loop formation on crossover frequency is observed in heterozygotes for pericentric inversion. One difference in this kind of pairing in heterozygotes for pericentric inversion and paracentric

inversion is that in the former centromeres may be paired whereas in the latter they are not.

3.5.2.11. Meiotic disturbance and male dependent infertility in heterozygotes for paracentric inversion

Meiotic disturbance may have a role in selection aganist recombinants by affecting gametogenesis. A hypothesis involving chromosomal and biochemical interaction of the rearranged chromosome and the X chromosome has been put forward by Forejt to explain male dependent infertility. It is postulated that rearranged chromosomes do not pair completely. The unpaired regions are thought to associate with the X chromosome. Under normal circumstances, the X chromosome is inactivated during meiosis (Lifschytz & Lindsley, 1972). Association of the unpaired region of the rearranged chromosome with the X chromosome is thought to interfere with the inactivation process. Consequently, X linked gene products not transcribed during meiosis are produced. X-linked enzymes glucose-6-phosphate dehydrogenase, hypoxanthine-guanine phosphoribosyltransferase and phosphoglycerate kinase-1 are reported to be more active than autosomal enzymes in cells in late prophase of sterile males heterozygous for X-autosome translocation T37H and for 1RK/12RK (Chandley, 1982b, Hotta & Chandley, 1982). Disruption of the normal pattern of pachytene DNA metabolism and abnormal pattern of endonuclease activity were observed in mouse strains that were sterile because of X-autosome or autosome-autosome translocations (Hotta, Chandley, Stern, Searle & Beechey, 1979). The activity of the X chromosome during meiosis is thought to lead to breakdown of spermatogenic differentiation (Forejt, 1982).

3.5.2.11.1. Incomplete synapsis and male dependent infertility

Incomplete synapsis of rearranged chromosomes (Forejt, 1981, de Boer & Searle, 1980) and association of the rearranged chromosome with the XY bivalent have been observed in pachytene spermatocytes in all male-sterile translocations studied in mice (Forejt, 1982). Association of rearranged chromosome and XY bivalent have also been observed in mice heterozygous for a Robertsonian translocation with a pericentric inversion (Davisson, Poorman, Roderick & Moses, 1981) and in mice heterozygous for two paracentric inversions (Chandley, 1982a). Heterozygosity for a paracentric inversion may affect synapsis and therefore may have a similar effect on male gametogenesis.

3.5.2.11.2. Male dependent infertility in paracentric inversion heterozygotes

Male-dependent infertility has a 'variegated' phenotype. That is, spermatogenesis is not arrested in all the germ cells (Forejt, 1982). The variegated phenotype may be identified as variable expression of impaired fertility. Absence of completely defective spermatogenesis in animals heterozygous for the various inversions may be an expression of the 'variegated' phenotype. As the extent of meiotic disturbance is consistent in heterozygotes of any particular inversion and appears to correlate with the physical characteristics of the inversion, it may be argued that the physical characteristics correlate with the extent to which 'variegated' phenotype is expressed in heterozygotes for a paracentric inversion.

3.5.2.11.3. Association of rearranged chromosomes with the XY bivalent in male dependent infertility

Regions of the rearranged chromosomes that are not synapsed are thought to play a role in the breakdown of spermatogenic differentiation by their association with the unpaired regions of the X chromosome. As mentioned in section 3.5.2.11.1, such an association is reported to be observed in mice heterozygous for two paracentric inversions (Chandley, 1982a). There is a difference in the meiotic disturbance observed in mice heterozygous for single paracentric inversions investigated in this thesis and that described by Chandley. The arrest reported by Chandley in compound heterozygotes is at pachytene, whereas in mice heterozygous for single inversions, the disturbance is a meiotic delay at metaphase I.

Interestingly, meiotic arrest similar to the one observed in the compound heterozygote 1RK/12RK is not observed in the compound heterozygotes 1RK/24RK and 12RK/24RK. The loop formed in 1RK/12RK is different from those formed in 1RK/24RK and 12RK/24RK because of the differences in the physical characteristics mentioned earlier (Refer section 3.5.2.9). This suggests that the arrest in the compound heterozygote 1RK/12RK may to some extent be a consequence of the complexity of the loop. Association of the rearranged chromosome in 1RK/24RK and 12RK/24RK with the XY bivalent, during pachytene, could be studied to determine if the disturbance in meiosis in these heterozygotes is associated with association of the rearranged chromosome with the XY bivalent.

3.5.2.11.4. Evidence against male dependent infertility in heterozygotes for paracentric inversion

Of the inversions investigated, asynapsis of the telomeric region of the chromosome is observed in heterozygotes for inversion 12RK (Figures 3-2 & 3-6). There is however, no significant meiotic disturbance in 12RK heterozygotes, compared to the corresponding homozygotes. This observation suggests that at least in the paracentric inversions studied, (all acrocentric chromosomes) meiotic disturbance does not result from the mechanism postulated by Forejt to explain male dependent infertility (Forejt, 1982). Electron microscopy of the loop formed in heterozygotes for 1RK suggests possible asynapsis at the region where the loop formation is initiated (Figure 3-7). As in the case of heterozygotes for 12RK, significant meiotic disturbance is not observed in heterozygotes for 1RK.

The documented relationship between the physical characteristics of the inversion and subsequent formation of loop and dicentric anaphase bridges suggests that the meiotic disturbance observed in these instances may not be a variable expression of male dependent infertility. The correlation between the physical characteristics of the inversion and extent of meiotic disturbance suggests the existence of variable expression, a characteristic of male dependent infertility (Refer section 3.5.2.11.2). To support this hypothesis, spermatocytes in pachytene may be studied for association between the rearranged chromosome and the XY bivalent, since the rearranged chromosome is reported to be associated with the XY bivalent during pachytene in male dependent infertility (Forejt, 1984). One difficulty in such a study is the identification of the rearranged chromosome

Figure 3-6

Loop formed by synapsis of the inverted segment in a male mouse heterozygous for the inversion 12RK. The asynapsed telomeric region (arrow) is thickened. Electron micrograph: Bar= 0.5μm.

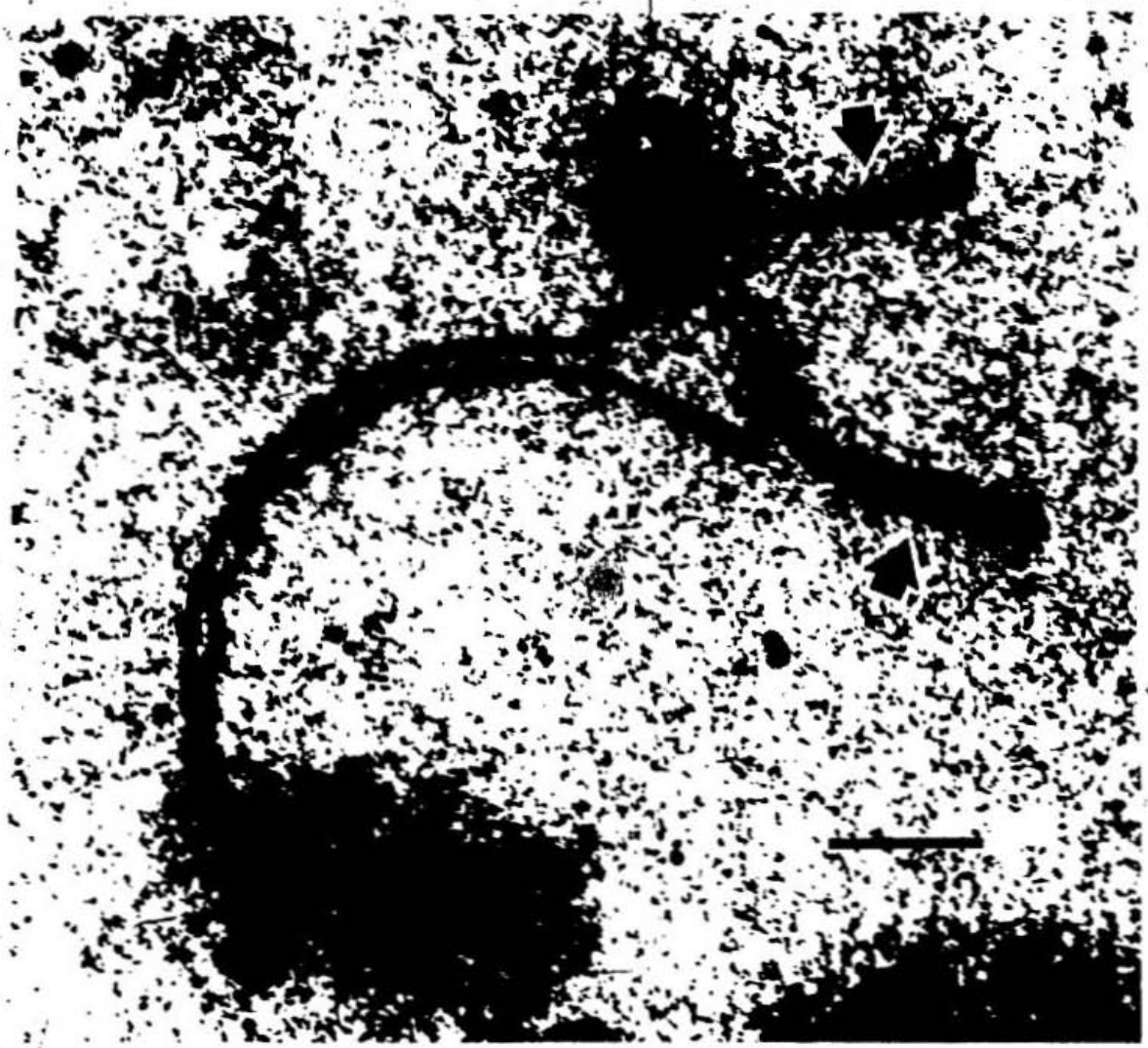


Figure 3-7

Loop formed by synapsis of the inverted segment in a male mouse heterozygous for the inversion IRK. The region where the loop starts to be formed (arrows) appears to be asynapsed, although the axis are not thickened. Electron micrograph: Bar= 0.5μm.

during pachytene. Inversions that show significant meiotic disturbance are those that do not show a loop during pachytene. In situ hybridisation using chromosome specific gene probes should make it possible to identify the rearranged bivalent.

3.5.2.12. Meiotic disturbance and selection against recombinants

Mice heterozygous for In(5)9RK (an inversion which involves a very large segment of the chromosome) have been observed to produce sperm with large heads which are presumed to have twice the usual nuclear volume (Hugenholtz & Bruce, 1979). It has been suggested that the uterotubal junction in female mice might as a partial barrier to the movement of such diploid sperm. (Ford, Evans & Burtenshaw, 1976). If diploid sperm are formed from the heteroploid secondary spermatocytes, carrying the recombinants, they may similarly be prevented from fertilizing an oocyte. This mechanism has been suggested in other species as well. In families like Chironomidae, Culicidae, Simuldiidae, Limoniidae and Ptychoteridae, despite chiasma formation in males, paracentric inversions have been observed in the natural population. This has been explained on the basis of selection against diploid sperm (White, 1973). In mammals if a diploid sperm fertilizes an oocyte the triploid offspring is inviable. This selects against recombinants at the post-fertilization stage of reproduction.

In summary, homologous pairing of the inverted segment by loop formation appears to decrease the probability of crossover. The bridge formed by the dicentric recombinant appears to prevent formation of haploid gametes carrying a recombinant. Thus the bridge has a role in the selection process.

Detailed analysis of sperm morphology and sperm count may be the next step in the investigations. The relationship of these aspects of male gametogenesis with physical characteristics of the inversions and the degree of meiotic disturbance can help in further substantiating the suggestion that the observed meiotic disturbance is a result of mechanical rather than physiological effects of the rearrangement. A reduction in sperm count associated with undisturbed fertility will substantiate the suggestion that meiotic disturbance in heterozygotes for paracentric inversions may have a role in selection against formation of gametes with recombinants.

Chapter 4

Female fertility

Differences in the constitution and behaviour of sex chromosomes during meiosis in males and females appear to influence the effect of chromosomal rearrangements on fertility in the two sexes. During meiosis, the X chromosome in males is in the inactive state (Lifschytz & Lindsley, 1972), whereas in females, the two X chromosomes are in the active state. In males heterozygous for chromosomal rearrangements, (those involving sex chromosomes as well as those involving autosomes) the unpaired regions of the rearranged chromosome are found to be associated with that of the X chromosome, preventing its inactivation. Abnormal transcription of X linked genes during meiosis is thought to be responsible for breakdown of spermatogenic differentiation (Forejt, 1982). As this interferes only with male gametogenesis, the infertility associated with it is confined to male heterozygotes, and could have a role in preventing the formation of gametes with abnormal recombinants in males.

Polar body formation is a feature of gametogenesis unique to females and may have a role in selection against gametes with abnormal recombinants in carriers of chromosomal rearrangements. Following meiosis each oogonium and spermatogonium results in four cells. In males, all four cells have the ability to

form sperm. In females only one of the four cells develops into the functional secondary oocyte. The other three cells, the polar bodies, degenerate. In heterozygotes for a chromosomal rearrangement, an abnormal recombinant may be preferentially or randomly excluded from fertilization by its inclusion in the polar body.

The role of the polar body in selection against recombinant offspring in heterozygotes for paracentric inversion was initially observed in Drosophila (Refer Section 1.8). Linear quartets, and inclusion of the dicentric recombinant in the two inner nuclei (or cell), a prerequisite for this mode of selection are not observed in mammals. It is possible however, that some other mechanism may result in selective elimination of the recombinant. In the absence of any particular selection mechanism, random inclusion of the recombinant in the polar body would eliminate the recombinant from the reproductive pathway in females with or without any noticeable effect as the case may be.

4.1. Chromosomal rearrangements and female fertility

Segregational impairment of fertility resulting from prenatal elimination of chromosomally unbalanced zygotes is a frequently observed effect of chromosomal rearrangement in balanced female carriers. In humans, prenatal elimination of chromosomally unbalanced zygotes may be recognised as spontaneous abortions (Refer sections 1.3.2.2.1 and 1.3.2.2.2). Observations suggesting segregational impairment of fertility have been reported in female mice heterozygous for the paracentric inversion 5RK and those heterozygous for the paracentric inversion 9RK (Ford, Evans & Buttonshaw, 1976).

Although segregational impairment of fertility is observed in male and female heterozygotes for rearrangements, females are found to be the carriers more frequently than males among carriers of balanced translocations ascertained following two or more spontaneous abortions (Lippman-Hand & Vekemans, 1983). Rearrangements that affect male, but not female fertility are suggested by these investigators as an explanation.

4.2. Data on human carriers of paracentric inversions

Data on human carriers for paracentric inversions suggest that female carriers have a greater potential for being identified because of a detrimental effect on reproduction. Of the 83 familial cases of paracentric inversions reported, the mother is the carrier in 51 (61.4%). (In addition, the mother has been found to be the carrier of a familial paracentric inversion of 7q (personal communication, Allderdice, 1986).) Of the 14 cases of balanced paracentric inversion associated with developmental abnormalities, the mother was the carrier in 10 (Fryns & Van den Berg, 1980, del Solar & Uchida, 1974, Orye & Van Bever, 1983, Peters-Slough, Plantevdt, Timmerman & Vooren, 1982, Callen, Woolatt & Sutherland, 1985, A French Collaborative Study, 1986b, Schmid, Haaf & Zorn, 1986) [personal communication, Allderdice, 1986]. In 7 cases in which recombinants have been suggested, the mother was the carrier in 6 cases (Valárcel, Benítez, Martínez, Rey & Sánchez - Casos, 1983, Mules & Stanberg, 1984, Speevak, Hunter, Hughes & Cox, 1985, Sparkes, Muller & Klisak, 1979, Hoo, Lorenz, Fischer & Furhmann, 1982) [personal communication, Daniel van Dyke, 1986]. The father was the

carrier of the inverted chromosome in the case reported by Kelly et al. (Kelly, Wyandt, Kasprzak, Ennis, Wilson, Koch & Schnatterly, 1979). The preponderance of female carriers in the above mentioned groups may be due to a small sample size. Another reason may be differences in the reproductive processes in males and females, with more efficient selection operating in male carriers of paracentric inversions.

4.3. Rationale for the investigations on female mice heterozygous for paracentric inversions

As in males, initial evaluation of the effect of chromosomal rearrangements on fertility may be made postnatally, by studying the ability of heterozygotes to produce live offspring, their litter size and frequency of liveborn offspring with congenital abnormalities. Association of congenital abnormalities with an unbalanced genome may be established by chromosomal analysis of the zygote. When a chromosome rearrangement is associated with reduced litter size or absence of liveborn offspring, various stages of reproduction may be investigated to determine the one at which reproduction is affected.

Certain stages of reproduction may be studied *in vivo*. Post-implantation loss, one index of loss due to genomic imbalance (Ford, 1975) may be estimated from counts of corpora lutea, moles (deciduomata), dead or abnormal embryos, and normal embryos.

The primary aim of this thesis was to determine if meiotic disturbances,

recombinant formation and selective elimination of recombinants occur in heterozygotes for paracentric inversions.

Increased pre-implantation and post-implantation embryonic losses have been observed in female mice heterozygous for In5 and In9 (Ford, Evans & Burtenshaw, 1976). Post-implantation death was more frequent than pre-implantation, suggesting genomic imbalance as the cause of death (Ford, 1975). This observation suggests formation and fertilization of oocytes with recombinants.

Significant loss during prenatal stages will result in reduced litter size. Litter sizes were therefore estimated for female mice heterozygous for the various inversions investigated to determine if there was significant prenatal loss.

Meiosis in female mice heterozygous for paracentric inversions was investigated for meiotic disturbance similar to that observed in comparable male mice (Refer section 3.4.3). The XY bivalent is thought to play a role in male dependent infertility (Forejt, 1974). If the meiotic disturbance observed in male mice heterozygous for certain paracentric inversions is observed in female carriers also, it would suggest that the disturbance in males is not a function of the XY bivalent.

Recombinant formation in heterozygotes for paracentric inversion results from uneven number of crossovers in the inverted segment. There is at least one report suggesting differences in the distribution of chiasma in male and female mice

(Polani, 1972) (Refer section 1.7.2). Second meiotic metaphases in females heterozygous for various paracentric inversions were investigated to determine if anaphase bridges and acentric fragments are formed in female heterozygotes as in males. Absence of expected recombinants would suggest that they are either not formed or are excluded by inclusion in the first polar body. Preparations of meiotic metaphases were obtained by *in vitro* techniques.

4.4. Maturation of mouse oocytes

4.4.1. *In vivo* maturation of mouse oocytes

Mammalian oogonia divide mitotically during early embryonic stages. In mice, around day 13 of embryonic life, mitosis is halted (Bronson, Dagg & Snell, 1975).The cells at this stage, the primary oocytes undergo meiosis and maturation. Maturation of an oocyte involves 2 successive meiotic divisions with release of a polar body at the conclusion of each division. In mammals the maturation process is long, commencing during the prenatal period and concluding after fertilization. As maturation is completed only on penetration by the sperm, the process of meiosis until second meiotic metaphase is known as the pre-maturation process.

Maturation of the oocyte in the mouse begins by 8 days before birth when the primary oocyte enters prophase. The first hold in the maturation process is reached approximately 5 days after birth, when the oocytes reach the diplotene stage. Primary oocytes in diplotene may be identified by presence of the germinal vesicle, a prominent nucleus. The maturation process resumes just prior to

ovulation in sexually mature female mice, some time after 4 weeks of age (Bronson, Dagg & Snell, 1975). Hours before ovulation the oocyte goes into the pre-maturation process by resumption of meiosis. After completion of first meiotic division the first polar body is extruded, and comes to lie beneath the zona pellucida (Bronson, Dagg & Snell, 1975). After completion of first meiotic metaphase, the oocyte is known as the secondary oocyte. The secondary oocyte proceeds immediately to the second meiotic division. The secondary oocyte remains in the second meiotic metaphase (second maturational hold) until it is penetrated by the sperm. After penetration by the sperm, the meiotic division is completed and the second polar body is released. (Calarco, 1972).

4.4.2. *In vitro* maturation of mouse oocytes

Maturation of mammalian oocytes *in vitro* has been reviewed by Donahue (Donahue, 1972). The follicular cells are thought to maintain oocytes in the germinal vesicle stage by producing a maturation inhibitor or by depriving the oocytes of nutrients required for maturation. Hormonal changes and calcium deficiency is observed to destabilize the association between the oocytes and the cumulus cells initiating maturation of oocytes (Masui and Clarke, 1979). Maturation is observed in follicle free oocytes cultured in suitable culture medium. *In vitro* culturing of oocytes takes advantage of this aspect of oocyte maturation. After 3.75 hours to 5 hours in culture oocytes reach metaphase I (Henderson & Edwards, 1968, Tschuida and Uchida, 1974), and after 12 hours in culture most oocytes reach second meiotic metaphase (Edwards, 1965). Maturation of oocytes in vitro is useful in studying meiotic chromosomes.

In this project oocytes were cultured *in vitro* and frequency of cells in second meiotic metaphase was estimated to determine the effect of heterozygosity for paracentric inversion on the meiotic process. Secondary oocytes (oocytes in second meiotic metaphase) were analysed for dicentric chromatid and/or acentric fragment, the possible recombinants in heterozygotes for paracentric inversions. Their absence could be either because they are not produced or because they are excluded by selective inclusion in the first polar body. Second meiotic metaphases were also analysed for aneuploidy. Some *in-vivo* data were collected to supplement the data from the *in vitro* analysis. This consisted of the number of oocytes in the germinal vesicle stage and the number in division among those released from the ovaries.

The materials and methods used in preparing and studying female meiotic metaphases are described in sections 2.3, 2.3.1 and 2.3.1.1.

4.5. Results

4.5.1. Litter size

The litter size of female inversion heterozygotes obtained by mating inversion homozygotes with C57Bl normals is reduced in the case of most inversions compared to that of homozygous females. The exception is 12RK/C57. [Table 4-1]. [Appendix K].

Significant reduction in litter size was observed in female mice heterozygous for 22RK (22RK/C57). This inversion involves a very long segment of the

Table 4-1: Litter size of female inversion homozygotes and heterozygotes

Genotype of females	No. of females	No. of litters	Mean litter size (% Fertility)
1RK/1RK	9	11	9.5 ± 0.41
1RK/C57	5	7	6.1 ± 0.88[a] (64.2%)
1RK/C3H	12	15	7.0 ± 0.46[a] (73.7%)
12RK/12RK	1	2	6.5 ± 1.50
12RK/C57	4	7[b]	5.9 ± 1.12 (90.1%)
	4	6[c]	6.8 ± 0.65
12RK/C3H	10	16	6.6 ± 0.42
24RK/24RK	10	22	6.5 ± 0.53
24RK/C57	4	7[b]	0.6 ± 0.57
	1	1[c]	4.0 (62.0%)
24RK/C3H	7	12	5.4 ± 0.66 (83.9%)
11RK/11RK	3	3	6.3 ± 1.67
11RK/C57	4	9[b]	4.7 ± 1.08
	3	7[c]	6.2 ± 0.82 (98.4%)
14RK/14RK	2	2	7.5 ± 1.50
14RK/C57	2	4	7.0 ± 0.00 (93.3%)

continued..

4.1 continued..

Genotype of females	No. of females	No. of litters	Mean litter size (% Fertility)
20RK/20RK	2	4	5.0 ± 0.58
20RK/C57	2	14[b]	2.4 ± 0.74 (48.6%)
	2	7[c]	4.9 ± 0.63 (97.1%)
22RK/22RK	4	8	7.3 ± 0.06
22RK/C57	4	9[b]	2.7 ± 1.01 (36.8%)
	4	7[c]	3.4 ± 1.15[a] (47.3%)
1RK/12RK	4	7[b]	2.4 ± 0.78
	3	5[c]	3.4 ± 0.68
1RK/24RK	2	3[b]	0.7 ± 0.67
	1	1[c]	2.0
12RK/24RK	4	6[b]	3.3 ± 0.84
	3	5[c]	4.0 ± 0.63

The values are the mean litter size (± S.E.M.). The litter size of heterozygotes were compared with that of the respective homozygotes by analysis of variance. Differences were considered significant (Tukey's test) at the $P<0.05$ level.
% Fertility is the mean litter size of the heterozygote mice divided by the mean litter size of the respective homozygote times 100.

[a] Significantly different compared to homozygotes at $P<0.05$

[b] Includes matings that did not result in pregnancy

[c] Includes only those matings that resulted in pregnancy

chromosome (Figure 2-1). Significant reduction is also observed in mice heterozygous for 1RK (1RK/C57 and 1RK/C3H), compared to the homozygote 1RK/1RK [Table 4-1]. 1RK is an inversion which involves a short segment of chromosome 1.

Only one of 7 matings involving 4 female 24RK/C57 resulted in pregnancy (14.3%), whereas all 12 matings involving 7, 24RK/C3H were successful. [Table 4-1].

4.5.2. Meiosis - *in vitro*

The proportion of oocytes resuming meiosis *in vitro* was significantly lower in 24RK/C57 and 20RK/C57 than in the respective homozygotes. The proportion of oocytes, from compound heterozygotes 1RK/12RK and 12RK/24RK, that resumed meiosis *in vitro* was low, whereas it was high in 1RK/24RK. [Table 4-2].

Compared to inversion homozygotes, significant meiotic disturbance is observed only in female mice heterozygous for 22RK, when individual mice were used as replicates and data analysed by analysis of variance [Table 4-3]. When data on oocytes from mice of each genotype was pooled and analysed by Student t-test, significant meiotic disturbance is observed in 12RK/C57 and 20RK/C57 [Table 4-3].

Table 4-2: Proportion of oocytes dividing in vitro

Genotype (n)	Oocytes set up	Oocytes dividing	Mean % dividing
C57BL (13)	345	277	81.43 ± 4.38
C3H (7)	96	67	64.58 ± 8.36
1RK/1RK (4)	105	83	78.22 ± 10.42
1RK/C57 (7)	168	134	79.69 ± 3.86
1RK/C3H (12)	246	185	73.00 ± 4.69
12RK/12RK (8)	105	88	81.83 ± 4.85
12RK/C57 (6)	143	111	78.09 ± 3.19
12RK/C3H (10)	170	129	73.8 ± 2.39
24RK/24RK (5)	90	82	91.16 ± 2.87
24RK/C57 (3)	84	59	71.00 ± 6.51*
24RK/C3H (8)	217	143	67.10 ± 4.49
11RK/11RK (4)	87	58	72.01 ± 10.30
11RK/C57 (6)	189	137	72.49 ± 10.86
14RK/14RK (5)	193	159	82.26 ± 4.13
14RK/C57BL (4)	151	128	83.80 ± 4.31
20RK/20RK (3)	39	39	100.0 ± 0.00
20RK/C57BL (5)	165	77	49.63 ± 9.09*
22RK/22RK (7)	125	91	72.18 ± 4.33
22RK/C57BL (8)	167	116	69.33 ± 3.88
1RK/12RK (5)	114	73	63.76 ± 5.41
1RK/24RK (4)	106	92	86.88 ± 1.65
12RK/24RK (2)	43	29	67.44 ± 1.00

The values are the mean proportion of oocytes dividing in vitro (± S.E.M.). Heterozygotes were compared with the respective homozygotes and normal controls by analysis of variance. Differences were considered significant (Tukey's w-procedure) at $P<0.05$.

* Significantly different from homozygotes by analysis of variance at $P<0.05$
(n) - Number of animals

Table 4-3: Data on oocytes dividing in vitro

Genotype	Age (days)	OC (n)	IM+IIM/OC %	IIM/IM+IIM %
C57Bl	61-341	437 (17)	36.85	24.8 ± 6.3
C3H	93-164	96 (7)	37.5	86.1 ± 5.2
1RK/1RK	81-156	105 (4)	47.62	41.3 ± 23.8
1RK/C57	76-133	402 (18)	25.62	10.7 ± 6.8
1RK/C3H	108-159	246 (12)	40.65	31.7 ± 3.6
12RK/12RK	109-178	95 (6)	42.11	44.0 ± 7.9
12RK/C57	63-125	108 (5)	46.30	14.4 ± 7.9[a]
12RK/C3H	120-157	176 (10)	52.27	60.1 ± 8.5
24RK/24RK	104-145	90 (5)	58.89	28.4 ± 9.8
24RK/C57	92-135	503 (19)	37.57	16.0 ± 4.9
24RK/C3H	63-169	217 (8)	35.02	93.0 ± 7.9
11RK/11RK	116-118	87 (4)	54.02	0.0 ± 0.0
11RK/C57	114-125	189 (6)	32.28	0.5 ± 0.5
14RK/14RK	105-127	162 (4)	28.40	8.3 ± 5.5
14RK/C57	119-127	185 (5)	57.30	20.6 ± 7.4
20RK/20RK	201-215	39 (3)	71.79	15.3 ± 5.0
20RK/C57	82-84	165 (5)	29.70	0.0 ± 0.0[a]
22RK/22RK	85-171	125 (7)	40.00	85.7 ± 5.1
22RK/C57	76-135	107 (8)	47.31	30.0 ± 7.9
1RK/12RK	119-121	114 (5)	31.58	14.2 ± 6.1
1RK/24RK	124-133	106 (4)	55.66	0.0 ± 0.0
12RK/24RK	63-64	43 (2)	39.53	5.9 ± 12.6

The values (IIM/IM+IIM) are the mean proportion of oocytes that proceeded to second meiotic metaphase (± S.E.M.). Heterozygotes were compared to the respective homozygotes and normal controls by analysis of variance. Differences were considered significant (Tukey's w-procedure) at $P<0.05$. They were also compared by Student t-Test and differences considered significant at $P<0.05$.

[a] Significantly different from homozygotes at $P<0.05$ (Analysis of variance)
[a] Significantly different from homozygotes at $P<0.05$ (Student t-test)
(n) - Number of animals
OC Oocytes in germinal vesicle stage set up in culture

4.5.3. Meiosis - *in vivo*

Heterozygotes for 12RK, 24RK and 20RK showed significantly more oocytes in the germinal vesicle stage, the first stage studied in the process of oocyte maturation [Table 4-4].

The number of oocytes in division *in vivo*, the second stage studied in the process of oocyte maturation, is higher in heterozygotes, than in homozygotes, for the various inversions. It is significantly higher in heterozygotes for 1RK (1RK/C57 and 1RK/C3H) and 20RK compared to the respective homozygotes [Table 4-4].

4.5.4. Cytogenetic results

Only a few well spread second meiotic metaphases were available for cytogenetic analysis in most instances [Table 4-5]. Heterozygotes for 12RK obtained by mating 12RK/12RK with C3HFeJ background was the only heterozygote that provided a sufficient number of second meiotic metaphases for cytogenetic analysis. It is also the only heterozygote in which a significant number of metaphases with chromosomes that appeared to be dicentric were observed (Figure 4-1) [Table 4-5].

Acentric chromatids were observed in heterozygotes for 1RK, 12RK and 24RK (Figure 4-2) [Table 4-5], but not above baekground except 12RK/C3H.

Compared to other heterozygotes, more metaphases with acentric chromatids were observed in 12RK/C3H.

Table 4-4: Data on mature oocytes and oocytes dividing in vivo

Genotype	Age (days)	MV/ animal (n)	MO/ animal (n)
C57Bl	61-341	2.3 ± 0.5 (18)	23.9 ± 2.2 (20)
C3H	93-164	2.9 ± 0.8 (7)	15.6 ± 3.2 (7)
1RK/1RK	81-156	4.6 ± 0.8 (14)	20.5 ± 1.9 (14)
1RK/C57	76-133	6.1 ± 0.7 (30)*	21.9 ± 1.3 (34)
1RK/C3H	103-159	6.3 ± 1.2 (12)*	23.2 ± 2.0 (12)
12RK/12RK	109-178	2.0 ± 0.7 (8)	15.3 ± 2.7 (9)
12RK/C57	63-125	4.5 ± 0.9 (12)	26.0 ± 1.9 (12)*
12RK/C3H	120-157	3.9 ± 1.1 (10)	19.5 ± 2.0 (10)
24RK/24RK	104-145	3.3 ± 1.0 (8)	19.3 ± 2.4 (8)
24RK/C57	92-135	4.3 ± 0.9 (19)	24.8 ± 1.7 (25)
24RK/C3H	63-169	5.3 ± 1.0 (8)	28.5 ± 2.6 (8)*
11RK/11RK	116-118	2.0 ± 1.4 (4)	21.8 ± 4.6 (4)
11RK/C57	114-125	4.2 ± 1.1 (6)	32.7 ± 1.7 (6)
14RK/14RK	105-127	5.0 ± 0.9 (7)	37.4 ± 3.6 (7)
14RK/C57	119-127	7.2 ± 3.1 (5)	38.0 ± 4.0 (5)
20RK/20RK	201-215	1.0 ± 0.0 (4)	13.0 ± 1.1 (4)
20RK/C57	82-84	6.4 ± 1.3 (5)*	35.8 ± 5.3 (5)*
22RK/22RK	85-171	3.7 ± 0.6 (9)	18.3 ± 1.9 (10)
22RK/C57	76-135	4.4 ± 0.8 (14)	20.4 ± 1.6 (14)
1RK/12RK	119-121	5.2 ± 0.8 (5)	25.6 ± 2.4 (5)
1RK/24RK	124-133	3.5 ± 1.0 (4)	26.5 ± 3.5 (4)
12RK/24RK	63-64	5.0 ± 1.0 (2)	23.5 ± 5.5 (2)

The values are the mean number of oocytes in the post-diplotene stage in vivo (MV) (± S.E.M) and the mean number of mature oocytes in the germinal vesicle stage (MO) (± S.E.M.). Heterozygotes were compared with the respective homozygotes and normal controls by analysis of variance. Differences were considered significant (Tukey's w-procedure) at $P<0.05$.

* Significantly different from homozygotes at $P<0.05$

Figure 4-1

Photomicrograph of a second meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK, with a dicentric chromosome (arrowhead).

Figure 4-2

Photomicrograph of a second meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK, with an acentric fragment (arrowhead).

Table 4-5: Data on possible recombinants in IIM metaphase

Genotype	No. in IIM	Dicentric	Acentric	Single chromatid
C57Bl	6	0	1	0
C3H	22	0	5	0
1RK/1RK	23	0	4	1
1RK/C57	10	0	4	0
1RK/C3H	12	2	4	1
12RK/12RK	2	0	0	0
12RK/C57	4	2	3	0
12RK/C3H	29	0	0	2
24RK/24RK	3	0	1	0
24RK/C57	5	0	6	0
24RK/C3H	6	0	5	2
11RK/11RK	0	0	0	0
11RK/C57	0	0	0	0
14RK/14RK	0	0	0	0
14RK/C57	6	0	0	0
20RK/20RK	0	0	0	0
20RK/C57	0	0	0	0
22RK/22RK	15	0	1	1
22RK/C57	5	0	0	1
1RK/12RK	4	0	1	0
1RK/24RK	0	0	0	0
12RK/24RK	1	0	0	0

A small number of metaphases from C57Bl, C3H and homozygotes for 1RK, 24RK and 22RK also show acentric fragments [Table 4-5].

Metaphases with chromosomes with heterochromatin in 1 end were observed in metaphases from 1RK/1RK, 1RK/C3H, 12RK/C3H, 24RK/C3H, 22RK/22RK and 22RK/C57 (Figure 4-3) [Table 4-5].

Some chromosomes with unequal chromatid lengths were observed in 1RK/1RK, 1RK/C3H, 12RK/12RK, 12RK/C3H and 24RK/C3H (Figure 4-4).

A number of first meiotic metaphases with chromosomes which appeared to have desynapsed is observed in many heterozygotes and some homozygotes [Table 4-6] (Figure 4-5). The mean frequency of desynapsed chromosomes is 1 or less than 1 in inbred strains and inversion homozygotes and more than 1 in heterozygotes for 1RK (both 1RK/C57 and 1RK/C3H), 12RK/C3H, 24RK/C57, 24RK/C3H and 14RK/C57, but not for 12RK/C57, 11RK/C57, 20RK/C57 and 22RK/C57 or any of the compound heterozygotes.

Table 4-6: Number of cells with desynapsed chromosomes in IM metaphase

Genotype	No. in IM	No. of chromosomes desynapsed								Desynapsis/ metaphase
		1	2	3	4	5	6	7	8	
C57Bl	22	4	1	4	1					1.0
C3H	3	1								0.3
1RK/1RK	4									0.0
1RK/C57	48	6	12	7	2	2	1			1.6
1RK/C3H	34		6	5	5	5	1		1	2.3
12RK/12RK	2			1						1 5
12RK/C57	5		1							0.4
12RK/C3H	27		5	3	4		4			2.2
24RK/24RK	19	4	3	1		1				0.9
24RK/C57	62	10	16	4	5	3	2	2		1.9
24RK/C3H	31	4	2	2	5	2		1		1.6
11RK/11RK	26			1	1		1			0.5
11RK/C57	16	1								0.1
14RK/14RK	6		2							0.7
14RK/C57	34	2	2	7	1					1.3
20RK/20RK	17	2	1	3						0.8
20RK/C57	16		1	2						0.5
22RK/22RK	2									0.0
22RK/C57	11	1	2	1						0.7
1RK/12RK	14									0.0
1RK/24RK	16	1								0.1
12RK/24RK	13									0.0

Figure 4-3 .

Photomicrograph of a second meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK, with a chromosome with heterochromatin at one end (arrowhead).

Figure 4-4

Photomicrograph of a second meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK, with a chromosome with unequal arms (arrowhead).

Above: Figure 4-5a

Photomicrograph of a first meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK with 3 desynapsed bivalents (arrowheads).

Below: Figure 4-5b

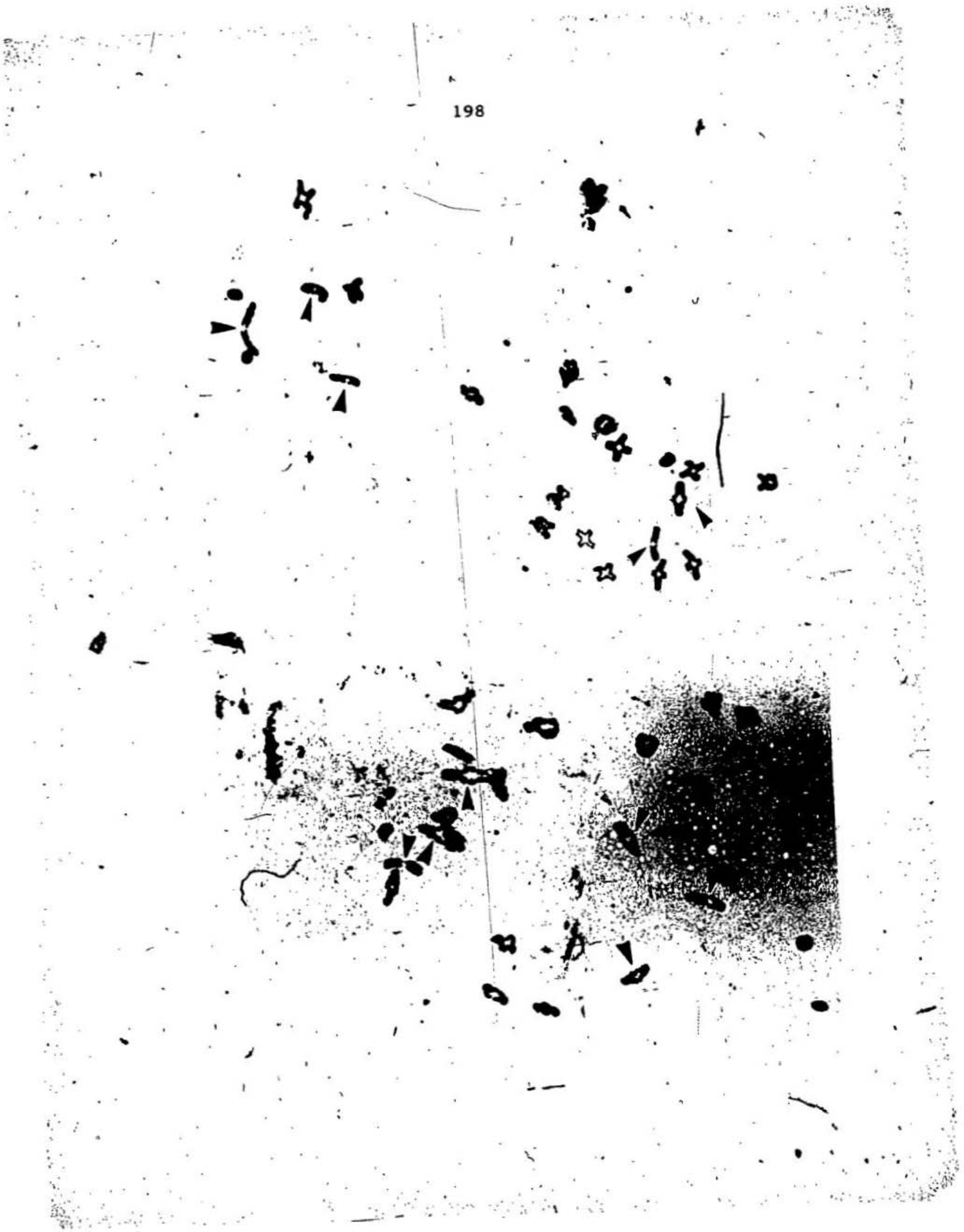
Photomicrograph of a first meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 1RK with 4 desynapsed bivalents (arrowheads).

Above: Figure 4-5c

Photomicrograph of a first meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 12RK with 5 desynapsed bivalents (arrowheads).

Below: Figure 4-5d

Photomicrograph of a first meiotic metaphase (centromere stained) from a female mouse heterozygous for the inversion 1R[illegible] with 6 desynapsed bivalents (arrowheads).



4.6. Discussions

4.6.1. Litter size

Reduced litter size of female mice heterozygous for paracentric inversions suggests (1) reduction in the number of oocytes available for fertilization or (2) increased prenatal mortality. If meiotic disturbance reduces gamete count, it would have a dramatic effect on oocyte count and female reproduction, since the number of oocytes available for fertilization is limited in females compared to the excess of sperm produced by males. The reduced litter size in heterozygotes for all the inversions investigated, significantly so for 22RK/C57, may be the consequence of meiotic disturbance. Estimation of litter size for 24RK/C57 female heterozygotes is impossible as only one litter is available for analysis. Based on the significant meiotic disturbance observed in male mice heterozygous for inversions 22RK and 24RK [Table 3-2], it may be assumed that heterozygosity in females has a similar effect. If so, the significant reduction in litter size of female mice heterozygous for 22RK may be attributed to the disturbance. Significant meiotic disturbance in female heterozygotes compared to homozygotes, as measured by a significant reduction in the proportion of oocytes that mature to first meiotic metaphase and proceed on to second meiotic metaphase, is observed only in inversion 22RK (Refer section 4.5.2). The number of oocytes available for fertilization (the number of oocytes that have proceeded on to second meiotic metaphase *in vivo*) may be estimated from the number of (1) corpora lutea and/or (2) oocytes in the fallopian tubes. (The number of oocytes dividing *in vivo* as tabulated in Table 4-1 is not a valid estimate as it includes oocytes in first meiosis.

Alternatively, prenatal elimination may have a role in the production of small litters. Increased prenatal elimination particularly in the post-implantation stage has been reported in female heterozygotes for inversions 5RK and 9RK (Ford, Evans & Burtenshaw, 1976). The reduced litter size observed in female heterozygotes for 22RK may be a consequence of similar prenatal elimination. *In vivo* observations may be informative in determining the extent of fetal loss due to genomic imbalance (Refer section 4.3).

The observed differences in rates of pregnancy [Appendix L] as measured by the number of matings that resulted in litters of 24RK heterozygotes obtained by crossing 24RK/24RK with C57BL and those with C3HFeJ [Table 4-1] requires cautious interpretation for three reasons. Although mating was not confirmed by vaginal plug, the male and female were separated after a minimum of 18 days. It may therefore be assumed that mating did take place before the animals were separated. If this is assumed and the matings that did not result in litters are considered in estimating litter size, the litter size for 24RK/C57 is 0.6. This value may be considered as a measure of fertility (or infertility). The 24RK heterozygotes obtained from the C3H crosses were younger (54-123 days) than those from C57 crosses (103-158 days) [Table 4-7]. There is some evidence suggesting increased post-implantation deaths in older female carriers of paracentric inversions (Ford, Evans & Burtenshaw, 1976). The decreased fertility in heterozygotes of 24RK from the C57Bl crosses may be the consequence of increased age and resulting increased post-implantation death. Reduction in the

Table 4-7: Age of female heterozygotes and litter size

Genotype of females	No. of litters	Age (days)	Mean litter size
1RK/C57	7	103-163	6.1 ± 0.88
1RK/C3H	15	57-104	7.0 ± 0.46
12RK/C57	7[b]	108-347	5.9 ± 1.12
	6[c]	108-347	6.8 ± 0.65
12RK/C3H	16	49-101	6.6 ± 0.42
24RK/C57	7[b]	103-158	0.6 ± 0.57[a]
	1[c]	107.0	4.0
24RK/C3H	12	54-123	5.4 ± 0.66

The values are the mean litter size (± S.E.M.). They were compared by analysis of variance. Differences were considered significant (Tukey's test) at the $P<0.05$ level.

[a] Significantly different from homozygotes at $P<0.05$

[b] Includes matings that did not result in pregnancy

[c] Includes only those matings that resulted in pregnancy

number of oocytes released with increase in age is another factor to be considered. Significant reduction is reported in the number of oocytes that matured to first meiotic metaphase after *in vitro* culture in female mice of two different strains, Q and CBA, in older animals (Speed, 1977). Age and proportion of oocytes that matured *in vitro* was not correlated in this study (regression coefficient=0.060 ± 0.085). Lack of correlation may be due to the heterogeneous background of the mice used in this study.

Another difference between 24RK/C57 and 24RK/C3H is with regard to the origin of inverted chromosome. The inverted chromosome was paternally derived in heterozygotes from the C3H crosses and maternally derived in heterozygotes from the C57 crosses. There are reports of differences in complementation of duplication/deficiency of certain chromosomal regions and severity of a trisomic effect attributable to differences in parental source (Searle & Beechey, 1985). The phenomenon of 'imprinting' (modification of chromosomes in the germ line that causes maternal and paternal chromosome to behave differently) is thought to be responsible for non-complementation lethality (Cattanach, 1986). In the diploid situation, however, this phenomenon may not be applicable.

Male partners for 24RK/C57 were normal C57Bl. The partners for 24RK/C3H were 24RK/C3H males. Meiotic disturbance is observed in 24RK/C57 and 24RK/C3H males [Table 3-2]. If meiotic disturbance in males was to effect fertility, it ought to be apparent in the mating between male and female

24RK/C3H. Observations to the contrary suggests that heterozygosity for the inversion in males is not the reason for the difference in fertility between 24RK/C57 and 24RK/C3H.

It may be speculated that genes contributed by the C3H parent might be responsible for the increase in fertility. However, fertility and litter sizes of normal C57 female and C3H female when mated with males homozygous for 1RK, 12RK and 24RK do not show any significant difference. There is also no difference between these fertility and litter sizes and those obtained when normal C3H females are mated with normal C3H males [Table 4-8].

Despite the difference in fertility of 24RK/C57 and 24RK/C3H females, their proportion of second meiotic metaphase (secondary oocytes) *in vitro* are not significantly different. [Table 4-3]. This observation once again suggests that the difference in age is the most probable reason for the difference in fertility. However it is not known if the immediate cause of reduced fertility in older females is a consquence of fewer oocytes available for fertilization, of increased post-implantation loss or both. Pre- and post-implantation data should be helpful in resolving this question.

Tease and Fisher have observed a decrease in the proportion of pachytene oocytes with a loop, with increase in age (Tease & Fisher, 1986). According to the "production line" model these oocytes mature and ovulate later in life. If the speculation made in males (with regard to negative relationship between loop

Table 4-8: Litter size of C57BL/6J and C3HFeJ females

Genotype of males	C57BL/6J Females (n)	C3HFeJ Females (n)
1RK/1RK	6.4 ± 0.77 (15)	6.4 ± 1.12 (5)
24RK/24RK	6.5 ± 0.53 (14)	7.7 ± 0.88 (3)
12RK/12RK	6.0 ± 1.00 (3)	8.0 ± 0.32 (5)
C3H		7.7 ± 1.20 (3)

The values are the mean litter size (± S.E.M.). The litter size of C57Bl- and C3HFeJ females were compared by analysis of variance. Differences were considered significant (Tukey's w-procedure) at the $P<0.05$ level.
n- Number of litters

formation and crossover) (Refer section 3.5.2.2) is applied to the females, absence of a loop in these oocytes will be associated with increased probability of crossover in the inverted segment. This would increase the probability of recombinant formation and subsequent prenatal selection in older compared to the young female heterozygotes.

It is also reported that after the period of drop in the proportion of pachytene oocytes with the loop, an increase is observed around the 19th day of post-conception (Tease & Fisher, 1986). Synaptic adjustment similar to that observed in male mice heterozygous for paracentric inversions has been observed in female mice heterozygous for paracentric inversions (Moses & Poorman, 1984). It is possible that the increase observed during the later part of fetal stage is because of inability to adjust the loop with increase in maternal age rather than being the result of differences in pairing with age as suggested by the investigators. Consequently the oocyte may be arrested at the pachytene stage and thus lower the number of oocytes available for fertilization. Interestingly, the litter size of seven 24RK/24RK female mice between 104 - 293 days, was between 5 and 11 pups in each litter (Appendix L). This observation in the homozygotes suggests that the association with age observed in 24RK/C57 may be a function of the inversion heterozygosity.

4.6.2. Meiotic disturbance

The mean number of oocytes in the germinal vesicle stage is consistently higher in heterozygotes than in the respective homozygotes. The difference is significant only in 12RK/C57, 24RK/C3H and 20RK/C57 [Table 4-4]. This increase in number may be a function of hybrid vigour. These heterozygotes were also younger than the respective homozygotes [Table 4-4]. Significant correlation, however, was not observed between age of the mouse and number of oocytes. (Regression coefficent of -0.302 ± 0.126 was obtained when data from the various genotypes was included. When data from 24RK/C57 (n=24) alone was used, the regression coefficient was 0.153 ± 0.413). The increase in the number of oocytes in the germinal vesicle stage (diplotene) is evidence for absence of meiotic arrest in the pre-diplotene stage.

The consistently increased number of oocytes in meiosis *in vivo*, in heterozygotes compared to that in homozygotes, significant in heterozygotes for 1RK and 20RK [Table 4-4], may be a consequence of the number of oocytes in maturation. The increased number of oocytes in maturation suggests (1)absence of meiotic arrest in stages prior to metaphase I or (2) retention of oocytes at first meiosis. There is no correlation between the number of oocytes in meiosis *in vivo* and number of oocytes in the germinal vesicle stage (R=0.284 ± 0.130). Therefore, there does not appear to be an arrest prior to first meiotic metaphase. Cytogenetic analysis of these oocytes is essential to determine if they are in first or second meiotic metaphase. A significantly greater frequency of first than second meiotic

metaphase would suggest meiotic disturbance or arrest at first meiotic metaphase *in vivo*.

Maximum proportion of oocytes that resumed meiosis *in vitro* was in 24RK/24RK and 20RK/20RK. [Table 4-2] The apparently decreased proportion of oocytes that resumed meiosis *in vitro* from 24RK/C57 and 20RK/C57 may be due to the unusually high proportion observed in the homozygotes [Table 4-2].

The location of breakpoints of the inversions involved in the compound heterozygotes may contribute to the varying proportions of oocytes that resumed meiosis *in vitro* from them. In 1RK/12RK and 12RK/24RK, two of the breakpoints are located close to one another. In 1RK/12RK, the inversions overlap. This may be true of 12RK/24RK also and may account for low proportions of oocytes that resumed meiosis *in vitro* as in 1RK/12RK. In 1RK/24RK, inversion 1RK is included within the region involving 24RK.

Despite the increased number of oocytes in the germinal vesicle stage and the increased number of oocytes in the metaphase stage (evident by the increased number of oocytes in division *in vivo* [Table 4-4]), fertility is significantly reduced in 24RK/C57 heterozygotes. The reduction in fertility suggests meiotic disturbance or arrest at first meiotic metaphase resulting in a decrease in the number of oocytes available for fertilization. Counts of corpora lutea and of oocytes in the fallopian tubes would provide an estimate of the number of oocytes that were available for fertilization. (Post-implantation death similar to that

observed in female mice heterozygous for 5RK and 9RK (Ford, Evans & Burtenshaw, 1976) is another possible cause for the reduced litter size. The extent of post-implantation death may be estimated from counts of corpora lutea and resorption sites.)

The small and insignificant regression coefficient of 0.120 (± 0.153) between age of mice and frequency of second meiotic metaphases indicates that the reduced frequency of second meiotic metaphases observed in heterozygotes for various inversions may not be the function of the age of the mice. This is substantiated by the observation in inversion 20RK. Although with increase in age there appears to be a decrease in the number of oocytes in the germinal vesicle stage in 20RK/20RK [Table 4-4], the frequency of second meiotic metaphases is not lower, but higher in the older homozygotes, compared to younger heterozygotes [Table 4-3]. Small number of oocytes in first meiotic metaphase after *in vitro* culturing of oocytes in older mice has been attributed to non-maturation or oocyte degeneration (Speed, 1977). In this study in the case of 20RK, despite increased age of 20RK/20RK, they show a higher proportion of oocytes which divided *in vitro* [Table 4-2] as well as proceeded to second meiotic metaphase than the younger 20RK/C57 [Table 4-3]. When data from the various genotypes were pooled and analysed, as mentioned in section 4.6.1, age and proportion of oocytes that matured *in vitro* was not found to be correlated (R=0.060 ± 0.084).

Compared to homozygotes, meiotic disturbance is observed only in heterozygotes

for the inversion 22RK [Table 4-3]. Similar meiotic disturbance is observed in male mice heterozygous for 22RK. However, unlike males, meiotic disturbance is not observed in female mice heterozygous for 24RK. Significantly increased frequency of second meiotic metaphases in homozygotes for 22RK compared to C57Bl need to be considered when interpreting the observations in heterozygotes (Refer section 4.6.3). Large variances may be one factor responsible for suppressing at the statistical level true biological differences in the proportion of second meiotic metaphases in some instances (eg. 12RK/12RK vs. 12RK/C57 and 20RK/20RK vs. 20RK/C57).

The absence of oocytes in second meiotic metaphase in 1RK/24RK [Table 4-3], suggests significant arrest of cells *in vitro*. (Although the numbers are small, the pregnancy rate appears to be reduced suggesting a similar effect *in vivo*. [Table 4-1]. The observation of oocytes in first meiotic metaphase suggests that arrest is between first and second meiotic metaphase. In contrast, oocytes in second meiotic metaphase and a small proportion of oocytes that resumed meiosis *in vitro* is observed in 1RK/12RK and 12RK/24RK. These observations suggest that arrest in 1RK/12RK and 12RK/24RK is probably between pachytene and first meiotic metaphase. The differences in location of breakpoints may be the reason for the differences in the stages of arrest (observed in 1RK/12RK and 12RK/24RK vs. 1RK/24RK).

4.6.3. Other observations of meiotic disturbance

The frequency of second meiotic metaphase in homozygotes and heterozygotes for 11RK and 22RK requires special mention [Table 4-3]. It is higher in female mice homozygous for 22RK than in any other homozygote or C3H, and significantly more than in normal C57 females. This is not the case in male mice homozygous for 22RK [Table 3-2]. The reduced proportion of second meiotic metaphase observed in female heterozygotes for 22RK may be entirely due to the high proportion of second meiotic metaphase in the homozygotes.

The frequency of second meiotic metaphase in homozygotes and heterozygotes for 22RK suggests the existence of a gene in 22RK which, in the homozygous state results in highly efficient maturation of oocytes. Homozygotes for 11RK appear to be homozygous for a gene which lowers the frequency of maturation dramatically. Heterozygosity for 11RK does not appear to change the situation significantly [Table 4-3]. Segregation analysis of this characteristic is essential for the support of this hypothesis.

Heterozygosity for 22RK lowers *in vitro* maturation of oocytes, and also reduces litter size (6.8 pups in homozygotes to 2.7 pups in heterozygotes). This reduction may be consequent to the meiotic disturbance due to heterozygosity for the structural rearrangement, rather than for a single gene. Post-implantation loss due to genomic imbalance (Refer section 4.6.1) is another possible reason for the reduced litter size. Both these reasons need to be excluded before the role of a

single gene in oocyte maturation is confirmed. Homozygosity for the gene in mice homozygous for the inversion 11RK does not affect litter size. Litter size of mice homozygous for inversion 11RK is not different from that of other homozygotes [Table 4-1] suggesting that the gene in 11RK affects *in vitro* maturation and not *in vivo* maturation.

Observations in homozygotes and heterozygotes for 11RK bear on the need to use homozygotes as a control when determining the effects of heterozygosity for a structural rearrangement on the kineties of meiosis. They also caution against the interpretation of data from *in vitro* studies in estimating meiosis *in vivo*.

The frequency of spontaneous maturation is reported to be positively correlated with the diameter of the oocyte up to a certain size (in the mouse it is 68 micrometers). Maturation of the smaller oocyte is often arrested at metaphase I (Masui and Clarke, 1979). This is a factor to be considered when interpreting the observations made in homozygotes and heterozygotes for 11RK.

As stated in section 4.5.4, heterozygosity for an inversion increases desynapsis. The apparent exceptions may have other explanantions. Interpretation of the low frequency of desynapsed chromosomes in C3H, 1RK/1RK, 12RK/12RK, 12RK/C57, 14RK/14RK and 22RK/22RK is not possible because of the small numbers of first meiotic metaphases available for analysis. Excluding the above, between 1.3 to 2.3 chromosomes are desynapsed per metaphase in heterozygotes 1RK/C57, 1RK/C3H, 12RK/C3H, 24RK/C57, 24RK/C3H and 14RK/C57. In

11RK/C57 and 20RK/C57 the frequency of chromosomes desynapsed per metaphase is lower than in the respective homozygotes. The unusual behaviour of 11RK (discussed at the beginning of this section) may be responsible for this observation. [Table 4-6]. Namely the low frequency of chromosomes desynapsed in 11RK/C57 may also support the suggestion that the meiotic disturbance observed in 11RK/11RK and 11RK/C57 may not be the result of the inversion. Contrary to expectation, males heterozygous for inversion 20RK do not show significant meiotic arrest. Possible reasons for this observation, discussed in section 3.5.2.8 may apply to the low frequency of desynapsed chromosomes observed in female mice heterozygous for 20RK.

Unlike heterozygotes for other inversions, the frequency of desynapsed chromosomes in 22RK/C57 is low (with a mean of 0.7 desynapsed chromosomes/metaphase). This may be the phenotypic effect of the gene which in 22RK/22RK results in a high maturation frequency. This observation may also be interpreted to substantiate the suggestion that the significant meiotic disturbance observed in 22RK/C57 is not a biological characteristic of the inversion but a reflection of the very high maturation frequency of oocytes from 22RK/22RK.

The very low frequency of desynapsed chromosomes in the compound heterozygotes may be because of arrest at pachytene. It may be concluded that there is no delay at first meiotic metaphase and therefore no desynapsis. That is, desynapsis of chromosomes is an effect of delay at first meiotic metaphase.

Even when the data on litter size and meiotic disturbance are considered, one cannot conclude confidently that meiotic disturbance similar to that observed in male heterozygotes may be operating in female heterozygotes. Lack of correlation between meiotic disturbance and litter size in homozygotes and heterozygotes for inversion 11RK and observations suggests that the biological effects of heterozygosity for 12RK and 20RK may be suppressed due to large variances. It is suggested that *in vivo* techniques need to be used in determining the effect of heterozygosity for paracentric inversion on the kinetics of female meiosis.

It would be worthwhile to determine if the growth and size of the ovaries of these female heterozygotes are affected. This effect has been observed in female heterozygotes for 2 chromosomal rearrangements, Is40II and t(11;19)42II and tertiary trisomy $Ts(5^{12})3II$. All these rearrangements in the males result in male specific sterility (Mahadeviah, Mittwoch & Moses, 1984, Mittwoch, Mahadeviah & Olive, 1981, Mittwoch, Mahadeviah & Setterfield, 1984). Therefore the information on ovarian growth will contribute to identifying the nature of meiotic disturbance in comparable male mice.

4.6.4. Cytogenetic observations

Interpretation of cytogenetic observations is limited by a number of factors. The frequency of analysable oocytes was between 26% and 72% [Table 4-3]. This includes first and second meiotic metaphases. The poor yield of second meiotic metaphases in heterozygotes, 0%-32%, (with only one at 60%) [Table 4-3] for most inversions is a serious obstacle for cytogenetic analysis. The significant numbers of dicentric chromatids and acentric fragments in 12RK/C3H, compared to other groups, may be because there are more second meiotic metaphases for analysis, (Refer section 4.5.4) [Table 4-5]. Dicentrics are observed only in heterozygotes. The number of acentric fragments in control (C57Bl and C3HFeJ) and various homozygotes (except 1RK/1RK) is low suggesting that those observed in 12RK/C3H are recombinants. The yield of second meiotic metaphases from heterozygotes for other inversions has to be improved before any attempt is made to correlate the physical characteristics of the inversion with recombinant formation. The observations on maturation rates in 11RK and 22RK (Refer section 4.6.3) suggest the existence of genes that influence in vitro maturation. If this is the case, heterozygosity for the inversion on a suitable background may be the answer to improving yield of second meiotic metaphases, provided heterozygosity does not have a significant effect on passage of the cell from first meiosis to second meiosis.

The observation that dicentric like structures are not observed in controls and homozygotes also suggests that these structures are dicentrics. If so, it follows that

dicentric chromosomes do not interfere with progress of oocyte from first meiosis to second meiosis. The size of the dicentric bridge is the sum of the length of the inverted segment and twice the length of the interstitial segment. Among all the inversions investigated, the longest dicentric chromosome ought to be formed in heterozygotes for 12RK. The size of the dicentric chromosome may be in favour of its persistence as it may be longer than the distance between the poles of the spindle formation. If the dicentric chromosome is shorter than the distance between the poles, it may break during anaphase and may not be observed as a dicentric chromosome during second meiotic metaphase.

If the small number of oocytes in second meiotic metaphase is due to the use of *in vitro* techniques, cytogenetic analysis of oocytes dividing *in vivo* may be informative, but only if it can be proved that the small litter size observed in some heterozygotes is due to post-fertilization loss and not due to decreased availability of oocytes for fertilization as a result of maturation arrest.

The chromosome with heterochromatin at one end may be interpreted as a chromatid separated prematurely after first meiotic metaphase (Figure 4-6). It could also be a chromatid prematurely separated in second anaphase. Normally separation in the latter instance occurs only after the oocyte has been penetrated by the sperm. Both these mechanisms have the potential to contribute to aneuploidy due to premature separation of the chromatid. As they are observed in 1RK/1RK and 22RK/22RK, the interpretation of this structure based on the suggested mechanism due to inversion heterozygosity is not possible.

Figure 4-6

Diagrammatic representation of the formation of a chromosome with heterochromatin at one end in second meiotic metaphase, from a female mouse heterozygous for a paracentric inversion, following unequal number of crossover in the inverted segment in an acrocentric chromosome.

Chromosomes with unequal arms may be the result of a break in the region between the two centromeres in the dicentric chromosome. It is difficult to interpret those observed in the heterozygotes on these lines as they are observed in some homozygotes as well (1RK/1RK and 12RK/12RK).

The absence of heterochromatin in the centromeric region of some chromosomes (Figure 4-7) interferes with the identification of acentric chromatids by C-staining.

It is apparent that observations from these investigations do not reveal a distinct pattern of effects of heterozygosity for paracentric inversions on meiosis and reproduction in females. Statistical and technical inadequacies place limitations on interpreting cytogenetic observations as well as interpretations related to the kinetics of meiosis in females. The limited number of oocytes in each animal and the factors that may be introduced by the *in vitro* techniques used must be considered when attempting to determine the effects of heterozygosity for paracentric inversion on the kinetics of meiosis. The possible existence of genes that effect maturation of oocytes (Refer section 4.6.3) need consideration in this context. The inability to identify specific chromosomes (the inverted chromosome in this instance) in meiotic preparations interferes with the interpretation of the structurally abnormal chromosomes in the preparations. Banded meiotic preparations or chromosome specific gene probes and in situ hybridization may be used to overcome this obstacle.

Evidence that oocytes carrying dicentric chromosomes and acentric fragments

Figure 4-7

Photomicrograph of second meiotic metaphase (centromere stained) from a female mouse homozygous for the inversion 22RK, with a chromosome with cytologically undetectable heterochromatin in the centromeric region.

may survive and be fertilized is provided by a report by Evans and Burgoyne (Evans & Burgoyne, 1984) on the fate of the acentric fragment and dicentric chromatid in female mice heterozygous for a paracentric inversion of the X chromosome In(X)III. In 2 - 8 cell embryo, up to 3 acentric fragments were reported. The observation of 2n/4n mosaic embryo were interpreted as the result of disturbed cytokinesis. Chromatids interpreted as products of breakage and fusion were also reported. The inverted segment in In(X)III involves 85% of the physical X chromosome. The female carrier of this inversion gives rise to an increased frequency of XO daughters (Evans & Phillips, 1975). In a collaborative study to determine the fate of XO mice, it was observed that some of the fertilized eggs exhibited developmental delay in culture. Cytogenetic evaluation of these embryos revealed dicentric and acentric fragments and 2n/4n mosaicism. These observations however await further confirmation as few dividing cells are obtained that are analysable. Meiotic disturbance in this stock has not been investigated. (Edward, P. Evans, personal communication 1986). The fact that this inversion is on the X chromosome, whereas the ones investigated in this study are on autosomes could account for differences in the fate of the embryos with the recombinants in the two studies.

Six individuals with paracentric inversions were ascertained and reported in the French Collaborative study following recurrent abortions. In 5 cases the female partner was heterozygous for the paracentric inversion (A French Collaborative Study, 1986b). It is difficult to determine the significance of this observation in view of the small sample size.

Chapter 5

Discussion

The observed frequency of any chromosomal rearrangement (or polymorphic variant) depends on the true frequency of the rearrangement in the population and the probability of its ascertainment. Most population screenings for chromosomal rearrangements and variants were carried out before high resolution banding techniques were available for cytogenetic evaluation. These techniques are particularly useful in detecting those rearrangements that result in subtle differences in the banding pattern of the chromosomes - some paracentric inversions and certain recombinants of some of them being two examples. Because these techniques are costly and laborious, they are carried out predominantly on clinically selected populations, and overt phenotypic effects of these rearrangements are therefore important in the ascertainment. The effects of chromosomal rearrangements are evident at various stages of reproduction and are discussed in section 1.10.1. One important phenotypic expression of heterozygosity for chromosomal rearrangements is impaired reproduction. Studies at various stages of reproduction on mice heterozygous for paracentric inversions were carried out in an attempt to determine the possible reasons for the under ascertainment of paracentric inversions.

The observations from this study suggest that the effects of heterozygosity for balanced chromosomal rearrangements on reproduction may vary with the nature of the rearrangement and with differences in their effect on gametogenesis in the two sexes.

5.1. Nature of rearrangement

Balanced rearrangements may be broadly classified into intrachromosomal (those which involve only one chromosome) and interchromosomal (those which involve more than one chromosome). Inversions (pericentric and paracentric) constitute the former and translocations the latter. Genomic imbalance resulting from segregation is largely responsible for impaired fertility in heterozygotes for translocations. In heterozygotes for inversions, uneven numbers of crossover in the inverted segment is a prerequisite for genomic imbalance. The frequency of crossover in the inverted segment is thought to depend on the length of the inverted segment, its location on the chromosome and the crossover characteristics of the individual(Refer section 1.7.2). The present studies suggest that, in male heterozygotes there is a negative relationship between loop formation and frequency of crossover in the inverted segment (as measured by frequency of anaphase bridge) (Refer section 3.5.2.7). If such a relationship is biologically true, loop formation may be a mechanism that prevents the formation of gametes with recombinants in heterozygotes for paracentric inversions.

5.1.1. Effectiveness of selection at different stages of reproduction

The kind of rearrangement and structure of recombinant formed may also determine the stage of reproduction when selection occurs, which may in turn influence the effectiveness of selection.

One reason for the difference in the observed effects of heterozygosity for pericentric and paracentric inversions may be the different stages at which the recombinants activate the selection process. In heterozygotes for a paracentric inversion, one possible recombinant is a dicentric chromosome which may interfere with cytokinesis and result in selection prior to fertilization. In heterozygotes for a pericentric inversion, crossover in the inverted segment results in a duplication/deficient chromosome and selection in the zygote. In these, as in translocations, genomic imbalance due to duplication/deficiency may result in selection after fertilization. Since selection after fertilization takes into consideration a variety of factors (extent of genomic imbalance in the zygote, fetal genotype, maternal genotype, maternal-fetal interaction and environmental factors) the degree of selection may vary from one rearrangement to another as well as from one case (individual and situation) to another. In humans, this range of variation in post-fertilization selection is apparent in the report on the large collaborative study by Boué et al. They report that when the rearrangement was ascertained through repeated spontaneous abortions, a wide range in the total length of the involved chromosome segments is observed (Boué & Gallano, 1984). In contrast, selection prior to gamete formation, that depends exclusively on the

physical (or structural) nature of the recombinant, would be a more reliable (efficient) form of selection.

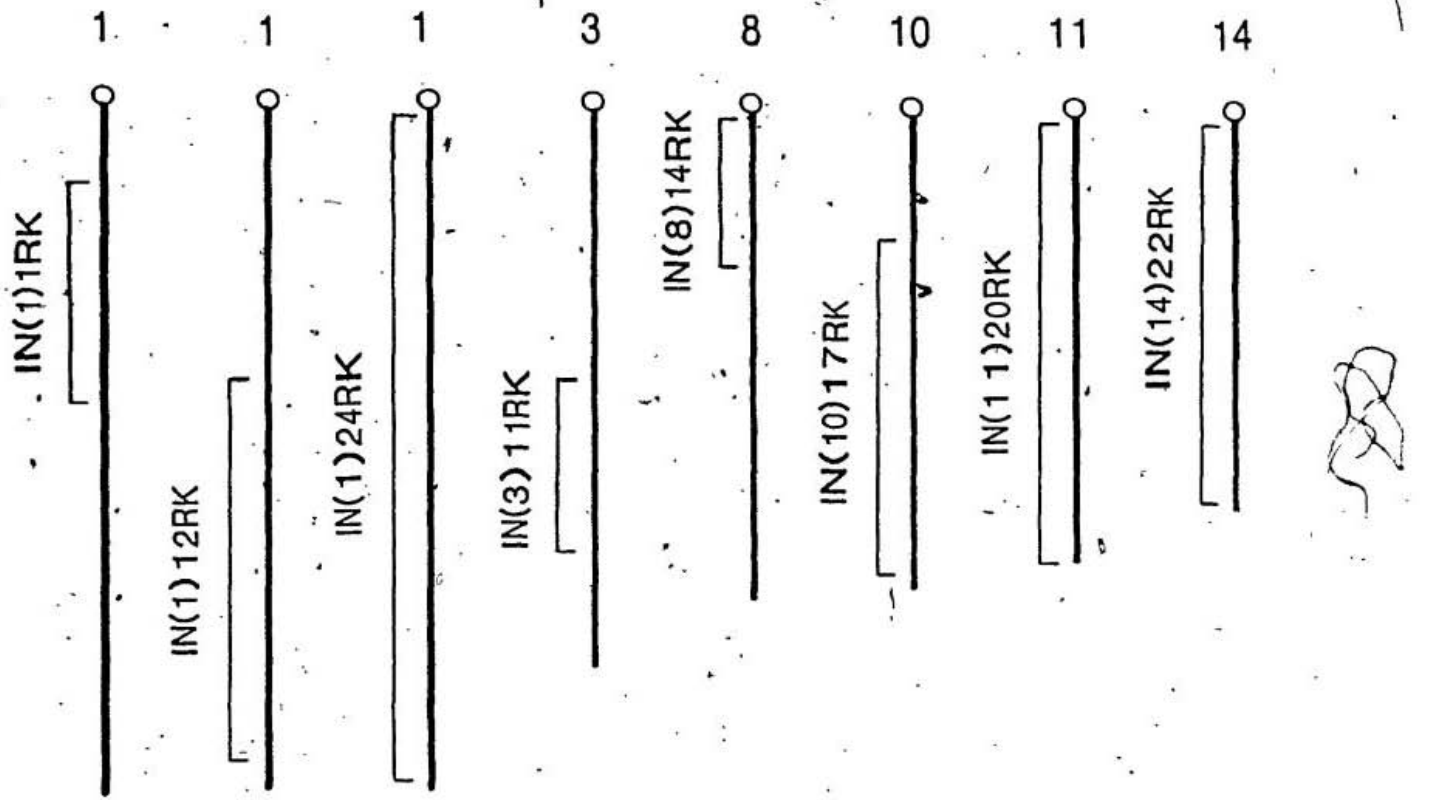
5.2. Differences in the effect of paracentric inversion heterozygosity on male and female meiosis and gametogenesis

Possible differences in the effect of structural heterozygosity on reproduction in the two sexes were introduced in section 1.10.2. It seems appropriate to start the evaluation of possible effects of paracentric inversion on meiosis and gametogensis at the pre-gametic stage. No conclusive evidence was found to suggest a difference in the effect of paracentric inversion heterozygosity on the kinetics of meiosis in the two sexes. In males a pattern is observed in the effect paracentric inversion heterozygosity has on spermatogenesis with decrease in the proportion of haploid secondary spermatocytes and increase in that of heteroploid secondary spermatocytes [Figure 5-1] (Refer section 3.4.3). Other observations suggest possible etiology for the observed disturbance in meiosis (Refer section 3.5.2.5.1.1) [Figure 5-2]. No such pattern could be discerned in females (Refer section 4.6.2) [Figure 5-3]. Attempts to compare meiotic disturbance in males and females are complicated by numerous factors. One of them may be existence of genes which could affect the maturation process of oocytes (as in 22RK/22RK and 11RK/11RK) but not of spermatocytes (Refer section 4.6.3). Other confounding difficulties are (1) female meiosis is studied *in vitro* and male meiosis *in vivo* and (2) the limited number of oocytes available from a single female compared to the large number of spermatocytes available from a male.

Figure 5-1

Proportion of haploid and heteroploid secondary spermatocytes, in paracentric inversion homozygotes and heterozygotes. The proportion of haploid secondary spermatocytes is significantly decreased in heterozygotes for inversions 22RK and 24RK. The proportion is small in heterozygotes for 17RK. Heterozygotes for 20RK is the exception. The proportion of heteroploid secondary spermatocytes is increased in the heterozygote.

Proportion of NIIM and HetIIM in male mice

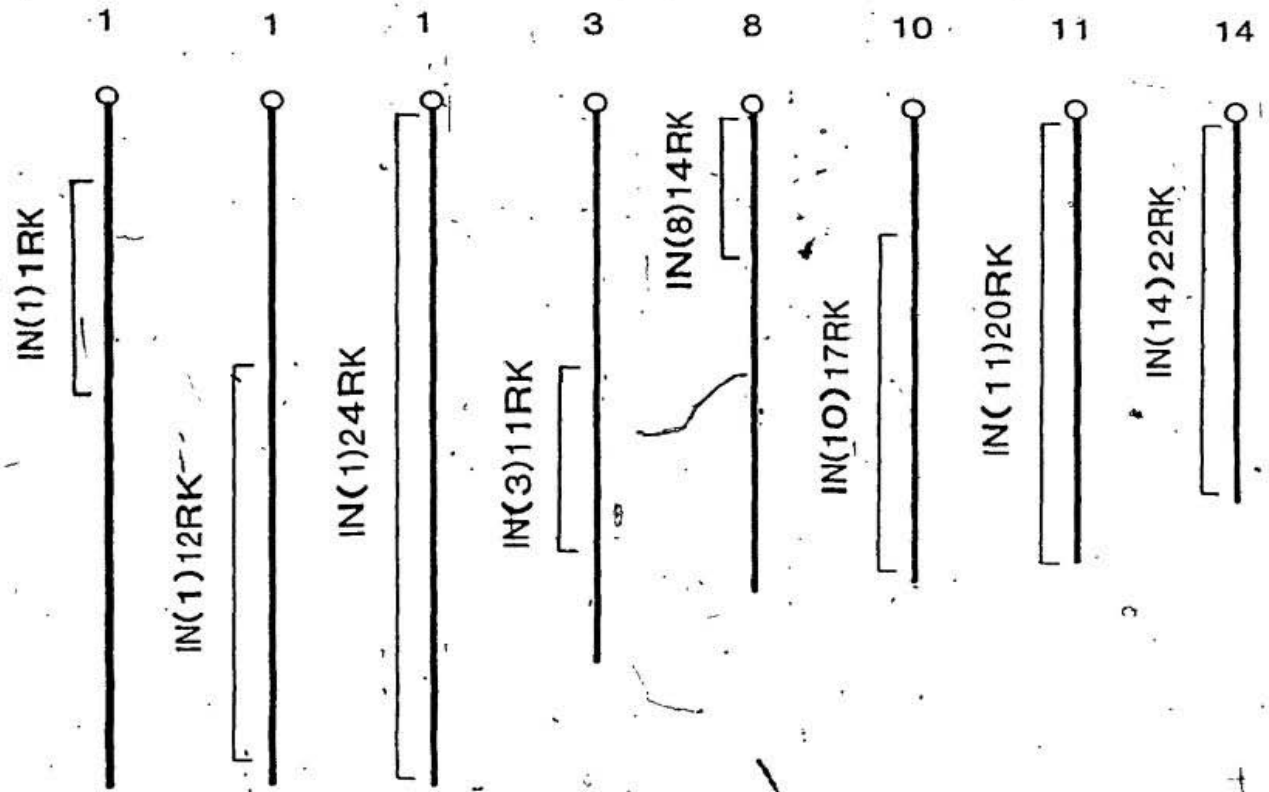


NIIM	Homo	50%	43%	44%	47%	42%		38%	31%
	Hetero	34%-37%	38%	16%-19%	50%	46%	17%	35%	16%
HetIIM	Homo	23%	16%	22%	17%	2[illegible]%		22%	27%
	Hetero	33%-40%	25%-32%	63%-65%	25%	40%	60%	37%	67%

Figure 5-2

Proportion of loop and anaphase bridges in male mice heterozygous for the paracentric inversions investigated. The frequency of cells with the loop is small in heterozygotes for inversions involving large segments (17RK, 20RK, 22RK and 24RK). With the exception of 20RK, the low frequency of cells with the loop, is associated with an increased frequency of anaphase bridge suggesting increased frequency of crossover.

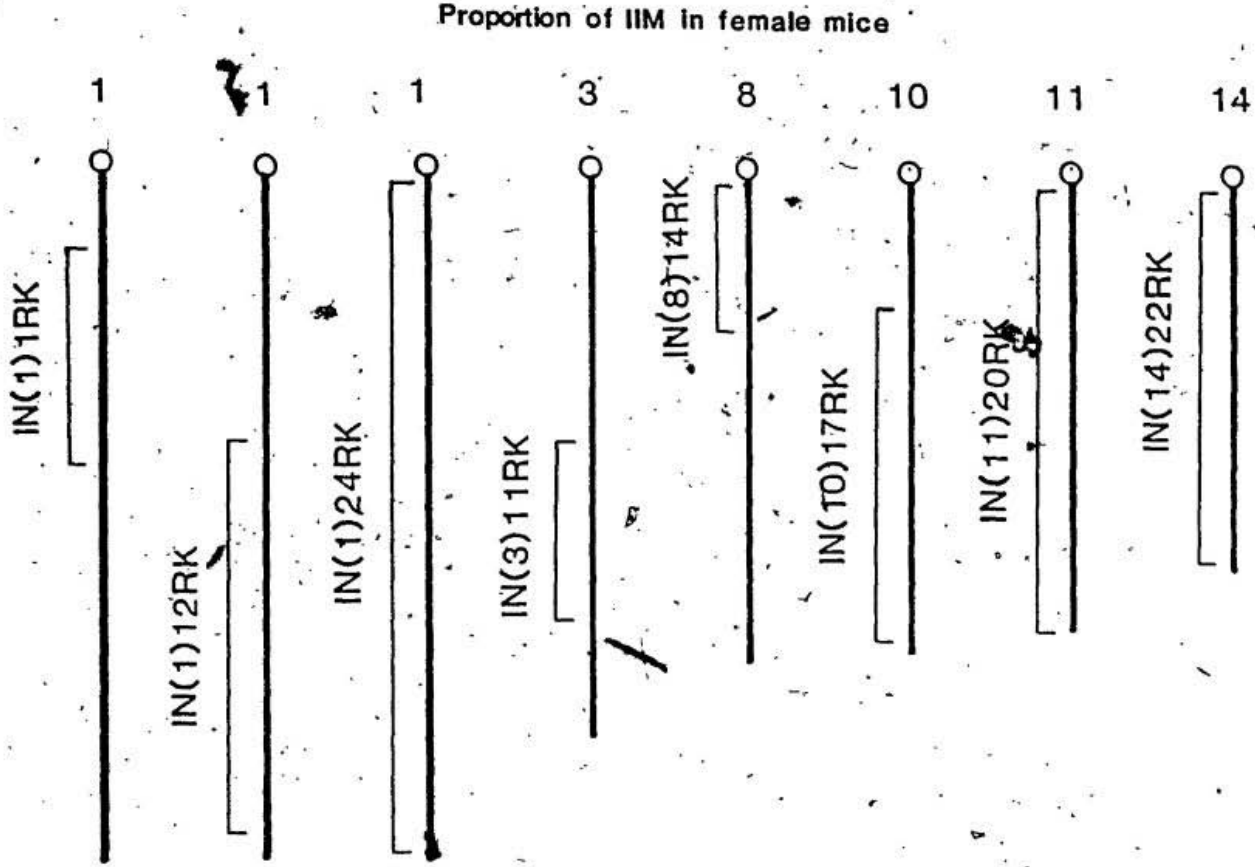
Proportion of loop and anaphase bridge



Loop	70%	50%	8%	82%	84%	21%	0%	2%
Anaphase bridge	34%	22%	73%	29%	35%	65%	47%	66%

Figure 5-3

Proportion of oocytes from homozygotes and heterozygotes for paracentric inversions in second meiosis following in vitro culture.


Proportion of IIM in female mice
1
1
1
3
8
10
11
14
IN(1)1RK
IN(1)12RK
IN(1)24RK
IN(3)11RK
IN(8)14RK
IN(10)17RK
IN(11)20RK
IN(14)22RK
Homo
41%
44%
28%
0%
8%
15%
86%
Hetero
13%-38%
18%-60%
16%-23%
1%
21%
0%
30%

5.2.1. Differences in the stages of reproduction when selection occurs in the two sexes and efficiency of selection

One can also attempt to determine if a differential risk, between sexes, results from difference in the stage of reproduction when selection operates. In the post-meiotic phase, post-implantation death is an important mode of selection against zygotes with genomic imbalance. Increased post-implantation death has been observed in male and female heterozygotes for 9RK and in female heterozygotes for 5RK. In the absence of a phenomenon similar to non-complementation (Searle & Beechey, 1985), post-implantation death ought to be apparent in pregnancies resulting from fertilization involving gametes from male as well as female carriers of paracentric inversions. Post-implantation death results in reduced litter size. In this study, the reduction in litter size is confined to the females. The possible reasons for reduction in litter size in female heterozygotes alone are discussed in section 4.6.1. One of them is a reduction in the number of oocytes available for fertilization i.e., not post-implantation death.

If, however, on the other hand post-implantation death due to genomic imbalance, as reported by Ford and Burtenshaw (Ford, Evans & Burtenshaw, 1976), is identified as the cause of reduced litter size, differences in the reproductive stages at which selection operates may be a reason for the differences in the litter size in male and female heterozygotes. Selection may be pre-gametic in males and post-implantation in females. The observed formation of heteroploid secondary spermatocytes, and the assumption that diploid (aneuploid) sperm are

excluded from fertilization (Refer section 3.5.2.12), suggests that selection prior to fertilization takes place in males heterozygous for paracentric inversions. As mentioned earlier, selection at the pre-gametic stage may be more effective than selection during the post-fertilization or post-implantation stage in paracentric inversion heterozygotes (Refer section 5.1.1). This hypothesis of differential risks in the two sexes due to differences in the stage at which selection occurs may explain the observations in human familial cases of paracentric inversions where the mother is found to be the carrier in those instances where possible recombinants have been identified (Refer section 5.4.1.).

Decreased risk for male heterozygotes compared to females, was first observed in D/G Robertsonian translocations (Hamerton, Cowie, Giannelli, Briggs & Polani, 1961, Stene, 1970). Preferential segregation and selective fertilization have been offered as explanations for this observation (Hamerton, Cowie, Giannelli, Briggs & Polani, 1961). Prezygotic selection or meiotic drive (defined as the preferential recovery of one or more classes of gametes resulting from meiosis in a heterozygote) in males heterozygous for t(DqGq) and t(DqDq) was suggested on the basis of an excess of balanced heterozygotes over chromosomally normal offspring (Hamerton, 1968). This suggestion may be questioned on the basis of recent reports on chromosomal analysis of male gametes from a male heterozygous for t(14;21), which showed a greater number of chromosomally normal than abnormal sperm (Balkan & Martin, 1983). Similar differences between sexes with regard to risk for certain types of segregation (3:1 disjunction and adjacent 2

segregation) have also been reported among carriers of some reciprocal translocations. This has been explained on the basis of non-disjunction of homologous centromeres, a phenomenon considered to occur more frequently among females than males (Stene & Stengel-Rutkowski, 1982). These observations, of decreased risk to male heterozygotes, and the suggestions put forward are compatible with those made in this thesis.

If the differences observed in litter sizes between male and female heterozygotes for the same paracentric inversion in this study represent variation in phenotypic expression, it may also be suggested that ascertainment of certain chromosomal rearrangements in humans based on effects apparent during the post-implantation stages of reproduction may be incomplete.

5.3. Low frequency of paracentric inversions in general and clinically defined populations

In contrast to other balanced rearrangements, the frequency of paracentric inversions in the general population and in clinically defined populations is low (Refer section 1.2). The frequency of any rearrangement depends on (1) Rate of mutation and ability to maintain itself in the population (2) Existence of a phenotype that leads to ascertainment for cytogenetic investigation and (3) Availability of technology to identify the rearrangement.

5.3.1. Rate of mutation

No significant difference is apparent between the proportion of *de novo* pericentric and paracentric inversions (Ref. sec. 1.3.1). Limitations in the ability to identify paracentric inversions by cytogenetic techniques, due to constraints placed on the use of high resolution techniques and alteration of banding pattern (Refer section 5.3.3), must be considered in this context. Those related to phenotype, ascertainment and reporting (Refer section 5.3.2) may also have contributed to the low rate of mutation reported.

5.3.1.1. The ability of inversions to maintain themselves in the population

Various factors may effect fixation of chromosomal rearrangements. Random genetic drift in small populations is one of them. Since inversions effectively suppress recombination around breakpoints and within the inverted regions, they may be selected by association with a favourable combination of linked alleles - another factor which affects fixation. Location of breakpoints and position effect may be advantageous or detrimental. Homozygosity for In(2)5RK is lethal and that for In(10)17RK results in small size and sterility (Roderick, 1979). These are examples of inversions in mice associated with detrimental effects. The high frequency of oocyte maturation observed in 22RK/22RK (if it is found to persist in different backgrounds) may suggest that the inversion is associated with a combination of alleles responsible for the high maturation rate of oocytes. This may be an example of a favourable effect associated with the inversion leading towards its fixation.

Meiotic drive is another phenomenon that may have an effect on fixation. The high frequency of bridges, a function of paracentric inversion heterozygosity, is observed in half of the sons and in half of the male offsprings of obligate heterozygous daughters (Roderick, 1979). The absence of deviation from 1:1 segregation is evidence for absence of meiotic drive in those inversions. Based on their own data and information available from literature, the French Collaborative Study has concluded that the ratio of segregation of normal and paracentric inversion is 1:1. This has been based on 13 inversion carriers and 8 normals in their sample and 11 inversion carriers and 11 normals reported in literature (A French Collaborative Study, 1986b).

5.3.2. The role of phenotype in ascertainment and reporting

The role of phenotype in the ascertainment of a chromosomal rearrangement and its reported frequency was introduced in section 1.10. From the observations made in this study it may be suggested that heterozygosity for paracentric inversions in males may not result in significant phenotypic abnormalities justifying cytogenetic investigations. On the other hand, the absence of an effective pre-gametic selection in female heterozygotes (compared to that in males) (Ref. sec. 5.2.1) and selection during post-fertilization stage may result in a recognisable phenotype in female heterozygotes. In female mice, reduction in litter size is the apparent effect of the heterozygosity on reproduction. As to be discussed in section 5.4.1. this may be the reason for the increased frequency of female compared to male carriers asertained and reported in human familial cases of paracentric inversions.

A bias in reporting may also influence the frequency. As paracentric inversions are balanced rearrangements, they may not be reported. This bias may disappear with increased awareness among investigators about the possible recombinants that have been reported recently following the introduction of more sophisticated cytogenetic techniques.

5.3.3. Availability of technology to identify paracentric inversions

One argument repeatedly put forward to explain the low frequency of paracentric inversions is the inability to identify them without the use of banding techniques. Even if paracentric inversions have an effect on development and/or reproduction, until lately, cytogenetic techniques essential for confirming their existence were not available. This situation has changed, and since the introduction of banding techniques in cytogenetics there is a gradual increase in the number of paracentric inversions reported each year since 1971. As mentioned earlier, (beginning of this chapter) high resolution techniques which may be useful in detecting paracentric inversons are costly and laborious. Therefore it is not feasible to use them for large scale population screening. The increased efficiency of high resolution banding techniques in identifying paracentric inversions is evident in the report on the French Collaborative Study in which the number of paracentric inversions ascertained following various indications suggesting cytogenetic investigations, were large compared to those previously reported. [Table 5-1]. Furthermore not all paracentric inversions result in recognisable alteration in banding pattern. The deficiency of intra-arm rearrangements (like

paracentric inversion) in lymphocytes following irradiation has been attributed to absence of band pattern disruption (Savage & Papworth, 1982).

5.4. Low frequency of recombinants

The frequency of recombinants of paracentric inversions is also apparently low compared to those of other rearrangements (Refer section 1.6). Dicentric chromosomes, one of the recombinants in a paracentric inversion heterozygote, and its derivatives have been reported in humans by various investigators. The first reports of chromatid bridges and fragments was that by Koller (Koller, 1037). Another observation of chromatid bridge was reported by Slifer and Beams (Slifer & Beams, 1949). In both these instances, details are not available regarding the karyotype of the individuals from whom the testicular material was obtained for histological investigations. The various recombinants that have been reported in humans are discussed in section 1.3.2.2.6. One dicentric recombinant has been reported (Mules & Stanberg, 1984) and another identified recently in the offspring of a carrier of inv(9)(q22.1q34.3) (personal communication, Daniel van Dyke 1986). A number of carriers of apparently balanced paracentric inversions, that are familial and found in individuals with developmental abnormalities have been reported (Refer section 1.4.3.1). Although genomic imbalance may exist in these instances they may not be identifiable for biological and technical reasons (Refer section 1.4.4). Further studies based on those carried out by Evans and Burgoyne (Evans & Burgoyne, 1984) (Refer section 4.6.4) may be informative in this regard.

The observation of anaphase bridges in histological sections of the testes of mice

Table 5-1: Basis of ascertainment of paracentric inversions by French Collaborative Study and those reported previously [A French Collaborative Study 1986]

Ascertainment	French study		Literature	
	n	%	n	%
Recurrent abortions	11	34	11	15
Sytematic	8	25	14	19
Malformations				
-normal karyotype	5	16	20	27
-aneusomie de recombinaison	0	0	7	9
Other aneuploidy	0	0	10	13
Stillbirth	2	6	1	1
Male sterility	3	9	2	3
Female sterility	1	3	1	1
Couple sterility	1	3	0	0
Other or unspecified	1	3	9	12

(Roderick & Hawes, 1974) clearly indicates that dicentric recombinants are formed in male mice heterozygous for paracentric inversions. Acentric fragments and dicentric chromatids have been observed in 2 - 8 cell embryos of female mice heterozygous for the paracentric inversion In(X)1H (Evans & Burgoyne, 1984). However these observations need to be confirmed by further investigations (Refer section 4.6.4).

5.4.1. Sex of human carriers of paracentric inversions and ascertainment of recombinants

One hundred and twenty one cases of paracentric inversions have been reported until 1986. Of 13 cases in the literature, in which the index case with abnormalities led to the ascertainment of familial cases of paracentric inversions, in only 4 four cases was the father found to be the carrier (Appendix G), a significant deviation from a 1:1 ratio suggesting that fewer males heterozygous for paracentric inversions are ascertained by phenotypic expression. Of seven that were ascertained following the identification of a proband with a possible recombinant in six the mother was the carrier of the rearrangement. This includes the paracentric inversion, inv(9)(q22.1q34.3), ascertained following identification of a proband with a possible recombinant, rec(9)dup(9) dicentric (personal communication, Daniel van Dyke, 1986). These observations suggest more efficient selection operating in the male compared to female carriers. The exception is the case reported by Kelly et al. (Kelly, Wyandt, Kasprzak, Ennis, Wilson, Koch & Schnatterly, 1979). Mules and Stanberg have speculated on the probability of

differential risks in male and female carriers of paracentric inversion (Mules & Stanberg, 1984).

These observations may be explained on the basis of increased probability of female functional gametes with recombinants available for fertilization compared to those of the male. This difference in the sex of the carrier ascertained may be a function of the effectiveness of selection in the two sexes, based on the different stages of reproduction when selection occurs (Ref. sec. 5.2.1).

5.5. Potential for further investigations

Many of the studies have the potential to be elaborated upon, and based on the observations, further investigations may be carried out in certain areas.

The observed correlation between lowered haploid secondary spermatocytes and increased heteroploid secondary spermatocytes suggests pre-zygotic selection in male mice heterozygous for paracentric inversion. Quantitative and qualitative analysis of sperm may provide information on the nature of selection of the secondary spermatocytes. Qualitative differences have been reported in heterozygotes for other inversions (Hugenholtz & Bruce, 1979) and the effect of such morphological changes on the function of the sperm discussed (Ford, Evans & Burtenshaw, 1976) (Refer section 3.5.2.12). Quantitative differences have not been reported for heterozygotes for paracentric inversions. A positive correlation of sperm count with haploid secondary spermatocytes frequency would imply pre-gametic selection.

Chromosome analysis of sperm following *in vitro* fertilization may help in further supporting the hypothesis that pre-gametic selection of gametes with recombinants, occurs in males heterozygous for paracentric inversions. These analyses will also help to determine if heterozygosity for paracentric inversion is associated with aneuploidy.

To determine if the unusual mode of pairing of the rearranged chromosomes suggested (Refer section 3.5.2.6) is similar to that in other rearrangements that result in male specific sterility (as discussed in section 3.5.2.11.3), contemporary techniques of in situ hybridization may be used to identify the rearranged chromosome accurately. Data from such an investigation will help to determine if the observed meiotic disturbance is an expression of male specific sterility with association of the rearranged chromosome with the XY bivalent.

It is apparent from these studies that, *in vitro* techniques to determine meiotic disturbance in females are clearly not reliable. Analysis of meiosis *in vivo* appears to be a better method. This is particularly recommended in view of the observation that 11RK/11RK females have a normal litter size, despite meiotic disturbance observed in vitro (Ref sec. 4.6.3). With regard to cytogenetic analysis, study of oocytes immediately after fertilization (analysis of female pronucleus) may be particularly useful. One advantage is that the *in vivo* environment for oocyte maturation is maintained. Another is that the extrusion of the second polar body occurs after sperm penetration. Hence the early embryo represents the

chromosomal contribution from the female carrier - after all possible pre-fertilization selection.

Statistical and technical factors may influence the extent of meiotic disturbance apparent in oocytes (Refer section 4.6.2. & 5.2). However, in view of the fact that meiosis in females is different from that in males in certain respects, it may be worthwhile to investigate pachytene in female heterozygotes and compare the findings with that in males.

Retarded ovarian growth pattern is considered as the phenotypic expression in females comparable to male specific sterility in male heterozygotes.(Refer section 4.7.3). Ovarian growth patterns in those inversion heterozygotes that show impaired fertility may help determining the nature of the meiotic disturbance observed in male heterozygotes. If the meiotic disturbance observed in males appears to be a variably expressed form of male specific sterility, studies on inversion heterozygotes may contribute to understanding the phenomenon of male specific sterility, particularly because the physical characteristics of the inversions correlate with the extent of disturbance. (Refer section 3.5.2.11.2).

Differences in the efficiency of selection in the two sexes have been suggested as a possible reason for the differences in risk between sexes, apparent during the post-fertilization stages of reproduction (Refer section 5.2.1). Cytogenetic analysis of male and female gametes will provide evidence for differences between sexes in selection against gametes carrying recombinants. Well designed experiments (the

stage of reproductive cycle, age of female mice and genetic backround are factors to be controlled) to determine post-implantation loss in male and female heterozygotes can also be of great value in determining differences in the stages of selection in the two sexes.

The analysis of early embryos are worthwhile in view of the observations by Evans and Burgoyne (Evans & Burgoyne, 1984). These investigations in embryos of male and female heterozygotes will provide evidence for differences in selection between them if they do exist. It will also explain the low frequency of recombinants.

Since the inversions are maintained in heterogeneous backgrounds, the use of littermates as controls in future investigations, seems to be important in view of the variability observed in the case of female heterozygotes, where a number of factors (genetic and environmental) may influence the meiotic and reproductive processes.

Conclusion

The physical characteristics of paracentric inversions appear to confer a variety of unique properties in the heterozygous state. Rearrangements involving only one arm of the chromosome (so there is no change in the arm ratio) are difficult to identify in mitotic metaphase chromosomes if the rearrangement does not result in a change of band pattern.

During meiosis, loop formation in the pachytene stage, which is another unique feature of inversions, appears to influence the frequency of crossover and recombinant formation in heterozygotes for paracentric inversions. Decrease in the frequency of crossover and recombinant formation may affect the probability of asceratainment of paracentric inversions.

Selection prior to fertilization also reduces the probability of ascertainment. Dicentric recombinants, when formed, appear to be effectively selected against prior to gamete formation in male heterozygotes. Selection during this stage of reproduction in paracentric inversion heterozygotes, may be more efficient than selection during the post-fertilization stage, which is a frequently identified mode of selection in heterozygotes for other rearrangements. These features may account for the low frequency of paracentric inversions and possible recombinants identified among livebirths.

Summary

In males, heterozygosity for a paracentric inversion affects spermatogenesis during meiosis. The apparent effects are:

1. There is a decrease in the frequency of haploid secondary spermatocytes in heterozygotes for large inversions.

2. There is an increase in the frequency of heteroploid secondary spermatocytes in heterozygotes for large as well as small inversions.

3. Loop formation facilitating synapsis of the inverted segment is less frequent in heterozygotes for large inversions compared to that for small inversions. A negative correlation between frequency of loop formation and anaphase bridge suggests that loop formation inhibits recombination.

4. The litter size is not affected; suggesting pre-gametic selection of abnormal recombinants.

In female heterozygotes the effects are:

1. The number of oocytes in diplotene is higher in heterozygotes for the various inversions investigated than in the respective homozygotes suggesting arrest in the pre-diplotene stage does not take place.

2. The number of oocytes in the post diplotene stage of meiosis is also higher in the heterozygotes than homozygotes suggesting meiotic arrest or delay.

3. On in vitro culturing of oocytes, the proportion of oocytes that proceeded to second meiotic metaphase is low in the case of heterozygotes for two of the three large inversions (20RK and 22RK) compared to the respective homozygotes. For one medium sized inversion (12RK) the proportion is low in one of two sets of heterozygotes.

4. The litter size is reduced in heterozygotes for one short inversion (1RK), one large inversion (22RK) and equivocal in the other (24RK).

Although paracentric inversion heterozygosity results in meiotic disturbance in males and females, the consequences of the disturbance is different in the two

sexes. In males meiotic disturbance leads to pre-gametic selection of recombinants, whereas in females the oocytes with the recombinants are reported to be functional and fertilized. Selection therefore appears to occur in the post-fertilization stage. Pre-gametic selection in male heterozygotes is so efficient that there is no opportunity for post-fertilization ascertainment.

References

A French Collaborative Study. (1986). Pericentric inversions in man. *Ann Génét, 29*, 129-168.

A French Collaborative Study. (1986). Paracentric inversions in man. *Ann Génét, 29*, 169-176.

Abramsson, L., G. Beckman, M. Duchek and I. Nordenson. (1982). Chromosomal aberrations and male infertility. *The Journal of Urology, 128*, 52-53.

Allderdice, P.W., U. Sreenivasan and B. Eales. (1980). 46,XY,inv(10)(q11q26) in a boy with normal intelligence and minor physical anomalies. *Can. J. Genet. Cytol., 22*, 655.

Ashley, T., M.J. Moses and A.J. Solari. (1981). Fine structure and behaviour of a pericentric inversion in the sand rat *Psammomys obeus*. *J. Cell Sci., 50*, 105-119.

Balkan, W. and R.H. Martin. (1983). Segregation of chromosomes into the spermatozoa of a man heterozygous for a 14;21 Robertsonian translocation. *American Journal of Medical Genetics, 16*, 169-172.

Bedford, J.M., J.C. Rodger and W.G. Breed. (1984). Why so many mammalian spermatozoa - a clue from marsupials? *Proc. R. Soc. London., B 221*, 221-233.

Blattner, W.A., M.L. Kistenmacher, S. Tsai, H.H. Punnett and E.R. Giblett. (1980). Clinical manifestations of familial 13;18 translocation. *Journal of Medical Genetics, 17*, 373-379.

Boué, J. and A. Boué. (1973). Chromosomal analysis of two consecutive abortuses in each of 43 women. *Humangenetik, 19*, 275-280.

Boué, J.G., A.Boué, P. Lazar and S. Gueguen. (1973). Outcome of pregnancies following a spontaneous abortion with chromosomal anomalies. *American Journal of Obstetrics and Gynecology, 116*, 806-812.

Boué, A. and P. Gallano. (1984). A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenatal Diagnosis, 4*, 45-67.

Brandham, P.E. (1969). Inversion heterozygosity and sub-chromatid exchange in *Agave stricta*. *Chromosoma (Berl.), 26*, 270-286.

Brandham, P.E. (1977). The meiotic behaviour of inversions in polyploid Aloineae. *Chromosoma(Berl.), 62*, 69-84.

Breg, W.R., D.A. Miller, P.W. Allderdice and O.J. Miller. (1972). Identification of translocation chromosomes by Quinacrine flouresccence. *Amer. J. Dis. Child., 123*, 561-564.

Bronson, F.H., C.P. Dagg and G.D. Snell. (1975). Reproduction. In E.L. Green (Eds.), *Biology of the Laboratory Mouse*. Dover Publications Inc., New York.

Brown, S.W. and D. Zohary. (1955). The relationship of chiasmata and crossing over in *Lilium formosanum*. *Genetics, 40*, 850-873.

Buckton, K.E., M.L. O'Riordan. S. Ratcliffe, J. Slight, M. Mitchell & S. McBeath. (1980). A G-band study of chromosomes in liveborn infants. *Ann. Hum. Genet, 43*, 227-239.

Burnham, C.R., J.T. Stout, W.H. Weinheimer, R.V. Kowles and R.L. Phillips. (1972). Chromosome pairing in maize. *Genetics, 71*, 111-126.

Cacheiro, N.L.A., L.B. Russell and M.S. Swartout. (1974). Translocations, the predominant cause of total sterility in sons of mice treated with mutagens. *Genetics, 76*, 73-91.

Calacro, P.G. (1972). The kinetochore in oocyte maturation. In Biggers, J.D. and A.W. Schuetz (Eds.), *Oogenesis*. University Park Press, Baltimore, Maryland.

Callan, H.G. and P.E. Perry. (1977). Recombination in male and female meiocytes contrasted. *Phil. Trans. R. Soc. Lond. B., 277*, 227-233.

Callen, D.F., E. Woollatt and G.R. Sutherland. (1985). Paracentric inversions in man. *Clinical Genetics, 27*, 87-92.

Canki, N. and B. Dutrillaux. (1979). Two cases of familial paracentric inversion in man associated with sex chromosome anomaly. 47,XXY.inv(5)(q21q32) and 45,X,inv(7)(q11.3q22.3). *Hum. Genet., 47*, 261-268.

Cantú, J.M., M. Díaz, D. Möller, M. Jiménez-Sáinz, L. Sandoval, G. Vaca and H. Rivera. (1985). Azoospermia and duplication 3qter as distinct consequences of a familial t(X;3) (q26;q13.2). *American Journal of Medical Genetics, 20*, 677-684.

Carson, H.I. (1946). The selective elimination of inversion dicentric chromatids during meiosis in the eggs of Sciara impatiens. *Genetics, 31*, 95-113.

Caspersson,T., L. Zech and C. Johansson. (1970). Differential binding of alkylating fluorochromes in human chromosomes. *Experimental Cell Research, 60*, 315-319.

Cattanach, B.M. (1986). Parental origin effects in mice. *J. Embryol. exp. Morph., 97 Supplement*, 137-150.

Chaganthi, R.S.K. and J. German. (1979). Human male infertility, probably genetically determined, due to defective meiosis and spermatogenic arrest. *Am J Hum Genet, 31*, 634-641.

Chandley, A.C. (1981). Male infertility and meiosis in man. In Frajese, G., C. Conti, E.S.E. Hafez and A. Fabbrini (Eds.), *Oligozoospermia: Recent progress in andrology*. Raven Press, New York.

Chandley A.C. (1982). A pachytene analysis of two male-fertile paracentric inversions in chromosome 1 of the mouse and in the male-sterile double heterozygote. *Chromosoma(Berl.), 85*, 127-135.

Chandley, A.C. (1982). Normal and abnormal meiosis in man and other mammals. In Crosignani, P.G. and B.L. Rubin (Eds.), *Genetic control of gamete production and function. Proceedings of the Serono Clinical Colloquia on Reproduction. Number 3.* Academic Press and Grune & Stratton.

Chandley, A.C. & J.M. Fletcher. (1973). Centromere staining at meiosis in man. *Humangenetik, 18*, 247-252.

Chandley, A.C., S. Christie, J. Fletcher, A. Frackiewicz and P.A. Jacobs. (1972). Translocation heterozygosity and associated subfertility in man. *Cytogenetics, 11*, 516-533.

Chandley, A.C., P. Edmond, S. Christie, L. Gowans, J. Fletcher, A. Frackiewicz and M. Newton. (1975). Cytogenetics and infertility in man I. Karyotype and seminal analysis. *Ann. Hum. Genet. Lond., 39*, 231-254.

Chandley, A.C., T.B. Hargreave and J.M. Fletcher. (1982). Translocation 21q22q in an infertile human male. *Journal of Medical Genetics, 10*, 366-369.

Chandley, A.C., N. Maclean, P. Edmond, J. Fletcher and G.S. Watson. (1976). Cytogenetics and infertility in man. II. Testicular histology and meiosis. *Ann. Hum. Genet., 40*, 165-176.

Chandley, A.C., H. Seuánez and J.M. Fletcher. (1976). Meiotic behaviour of five human reciprocal translocations. *Cytogenet. Cell Genet., 17*, 98-111.

Daniel, A. (1979). Structural differences in reciprocal translocation. Potential for a model of risk in Rep. *Hum Genet, 51*, 171-182.

Daniel, A. (1981). Structural differences in pericentric inversions. Application to a model of risk of recombinants. *Hum Genet, 56*, 321-328.

Darlington, C.D. (1938). Chromosome behaviour and structural hybridity in the Tradescantiae II. *Journal of Genetics, XXXV*, 250-280.

Das, K. (1955). Cytogenetic studies of partial sterility in X-ray irradiated barley. *Indian Jour. Genetics and Plt. Breeding, 15*, 99-111.

Davisson, M.T. and T.H. Roderick. (1973). Chromosomal banding pattern of two paracentric inversions in mice. *Cytogenet. Cell Genet., 12*, 398-403.

Davisson, M.T., P.A. Poorman, T.H. Roderick and M.J. Moses. (1981). A pericentric inversion in the mouse. *Cytogenet. Cell Genet., 30*, 70-76.

de Boer, P. and A.G. Searle. (1980). Summary and synthesis. Workshop on chromosomal aspects of male sterility in mammals. *J. Reprod. Fert., 60*, 257-265.

del Porto, G., E. d'Alessandro, C. de Matteis, R. d'Innocenzo, M. Baldi, A. Pachi and F. Cappa. (1984). Familial paracentric inversion of chromosome 15 (q15q24). *Journal of Medical Genetics, 21*, 451-453.

del Solar, C. and I.A. Uchida. (1974). Identification of chromosomal abnormalities by Quinacrine-staining technique in patients with normal karyotypes by conventional analysis. *The Journal of Pediatrics, 84*, 534-538.

Deroover, J., J.P. Fryns, J. Haegeman and H. Van Den Berghe. (1979). Paracentric inversion in the short arm of chromosome 1. *Hum. Genet., 49*, 117-121.

Dietrich, A.J.J. and R.J.P. Mulder. (1981). A light microscopic study of the development and behaviour of the synaptonemal complex in spermatocytes of the mouse: *Chromosoma (Berl.), 83*, 400-418.

Djalali, M., P.Steinbach and G. Barbi. (1984). Familial paracentric inversion inv(3)(q21q25.1) Case report and review of literature. *Ann. Génét., 27*, 41-44.

Donahue, R.P. (1972). The relation of oocyte maturation to ovulation in mammals. In Biggers, J.D. and A.W. Schuetz (Eds.), *Oogenesis*. University Park Press, Baltimore, Maryland.

Dutrillaux, B., J. Couturier, L. Sabatier, M. Muleris and M. Prieur. (1986). Inversions in evolution of man and closely related species. *Ann Génét, 29*, 195-202.

Dutrillaux, B, M. Prieur and A. Aurias. (1986). Theoretical study of inversions affecting human chromosomes. *Ann Génét, 29*, 184-188.

Edwards, R.G. (1965). Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature,* , 349-351.

Egozcue, J., C. Templado, F. Vidal, J. Navarro, F. Morer-Fargas and S. Marina.

(1983). Meiotic studies in a series of 1100 infertile and sterile males. *Hum Genet, 65,* 185-188.

Ekberg, I. (1969). Different types of sterility induced in barley by ionizing radiations and chemical mutagens. *Heredity, 63,* 257-278.

Eliasson, R. (1981). Sperm count and fertility: Facts and myths. In Frajese, G., C. Conti, E.S.E. Hafez and A. Fabbrini (Eds.), *Oligozoospermia: Recent progress in andrology.* Raven Press, New York.

Engle, E.T. and J. Rosasco. (1927). The age of the albino mouse at normal sexual maturity. *Anat. Rec., 36,* 383-388.

Evans, E.P. (1979). Cytological methods for the study of meiotic properties in mice. *Genetics, 92: Supplement,* 97s-103s.

Evans, E.P. and P.B. Burgoyne. (1984). The chromosome unbalance observed in early preimplantation embryos from mice heterozygous for the paracentric inversion of the X chromosome, In(X)1H. *Chromosomes today, 8,* 305.

Evans, E.P. and C.E. Ford. (1976). Some cytological properties of paracentric inversions in the mouse. In Jones, K and P.E. Brandham (Eds.), *Current chromosome research.* Elsvier/North-Holland Biomedical Press, Amsterdam- The Netherlands.

Evans, E.P. & R.J.S. Phillips. (1975). Inversion heterozygosity and the origin of daughters of Bpa/+ female mice. *Nature, 256,* 40-41.

Evans, E.P., G. Breckon and C.E. Ford. (1964). An air-drying method for meiotic preparations from mammalian testes. *Cytogenetics, 3,* 289-294.

Faed, M.J.W., M.A. Lamont and K. Baxby. (1982). Cytogenetic and histological

studies of testicular biopsies from subfertile men with chromosome anomaly. *Journal of Medical Genetics, 19*, 49-56.

Faed, M.J.W., Jr Robertson, M.A. Lamont, W. MacIntosh, J. Grieve, K. Baxby, G.B. James and A.M. Crowder . (1979). A cytogenetic survey of men being investigated for subfertility. *J. Reprod. Fert., 56*, 209-216.

Ferguson-Smith, M.A. and J.R.W. Yates. (1984). Maternal age specific rates for chromosome aberrations and factors influencing them: Report of a collaborative European study on 52965 amniocentesis. *Prenatal Diagnosis, 4*, 5-44.

Finley, S.C., P.R. Scarbrough, A.J. Carroll and T.L. Feng. (1985). Paracentric inversion of chromosome 13: Possible implications in repeated fetal loss. *American Journal of Human Genetics, 37*, A92.

FitzSimmons, J, R.J. Wapner and L.G. Jackson. (1983). Repeated pregnancy loss. *American Journal of Medical Genetics, 16*, 7-13.

Fogwill, M. (1958). Differences in crossing over and chromosome size in the sex cells of Lilium and Fritillaria. *Chromosoma (Berl.), 9*, 493-504.

Ford, C.E. (1975). The time in development which gross genome unbalance is expressed. In Balls, M & A.E. Wild (Eds.), *The Early development of mammals*. Cambridge University Press.

Ford, C.E., E.P. Evans and M.D. Burtenshaw. (1976). Meiosis and fertility in mice heterozygous for paracentric inversions. In Jones, K. and P.E. Brandham (Eds.), *Current chromosome research*. Elsevier/North-Holland Biomedical Press, Amsterdam-The Netherlands.

Ford, C.E., A.G. Searle, E.P. Evans and B. Jean West. (1969). Differential transmission of translocations induced in spermatogonia of mice by irradiation. *Cytogenetics, 8,* 447-470.

Forejt, J. (1974). Nonrandom association between a specific autosome and the X chromosome in meiosis of the male mouse: possible consequence of the homologous centromeres' separation. *Cytogenet. Cell Genet., 13,* 369-383.

Forejt, J. (1976). Spermatogenic failure of translocation heterozygotes affected by H-2 linked gene in mouse. *Nature, 260,* 143-145.

Forejt, J. (1981). Hybrid sterility gene located in the *T/H-2* supergene on chromosome 17. In Reisfeld, R.A. & Ferrone, S. (Eds.), *Current trends in Histocompatibility. Immunogenetic and molecular profiles.* Plenum Press. New York and London.

Forejt, J. (1982). X-Y involvement in male sterility caused by autosome translocations - a hypothesis. In Crosignani, P.G. and B.L. Rubin (Eds.), *Genetic control of gamete production and function. Proceedings of the Serono Clinical Colloquia on Reproduction. Number 3.* Academic Press and Grune & Stratton.

Forejt, J. (1984). X-inactivation and its role in male sterility. *Chromosomes today, 8,* 117-127.

Fox, D.P. (1973). The control of chiasma distribution in the Locust, *Schistocerca gregaria*(Forskal). *Chromosoma (Berl.), 43,* 280-328.

Fryns, J.P. and H. Van den Berg. (1980). Paracentric inversion in man: Personal experience and review of the literature. *Hum Genet, 54,* 413-416.

Fryns, J.P., A. Kleckowska and H. Van den Berghe. (1986). Paracentric inversions in man. *Hum Genet, 73*, 205-213.

Fryns, J.P., A. Kleckowska, E. Kubién, P.Petit and H. Van den Berghe. (1984). Cytogenetic survey in couples with recurrent fetal wastage. *Hum Genet, 65*, 336-354.

Fryns, J.P., A. Kleczkowska, E. Kubién and H. Van den Berghe. (1984). Cytogenetic findings in moderate and severe mental retardation. A study of an institutionalized population of 1091 patients. *Acta Paediatrica Scandinavica, Supplement 313*, 1-23.

Funderburk, S.J., M.A. Spence and R.S. Sparkes. (1977). Mental retardation associated with "balanced" chromosome rearrangement. *Am. J. Hum. Genet., 29*, 136-141.

Giraldo, A., E. Silva, I. Martínez, C. Campos and J. Guzmán. (1981). Pericentric inversion of chromosome 1 in three sterile brothers. *Hum Genet, 58*, 226-227.

Gonzales, J., S. Lesourd and B. Dutrillaux. (1981). Mitotic and meiotic analysis of a reciprocal translocation t(Y;3) in an azoospermic male. *Hum Genet, 57*, 111-114.

Greenbaum, I.F. and M.J Reed. (1984). Evidence for heterosynaptic pairing of the inverted segment in pericentric inversion heterozygotes of the deer mouse (*Peromyscus maniculatus*. *Cytogenet. Cell Genet., 38*, 106-111.

Grell. R.F. (1962). A new hypothesis on the nature and sequence of meiotic events in the female of *Drosphila melanogaster. Proc.Nat. Acad. Sci., 48*, 165-172.

Grell, E.H. (1970). Distributive pairing: Mechanism for segregation of compound autosomal chromosomes in oocytes of *Drosophila melanogaster*. *Genetics*, *65*, 65-74.

Grell, R.F. and J.I. Valencia. (1964). Distributive pairing and aneuploidy in man. *Science*, *145*, 66-67.

Gropp, A. and H. Winking. (1981). Fertility vs. sterility in males with double Robertsonian heterozygosity and monobrachial homology. *Mouse News Letter*, *65*, 33.

Gropp, A., H. Winking and C. Redi. (1982). Consequences of Robertsonian heterozygosity: Segregational impairment of fertility versus male-limited sterility. In Crosignani, P.G. and B.L. Rubin (Eds.), *Genetic control of gamete production and function. Proceedings of the Serono Clinical Colloquia on Reproduction. Number 3.* Academic Press and Grune & Stratton.

Hamerton, J.L. (1968). Robertsonian translocations in man: evidence for prezygotic selection. *Cytogenetics*, 7, 260-276.

Hamerton, J.L., V.A. Cowie, F. Giannelli, S.M. Briggs, and P.E. Polani. (1961). Differential transmission of Down's syndrome (mongolism) through male and female translocation carriers. *Lancet*, *ii*, 956-957.

Hartley, S.E. & H.G. Callan. (1977). Inversion heterozygosity in females of the newt *Notophthalmus viridescens* and its influence on chiasma distribution. *J. Cell Sci.*, *24*, 131-141.

Henderson, S.A. and R.G. Edwards. (1968). Chiasma frequency and maternal age in mammals. *Nature*, *218*, 22-28.

Hendry, W.P.; P.E. Polani, R.C.B. Pugh, I.F. Sommerville and D.M. Wallace. (1976). 200 infertile males: Correlation of chromosome, histological, endocrine and clinical studies. *British Journal of Urology, 47*, 899-908.

Herr, H.M., S.J. Horton and C.I. Scott Jr. (1985). De novo paracentric inversion in an X chromosome. *Journal of Medical Genetics, 22*, 140-142.

Hoo, J.J., R. Lorenz, A. Fischer and W. Fuhrmann. (1982). Tiny interstitial duplication of proximal 7q in association with a maternal paracentric inversion. *Hum Genet, 62*, 113-116.

Hook, E.B., D.M. Schreinemachers, A.M. Wiley and P.K. Cross. (1984). Inherited cytogenetic abnormalities detected incidentally in fetuses diagnosed prenatally: Frequency, parental-age associations, sex-ratio trends and comparisons with rates of mutants. *Am J Hum Genet, 36*, 422-443.

Hotta, Y. and A.C. Chandley. (1982). Activities of X-linked enzymes in spermatocytes of mice rendered sterile by chromosomal alterations. *Gamete Research, 6*, 65-72.

Hotta, Y., A.C. Chandley, H. Stern, A.G. Searle and C.V. Beechey. (1979). A disruption of pachytene DNA metabolism in male mice with chromosomally-derived sterility. *Chromosoma (Berl.), 73*, 287-300.

Hugenholtz, A.P. and W.R. Bruce. (1979). Sperm size abnormalities in homozygous and heterozygous *In(5)9RK* mice. *Can. J. Genet. Cytol., 21*, 115-119.

Hultén, M. (1974). Chiasma distribution in diakinesis in the normal human male. *Hereditas, 76*, 55-78.

Jacobs, P.A. (1974). Correlation between euploid structural chromosome rearrangements and mental subnormality in humans. *Nature, 249*, 164-165.

Jacobs, P.A. (1981). Mutation rates of structural chromosome rearrangements in man. *Am. J. Hum. Genet., 33*, 44-54.

Jaeken, J., J.P. Fryns, L. Standaert, P. de Cock and H. Van Den Berghe. (1980). De novo paracentric inversion in a microcephalic boy : 46,XY, inv(14)(q13q24). *Ann. Génét., 23*, 105-107.

Johnson, D.D., W.B. Dobyns, G.W. Dewald and H. Gordon. (1985). Family studies of pericentric and paracentric inversions. *American Journal Of Human Genetics, 37*, A100.

Kaelbing, M. and N.S. Fechheimer. (1985). Synaptonemal complex analysis of a pericentric inversion in chromosome 2 of domestic fowl, *Gallus domesticus*. *Cytogenet. Cell Genet., 39*, 82-86.

Kaiser, P. (1984). Pericentric inversions. Problems and significance for clinical genetics. *Hum Genet, 68*, 1-47.

Kajii, T. and A. Ferrier. (1978). Cytogenetics of aborters and abortuses. *Am. J. Obstet. Gynecol., 131*, 33-38.

Kelly, T.E., H. Wyandt, R. Kasprzak, J. Ennis, K. Wilson, V. Koch and Schnatterly. (1979). Paracentric inversion: Probable mechanism for an interstitial 3p deletion in a patient with multiple anomalies. *Am. J. Hum. Genet., 31*, 100A.

Kitzmiller, J.B. (1976). Genetics, cytogenetics and evolution of mosquitoes. *Advances in Genetics, 18*, 315-433.

Koller, P.C. (1937). The genetical and mechanical properties of sex chromosomes. III. Man. . *Proc. R. Soc. Edinburgh, 57*, 194-214.

Komai, T., and T. Takaku. (1942). On the effect of the X-chromosome inversion on crossing over in *Drosophila virilis*. *Cytologia, 12*, 357-365.

Koulischer, L., R. Schoysman, Y. Gillerot and J.M. Debry. (1982). Meiotic chromosome studies in human male infertility. In Crosignani, P.G. and B.L. Rubin (Eds.), *Genetic control of gamete production and function. Proceedings of the Serono Clinical Colloquia on Reproduction. Number 3.* Academic Press and Grune & Stratton.

Kuleshov, N.P. (1976). Chromosome anomalies of infants dying during the perinatal period and premature newborn. *Hum Genet, 31*, 151-160.

Léonard,C., J.P. Bisson & G. David. (1979). Male sterility associated with familial heterozygosity: t(8;15)(q22;p11). *Archives of Andrology, 2*, 269-275.

Laurent, C., M.-Cl. Biemont, M. Cognat and B. Dutrillaux. (1977). Studies of the meiotic behaviour of a translocation t(10;13)(q25;q11) in an oligospermic man. *Hum Genet, 39*, 123-126.

Laurent, C., A.C. Chandley, B. Dutrillaux and R.M. Speed. (1982). The use of surface spreading in the pachytene analysis of a human t(Y;17) reciprocal translocation. *Cytogenet. Cell Genet., 33*, 312-318.

Lifschytz, E. and D.L. Lindsley. (1972). The role of X-chromosome inactivation during spermatogenesis. *Proc. Nat. Acad. Sci. USA, 69*, 182-186.

Lin, C.C., M.M. Gedeon, P. Griffith, W.K. Smink, D.R. Newton, L. Wilkie and L.M. Sewell. (1976). Chromosome analysis on 930 consecutive newborn

children using Quinacrine flourescent banding technique. *Hum. Genet, 31,* 315-328.

Lindenbaum, R.H., M. Hultén, A. McDermott and M. Seabright. (1985). The prevalence of translocations in parents of children with regular trisomy 21: a possible interchromosomal effect? *Journal of Medical Genetics, 22,* 24-28.

Lippman-Hand,A. and M. Vekemans. (1983). Balanced translocations among couples with two or more spontaneous abortions: Are males and females equally likely to be carriers? *Hum Genet, 63,* 252-257.

Lyon, M.F. and R. Meredith. (1966). Autosomal translocations causing male sterility and viable aneuploidy in the mouse. *Cytogenetics, 5,* 335-354.

Micic, M. and S. Micic . (1984). Meiotic studies in two infertile males with autosomal translocations. *Hum Genet, 65,* 308-310.

Micic, M.D. and S.R. Micic. (1981). Meiotic findings in human reciprocal 1;3 translocation. *Hum Genet, 57,* 442-443.

Machin, G.A. and J.A. Crolla. (1974). Chromosome constitution of 500 infants dying during the perinatal period. *Humangenetik, 23,* 183-198.

Madan, K., M. Seabright, R.H. Lindenbaum and M. Bobrows. (1984). Paracentric inversions in m[illegible] *Journal of Medical Genetics, 21,* 407-412.

Maguire, M.P. (1977). Homologous chromosome pairing. *Phil. Trans. R. Soc. Lond. B., 277,* 245-258.

Mahadeviah, S., U.Mittwoch and M.J.Moses. (1984). Pachytene chromosomes in male and female mice heterozygous for the Is(7;1)40H insertion. *Chromosoma(Berl.), 90,* 163-166.

Marmor, D., J. Taillemite, J. van den Akker, M. Portnoi, N. Joyce, D. Delafontaine and C. Roux. (1980). Semen analysis in subfertile balanced-translocation carriers. *Fertility and Sterility, 34*, 496-502.

Masui, Y. and H.J. Clarke. (1979). Oocyte Maturation. *International Review of Cytology, 57*, 185-282.

Maudlin, I. and E.P. Evans. (1980). Chiasma distribution in mouse oocytes during diakinesis. *Chromosoma (Berl.), 80*, 49-56.

McClintock, B. (1933). The association of non-homologous parts in chromosomes in the mid-prophase of meiosis in Zea mays. *Zeitschrift fur Zellforschung and mikroskopische anatomie, 19*, 191-237.

McIlree, M.E., W.H. Price, W.M.C. Brown, W.S. Tulloch, J.E. Newsam and N. Maclean. (1966). Chromosome studies on testicular cells from 50 subfertile men. *The Lancet, 2*, 69-71.

Menkveld, R., J.A. van Zyl and A.E. Retief. (1983). A possible increase in the number of metaphase I spermatocytes in testicular biopsy of infertile men with chromosomal aberrations. *Andrologia, 15*, 233-235.

Mittwoch, U., S. Mahadevaiah and M.B. Olive. (1981). Retardation of ovarian growth in male-sterile mice carrying an autosomal translocation. *Journal of Medical Genetics, 18*, 414-417.

Mittwoch, U., S. Mahadevaiah and L.A. Setterfield. (1984). Chromosomal anomalies that cause male sterility in the mouse also reduce ovary size. *Genet. Res. Camb., 44*, 219-224.

Moreau, N. and M. Teyssier. (1984). Whole chromosome translocation t(1;3) in an infertile man. *Journal of Medical Genetics, 21*, 234-235.

Morgan, D.H. (1950). A cytogenetic study of inversions in *Zea mays*. *Genetics, 35*, 153-174.

Morton, N.E., P.A. Jacobs, A. Frackiewicz, P. Law and C.J. Hilditch. (1975). The effect of structural aberrations of the chromosomes on reproductive fitness in man. I. Methodology. *Clinical Genetics, 8*, 159-168.

Moses, M.J. (1977). Synaptonemal complex karyotyping in spermatocytes of the chinese hamster (Cricetulus griseus) I. Morphology of the autosomal complement in spread preparations. *Chromosoma(Berl), 60*, 99-125.

Moses, M.J. (1980). New cytogenetic studies on mammalian meiosis. In Serio, M. and L. Martini (Eds.), *Animal model in Human reproduction*. Raven Press, New York.

Moses, M.J. and P.A. Poorman. (1984). Synapsis, synaptic adjustment and DNA synthesis in mouse oocytes. *Chromosomes today, 8*, 99-103.

Moses, M.J., P.A. Poorman, T.H. Roderick and M.T. Davisson. (1982). Synaptonemal complex analysis of mouse chromosomal rearrangement IV. Synapsis and synaptic adjustment in two paracentric inversions. *Chromosoma(Berl.), 84*, 457-474.

Mules, E.H. and J. Stanberg. (1984). Reproductive outcome of paracentric inversion carriers: Report of a liveborn dicentric recombinant and literature review. *Hum Genet, 67*, 126-131.

Muller, H.J. (1952). Bearings of the "*Drosophila*" work on systematics. In Julian Huxley (Eds.), *The New Systematics*. Oxford University Press.

Navarro, J., F. Vidal, M. Guitart and J. Egozcue. (1981). A method for the

sequential study of synaptonemal complexes by light and electron microscopy. *Hum Genet, 59*, 419-421.

Neri, G., A. Serra, M. Campana and B. Tedeschi. (1983). Reproductive risks for translocation carriers: Cytogenetic study and analysis of pregnancy outcome in 58 families. *Am J Hum Genet, 16*, 535-561.

Nielsen, J. and B. Krag-Olsen. (1981). Follow-up of 32 children with autosomal translocations found among 11,148 consecutively newborn children from 1969 to 1974. *Clinical Genetics, 20*, 48-54.

Nur, U. (1968). Synapsis and crossing over within a paracentric inversion in the grasshopper *Camnula pellucida. Chromosoma (Berl.), 25*, 198-214.

Orye, E. and H. Van Bever. (1983). Paracentric inversions: two new familial cases, inv (7)(q22q11) and inv (11)(q23q13). *Journal of Medical Genetics, 20*, 231-232.

Pantzar, J.T., J.E. Allanson, D.K. Kalousek and B.J. Poland. (1984). Cytogenetic findings in 318 couples with repeated spontaneous abortion: A review of experience in British Columbia. *American Journal of Medical Genetics, 17*, 615-620.

Peters-Slough, M.F., H.T. Planteydt, M.J. Timmerman and M.J.V.D. Vooren. (1982). A familial paracentric inversion in the short arm of chromosome 3: a case report. *Clinical Genetics, 22*, 102-104.

Plymate, S.R., M.J. Bremner and C.A. Paulsen. (1976). The association of D-group chromosomal translocations and defective spermatogenesis. *Fertility and Sterility, 27*, 130-144.

Polani, P.E. (1972). Centromere localization at meiosis and the position of chiasmata in the male and female mouse. *Chromosoma (Berl.), 96*, 343-374.

Polani, P.E., J.A. Crolla, M.J. Seller and F. Moir. (1979). Meiotic crossing over exchange in the female mouse visualised by BUdR substitution. *Nature, 278*, 348-349.

Retief, A.E., J.A. Van Zyl, R. Menkveld, M.F. Fox, G.M. Kotzèand J. Brusnicky. (1984). Chromosome studies in 496 infertile males with a sperm count below 10 million/ml. *Hum Genet, 66*, 162-164.

Rhoades, M.M. and E. Dempsey. (1953). Cytogenetic studies of deficient-duplication chromosomes derived from inversion heterozygotes in maize. *Amer. Jour. Bot., 40*, 405-424.

Riccardi, V.M. and G.P. Holmquist. (1979). De novo 13q paracentric inversion in a boy with cleft palate and mental retardation. *Hum. Genet., 52*, 211-215.

Ridler, M.A.C., S.D. Sutton. (1981). A case of a paracentric inversion inv(7) (q11q22). Prenatal detection and counselling. *Prenatal Diagnosis, 1*, 81-84.

Rivera, H., M.C. Alvarez-Arratia, M. Moller, M. Díaz and J.M. Cantú. (1984). Familial inv(1)(p3500q2.3) associated with azoospermia. *Hum Genet, 66*, 165-167.

Roderick, T.H. (1971). Producing and detecting paracentric chromosomal inversions in mice. *Mutation Reasearch, 11*, 59-69.

Roderick, T.H. (1979). Chromosomal inversion in studies of mammalian mutagenesis. *Genetics, 92 Supplement*, s121-s126.

Roderick, T.H. (1983). Using inversions to detect and study recessive lethals and

detrimentals in mice . In de Serres, F.J. and W. Sheridan (Eds.), *Utilization of mammalian specific locus in hazard evaluation and estimation of genetic risk.* Plenum Publishing Corporation.

Roderick, T.H. and N.L. Hawes. (1974). Nineteen paracentric chromosomal inversions in mice. *Genetics, 76,* 109-117.

Rodriguez, M.T., M.J. Martin and J.A. Abrisqueta. (1985). A complex balanced rearrangement involving four chromosomes in an azoospermic man. *Journal of Medical Genetics, 22,* 66-67.

Román, C.S., M. T. Sordo and J.M. García-Sagredo. (1979). Meiosis in two human reciprocal translocations. *Journal of Medical Genetics, 16,* 56-59.

Roosen-Runge, E.C. (1962). The process of spermatogenesis in mammals. *Biol. Rev., 37,* 343-377.

Rothfels, K. and R. Nambiar. (1975). The origin of meiotic bridges by chiasma formation in heterozygous inversions in *Prosimulium multidentatum* (Diptera:Simuliidae). *Chromosoma (Berl.), 52,* 283-292.

Savage, J.R.K. and D.G. Papworth. (1982). Frequency and distribution studies of asymmetrical versus symmetrical chromosome aberrations. *Mutation Research, 95,* 7-18.

Schmid, M., T. Haaf and M. Zorn. (1986). Paracentric inversions in human chromosome 7. *Hum Genet, 74,* 197-199.

Schulz-Schaeffer, J. (1980). In (Eds.), *Cytogenetics. Plants, Animals, Humans.* Springer-Verlag, New York, Heidelberg, Berlin.

Searle, A.G. (1974). Nature and consequences of induced chromosome damage in mammals. *Genetics, 78,* 173-186.

Searle, A.G. (1981). Chromosomal variants. In Green, M.C. (Eds.), *Genetic variants and strains of the laboratory mouse.* Gustav Fischer Verlag Stuttgart New York.

Searle, A.G. (1982). The genetics of sterility in the mouse. In Crosignani, P.G. and B.L. Rubin (Eds.), *Genetic control of gamete production and function. Proceedings of the Serono Clinical Colloquia on Reproduction. Number 9.* Academic Press and Grune & Stratton.

Searle, A.G. and C.V. Beechey. (1985). Noncomplementation phenomena and their bearing on non disjunctional effects. In Dellarco, V.L., P.E. Voytek ans A. Hollaender (Eds.), *Aneuploidy. Etiology and Mechanisms.* Basic Life Sciences.

Searle, A.G., C.V. Beechey & E.P. Evans. (1978). Meiotic effects in chromosomally derived male sterility of mice. *Ann. Biol. anim. Bioch. Biophys., 18,* 391-398.

Searle, A.G., C.E. Ford and C.V. Beechey. (1971). Meiotic disjunction in mouse translocations and the determination of centromere position. *Genet. Res. Camb., 18,* 215-235.

Shabtai, F., U. Sandowski, R. Nissimov, D. Klar and I. Halbrecht. (1985). Familial syndrome with some features of the Langer-Giedion syndrome, and paracentric inversion of chromosome 8, inv 8 (q11.23-q21.1). *Clinical Genetics, 27,* 600-605.

Simonsen, O. (1973). Cyto-genetic investigations in diploid and autotetraploid populations of *Lolium perenne* L. . *Hereditas, 75,* 157-188.

Simonsen, O. (1975). Cytogenetic investigations in diploid and autotetroploid populations of *Festuca pratenis*. *Hereditas, 79*, 73-108.

Singh, R.P. (1981). Klinefelter's syndrome with a 47,XXY; inv (12) (q15q24) karyotype. *Clinical Genetics, 19*, 188-190.

Slifer, E.H. and H.W. Beams. (1949). Chromatid bridges and fragments in human spermatocytes. *American Journal of Human Genetics, 1*, 79-82.

Smith, S.G. (1935). Chromosome fragmentation produced by crossing over in *Trillium erectum L.*. *Journal of Genetics, XXX*, 227-232.

Sparkes, R.S., H. Muller and I. Klisak. (1979). Retinoblastoma with 13q- chromosomal deletion associated with maternal paracentric inversion of 13q. *Science, 203*, 1027-1029.

Speed, R.M. (1977). The effects of ageing on the meiotic chromosomes of male and female mice. *Chromosome (Berl.), 64*, 241-254.

Speevak, M., A.G.W:, Hunter, H. Hughes & D.M. Cox. (1985). A familial paracentric inversion inv (1) (q42q44) resulting in a child with a del (1)(q42) karyotype. *Ann Génét., 28*, 177-180.

Stebbins, G.L. and S. Ellerton. (1939). Structural hybridity in *Paeonia californica* and *P. Brownii*. *Journal of Genetics, XXXVIII*, 1-36.

Stein, Z., M. Susser, D. Warburton, J. Wittes and J. Kline. (1975). Spontaneous abortions as a screening device. The effect of fetal survival on the incidence of birth defects. *American Journal of Epidemiology, 102*, 275-200.

Stene, J. (1970). Statistical inference on segregation ratios for D/G-translocations, when the families are ascertained in different ways. *Ann. Hum. Genet, Lond., 34*, 93-115.

Stene, J. and S. Stengel-Rutkowski. (1982). Genetic risks for familial reciprocal translocations with special emphasis on those leading to 9p, 10p and 12p trisomies. *Ann. Hum. Genet., 46,* 41-74.

Stetten, G. and J.A. Rock. (1983). A paracentric chromosomal inversion associated with repeated early pregnancy wastage. *Fertility and sterility, 40,* 124-126.

Sturtevant, A.H. (1919). Inherited linkage variations in the second chromosome. *Carnegie Inst. Wash. Pub., 278,* 305-341.

Sturtevant, A.H. (1931). Known and probable inverted sections of the autosomes of *Drosophila melanogaster. Carnegie Inst. Wash. Pub., 421,* 1-27.

Sturtevant, A.H. and G.W. Beadle. (1936). The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. *Genetics, 21,* 554-604.

Sung, W.K., M. Komatsu and G. Jagiello. (1983). A method for obtaining synaptonemal complexes of human pachytene oocytes. *Caryologia, 36,* 315-324.

Sutherland, G.R., R.F. Carter, R. Bauld, I.I. Smith and A.D. Bain. (1978). Chromosome studies at the pediatric necropsy. *Ann. Hum. Genet., 42,* 173-181.

Swanson, C.P., T. Merz and W.J. Young. (1981). In (Eds.), *Cytogenetics. The chromosome in division, inheritance and evolution.* Prentice-Hall, Inc., New Jersey.

Tóth, A., M. Gaál, G. Sára and J. László. (1982). Pericentric inversion of

chromosome 1 in an azoospermic man. *Journal of Medical Genetics, 19,* 303-305.

Tarkowski. (1966). An air-drying method for chromosome prepararations from mouse eggs. *Cytogenetics, 5,* 394-400.

Tease, C. (1978). Cytological detection of crossing-over in BUdR substituted meiotic chromosomes using the flourescent plus Giemsa technique. *Nature, 272,* 253-254.

Tease, C. and G. Fisher. (1986). Further examination of the production line hypothesis in mouse foetal oocytes. I. Inversion heterozygotes. *Chromosoma (Berl), 93,* 447-452.

Tease, C. and G.H. Jones. (1978). Analysis of exchanges in differentially stained meiotic chromosomes of *Locusta migratoria* after BrdU-substitution and FPG staining. *Chromosoma (Berl.), 69,* 163-178.

Tharapel, A.T., R.L. Summitt, R.S. Wilroy and P. Martens. (1977). Apparently balanced *de novo* translocations in patients with abnormal phenotypes: Report of 6 cases. *Clinical Genetics, 11,* 255-269.

Therman, E. (1986). In (Eds.), *Human chromosomes. Structure Behaviour Effects.* Springer Verlag.

Tiepolo, L., O. Zuffardi, M. Fraccaro and A. Giarola. (1981). Chromosome abnormalities and male infertility. In Frajese, G., C. Conti, E.S.E. Hafez and A. Fabbrini (Eds.), *Oligozoospermia: Recent progress in andrology.* Raven Press, New York.

Tschuida, W.S. and I.A. Uchida. (1974). Chromosome aberrations in

spermatocytes and oöcytes of mice irradiated prenatally. *Mutation Research, 22*, 277-280.

Valárcel, E., J. Benítez, P. Martínez, J.A. Rey and A. Sánchez Cascos. (1983). Cytogenetic recombinants from a female carrying a paracentric inversion of a short arm of chromosome number 5. *Hum Genet, 63*, 78-81.

Van Dyke, D.L., L. Weiss, J.R. Roberson and V.R. Babu. (1983). The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. *Am J Hum Genet, 35*, 301-308.

van Zyl, J.A., R. Menkveld, T.J. van Kotzé, A.E. Retief and W.A. van Niekerk (1975). Oligozoospermia: A seven-year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate. *Int J Fertil, 20*, 129-132.

Ved Brat, S. (1966). Genetic systems in *Allium* II. Sex differences in meiosis. In Darlington, C.D. and K.R. Lewis (Eds.), *Chromosomes Today*. Plenum Press.

Venter, P.A., B. Dawson, J.L. Du Toit, E.L. Smith, N. Kritzinger, A.S. Landman, A.S. Cronje and J.O. Hof. (1981). A familial paracentric inversion: A short review of the current status. *Hum Genet, 67*, 121-125.

Vidal, F., C. Templado, J. Navarro, S. Brusadin, S. Marina and J. Egozcue. (1982). Meiotic and synaptonemal complex studies in 45 subfertile males. *Hum Genet, 60*, 301-304.

Vidal, F., C. Templado, J. Navarro, S. Marina and J. Egozcue. (1982). Meiotic

and synaptonemal complex studies in a 14/21 translocation carrier. *International Journal of Andrology, 5*, 21-26.

Viguié, F., F. Romani and J.P. Dadoune. (1982). Male infertility in a case of (Y;6) balanced reciprocal translocation. Mitotic and meiotic study. *Hum Genet, 62*, 225-227.

Warburton, D. (1982). *De novo* structural rearrangements: implications for prenatal diagnosis. In Wiley, A.M., T.P. Carter, S. Kelly and I.H. Porter (Eds.), *Clinical Genetics: Problems in diagnosis and counselling.* Academic Press.

Warburton, D. (1984). Outcome of cases of *de novo* structural rearrangements diagnosed at amniocentesis. *Prenatal Diagnosis, 4*, 69-80.

Warburton, D. and F.C. Fraser. (1964). Spontaneous abortion risks in man: Data from reproductive histories collected in a Medical Genetics Unit. *Human Genetics, 16*, 1-25.

Warburton, D., V. Grady and G. Jagiello. (1981). The relationship of parental chromosome anomalies to fetal karyotype in spontaneous abortions. *Birth Defects Original Article Series, XVII*, 72-73.

Watson, I.D. and H.G. Callan. (1963). The form of bivalent chromosomes in newt oocytes at first metaphase of meiosis. *Quart. J. micr. Sci.,, 104*, 281-295.

Watt, J.L. and D.A. Couzin. (1984). A case of interchromosomal effect? *Journal of Medical Genetics, 21*, 375-376.

Westergaard, M. and D. von Wettstein. (1972). The synaptinemal complex. *Annual Review of Genetics, 6*, 71-110.

White, M.J.D. (1973). In (Eds.), *Animal cytology and evolution*. Cambridge University Press.

Winking, H. (1980). Cytogenetic and histological observations in sterile males with Robertsonian translocations. *Cytogenetics and Cell Genetics, 27*, 213.

Winsor, E.J.T., C.G. Palmer, P.M. Ellis, J.L.P. Hunter and M.A. Ferguson-Smith. (1978). Meiotic analysis of a pericentric inversion, inv(7) (p22q32), in the father of a child with a duplication-deletion of chromosome 7. *Cytogenet. Cell Genet, 20*, 169-184.

Yamada, K., S. Nanko, S. Hattori and K. Isurugi. (1982). Cytogenetic studies in a Y-to-X translocation observed in three members of one family, with evidence of infertility in male carriers. *Hum Genet, 60*, 85-90.

Yang-Feng, T.L., S.C. Finley, W.H. Finley and U. Franke. (1985). High resolution cytogenetic evaluation of couples with recurring fetal wastage. *Hum Genet, 69*, 246-249.

Yunis, J.J. (1976). High resolution of human chromosomes. *Science, 191*, 1268-1270.

Appendix A

Paracentric inversions in subfertile men.

Inv(7) (q22q34) Sperm count of 43 million/ml. [Faed, M.J.W. et al., 1979]
Inv(11) (q21q23) Azoospermia and increased gonadotrophins. [Madan, K. et al., 1984]

Appendix B

Paracentric inversions identified due to fetal wastage

Inv(7) (p15p22) [Stetten, G. et al., 1983]
Inv(13) (q14q22) [Finley, S.C. et al., 1985]
Inv(12)(q12q24) [Fryns, J.P. et al., 1980]
Inv(5)(q13.2q2100) [French Collaborative Study 1986]
Inv(5)(q13.2q2100) [French Collaborative Study 1986]
Inv(5)(q1200q3400) [French Collaborative Study 1986]
Inv(8)(q1200q2300) [French Collaborative Study 1986]
Inv(11)(q1200q2200) [French Collaborative Study 1986]
Inv(11)(q1200q2200) [French Collaborative Study 1986]
Inv(13)(q1200(qter) [French Collaborative Study 1986]
Inv(14)(q13.2q24.2) [French Collaborative Study 1986]
Inv(14)(q1200q1309) [French Collaborative Study 1986]
Inv(13)(q13.1q22.3) [Yang-Feng, T.L., et al. 1985]

Appendix C

Familial paracentric inversions in which fetal wastage has been reported.

Inv(5) (pterp13) [Valearcel, E. et al., 1983]
Inv(1) (q42q44) [Speevak, M. et al., 1985]
Inv(11) (q21q23) [Madan, K. et al., 1984]
Inv(5) (p13p15) [Madan, K. et al., 1984]
Inv(7) (q11q22) [Ridler, M.A.C. et al., 1981]
Inv(15) (q15q24) [del Porto, G. et al., 1984]
Inv(10) (q11q26) (Venter, P.A. et al., 1984]
Inv(10) (p15p22) [Stetten, G. et al., 1983]
Inv(3) (p13p25) [Peters-Slough, M.F. et al., 1982]
Inv(3) (q21q25.1) [Djalali, M. et al., 1984]
Inv(12) (q12q24), mosaic for inversion. [Fryns, J.P. et al., 1984]
Inv(3) (p13p25) [Fryns, J.P. et al., 1984]
Inv(2) (q13q24), mosaic for invefsion. [Fryns, J.P. et al., 1984]

Appendix D

Familial paracentric inversions in which abnormal recombinants have been reported.

Inv(5) (pterp13) [Valcarcel, E. et al., 1983]
Inv(1) (q42q44) [Speevak, M. et al., 1985]
Inv(7) (q11q22) [Hoo, J.J. et al., 1982]
Inv(13) (q12q22) [Sparkes, R.S. et al., 1979]
Inv(14) (q24.2q32.3) [Mules, E.H. et al., 1984]
Inv(3)(p14p22) [Kelly, T.E. et al., 1979]

Appendix E

Paracentric inversions associated with congenital abnormalities.

Inv(3) (p13p25) [Fryns, J.P. et al., 1980]
Inv(3) (p13p25) [Fryns, J.P. et al., 1980]
Inv(1) (q25q42) [Madan,K. et al., 1984]
Inv(16) (q) [del Solar. C. et al., 1974]
Inv(15) (q15q24) [del Porto, G. et al., 1984]
Inv(8) (q11.23-q21.1) [Shabtai, F. et al., 1985]
Inv(3) (p13p25) [Peters-Slough, M.F. et al., 1982]
Inv(1) (p22p36) [Deroover, J. et al., 1979]
Inv(3) (p21.1p25) [Callen, D.F. et al., 1985]
Inv(10) (q11q26) [Allderdice, P.W. et al., 1980]
Inv(X)(q24q48) [Shabtai, F. et al., 1983]
Inv(7)(q11q22) [Schmid, M. et al., 1986]
Inv(4)(p1309p16.2) [French Collaborative Study, 1986]
Inv(5)(q1500q3400) [French Collaborative Study, 1986]
Inv(7)(q11.1q33) [French Collaborative Study, 1986]
Inv(7)(q2100q22.2) [French Collaborative Study, 1986]
Inv(7)(q22.2q3509) [French Collaborative Study, 1986]
Inv(12)(q1209q21.3) [French Collaborative Study, 1986]

Appendix F

Paracentric inversions associated with aneuploidy

Inv (7)(q22q31)-XXY [Madan, K. et al., 1984]
Inv (21)(q21q22)-+21 [Madan, K. et al., 1984]
Inv (12)(q15q24)-XXY [Singh, R.P. et al., 1981]
Inv (5)(q21q32)-XXY [Canki, N. et al., 1979]
Inv(7)(q11.3q22.2)-XO [Canki, N. et al., 1979]

Appendix G

Familial paracentric inversions ascertained on the basis of an index case with abnormalities

Inv(7)(q2100q22.2)-Paternal [French Collaborative Study 1986]
Inv(7)(q22.2q3509)-Maternal [French Collaborative Study 1986]
Inv(12)(1209(21.300)-Paternal [French Collaborative Study 1986]
Inv(3)(p13p25)-Maternal [Fryns, J.P. et al., 1980]
Inv(3)(p13p25)-Maternal [Fryns, J.P. et al., 1980]
Inv(3)(p13p25)-Maternal [Peters-Slough, M.F. et al., 1982]
Inv(1)(q25q42)-Paternal [Madan, K. et al., 1984]
Inv(3)(p21.2p25)-Maternal [Callen, D.F. et al., 1985]
Inv(1)(p22p36)-Paternal [Deroover, J. et al., 1979]
Inv(16)(q11q22)-Maternal [del Solar, C. et al., 1974]
Inv(11)(q13q23)-Maternal [Orye, E. et al., 1983]
Inv(7)(q11q22)-Materanl [Schmid, M. et al., 1986]
Inv(X)(q24q28)-Maternal [Shabtai, F. et al., 1983]

Appendix H

Data on spermatogenesis

DBA

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
DBA/A	63	75	74	24	49.7	56.7	24.5
DBA/B	63	88	38	17	30.2	38.5	30.9
DBA/C	63	98	68	24	41.0	48.2	26.1
DBA/D	63	67	43	12	39.1	45.1	21.8

C3HFeJ

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
C3H	146	94	31	19	24.8	34.7	38.0
C3HM1	110	46	54	11	54.0	58.6	16.9
C3HM2	110	65	35	15	35.0	43.5	30.0
C3HM3	110	71	43	09	37.7	42.3	17.3
C3HM4	110	53	47	14	47.0	53.5	23.0
C3HM5	110	69	31	11	31.0	37.8	26.2

continued...

Appendix II continued...

C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
45	-	60	40	06	40.0	43.4	13.0
MI/M1/M2	80	51	49	06	49.0	51.9	10.9
MG/M1/M2	110	62	38	15	38.0	46.1	28.3
MG/M1/M5	110	61	38	07	38.4	42.5	15.6

1RK/1RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
1F5/15/M1	-	54	49	12	47.6	53.0	19.7
1F5/15/M2	-	51	43	21	45.7	55.6	32.8
1F4/14/M2	-	39	61	13	61.0	65.5	17.6
1F4/14/M1	93	56	44	12	44.0	50.0	21.4

continued...

Appendix H continued...

1RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
FD/15/M1	80	45	27	23	37.5	52.6	46.0
FD/15/M2	80	47	32	16	40.5	50.5	33.3
dF7/M1/M1	92	80	20	20	20.0	33.3	50.0
FN/MG/F2/1F5/ 15/M1/M1	45	79	21	20	21.0	34.2	48.8
FN/MG/F2/1F5/ 15/M1/M2	45	70	30	33	30.0	47.4	52.4
60/13	-	62	28	18	31.1	42.6	39.1
16/35/A	-	48	36	13	42.9	50.5	26.5
FN/MG/F1/1F5/ 15M1/M1	81	57	22	18	27.9	41.2	45
FN/MG/F1/1F5/ 15M1/M2	81	48	50	23	51.0	60.3	31.5
FN/MG/F1/ 1F5/15M1/M3	81	64	35	14	35.4	43.4	28.6

continued....

Appendix II continued...

1RK/C3HFeJ

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
C3HF5/1M1/M2	123	60	40	16	40.0	48.3	28.6
C3HF3/1M2/M1	120	72	28	17	28.0	38.5	37.8
C3HF3/1M2/M2	120	58	42	19	42.0	51.3	31.2

12RK/12RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
12F3/12M1/M1	96	74	64	12	46.4	50.7	15.8
12F3/12M1/M2	96	46	54	06	54.0	56.6	10.0
12M1	63	77	48	9	38.4	42.5	15.8
12M2	63	86	45	12	34.4	39.9	21.1

continued....

Appendix H continued...

12RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
F1/12A/M1	66	74	82	17	52.6	57.2	17.2
FC/12M1/M3	65	40	25	11	38.5	47.4	30.6
FC/12M1/M2	65	40	28	11	41.2	49.4	28.2
FC/12M1/M1	60	91	79	30	46.5	54.5	27.5
FR/12M2/M1	90	55	15	15	21.4	35.3	50.0
FR/12M2/M2	90	70	30	18	30.0	40.7	37.5

continued....

Appendix H continued...

12RK/C3HFeJ

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
C3HF12/12M1/M1	146	67	33	07	33.0	37.4	17.5
C3HF12/12M1/M2	146	61	39	11	39.0	45.1	22.0
C3HF12/12M1/M3	146	78	22	08	22.0	27.8	26.7
C3HF12/12M1/M4	146	69	31	11	31.0	37.8	26.2
C3HF10/12M3/M1	100	58	37	14	39.0	46.8	27.5
C3HF10/12M3/M2	100	52	48	20	48.0	56.7	29.4
C3HF10/12M3/M3	100	54	46	10	46.0	50.9	17.9
C3HF10/12M3/M4	100	58	42	17	42.0	50.4	28.8

continued....

Appendix H continued..

24RK/24RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
24M1	107	66	67	18	50.4	56.3	21.2
24F6/21/M4	-	46	54	22	54.0	62.3	29.0
24F6/21/M4	-	44	36	10	45.0	51.1	21.7
24F9/12M6/ 21/M2/M1	171	62	57	16	47.9	54.1	21.9
24F9/12M6/ 21/M2/M2	171	77	48	13	38.4	44.2	21.3
24F9/12M6/ 21/M2/M3	171	77	48	14	38.4	44.6	22.6
16/70/M1	88	68	32	05	32.0	35.2	13.5

continued....

Appendix H continued...

24RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
24F1/C57BL/M1	60	66	22	26	25.0	42.1	54.2
24F1/C57BL/M2	60	81	16	42	16.5	41.7	72.4
24F1/C57BL/M4	69	60	14	36	18.9	45.5	72.0
24F1/C57BL/M5	69	40	06	30	13.0	47.4	83.3
30/32/A	-	127	21	30	14.2	28.7	58.8
24F10/M1/M1	73	65	23	18	26.1	38.7	43.9
24F10/M1/M2	73	73	15	17	17.1	30.5	53.1

continued....

Appendix H continued...

24RK/C3HFeJ

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
C3HF8/24M1/M1	207	72	23	33	24.2	43.8	58.9
C3HF7/24M1/M2	208	85	15	22	15.0	30.3	59.5
C3HF7/24M1/M3	208	91	9	26	9.0	27.8	74.3
C3HF7/24M1/M1	208	84	16	30	16.0	35.4	65.2

continued....

Appendix II continued...

11RK/11RK

Mouse no.	Age (days)	IM. (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
11F5/11M3/M1	70	60	40	11	40.0	46.0	21.6
11F5/11M3/M2	70	49	51	08	51.0	54.6	13.6
11F4/11M3/M1	75	45	55	06	55.0	57.6	9.8
8734/M1	147	58	42	12	42.0	48.2	22.2

11RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
FR/11M1/M1	61	47	65	23	58.0	65.2	26.1
FR/11M1/M2	61	66	73	15	52.5	57.1	17.1
11F2/ME/M2	61	71	55	26	43.7	53.3	32.1
11F2/ME/M1	61	69	61	18	46.9	53.4	22.8

continued....

Appendix H continued...

14RK/14RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
14M1	135	77	68	14	46.9	51.6	17.1
14M3	135	62	38	15	38.0	46.1	28.3
14M4	135	61	39	10	39.0	44.6	20.4
14F5/14M3/M3	107	68	57	14	45.6	51.1	19.7

14RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
66/14M2/M1	62	93	69	24	42.6	50.0	25.8
66/14M2/M2	64	50	33	34	39.8	57.3	50.8
66/14M2/M3	70	23	23	21	50.0	65.7	47.7
14F1/MJ/M2	69	50	38	29	43.2	57.3	43.3
14F2/MJ/M1	69	57	38	21	40.0	50.9	35.6
14F1/MJ/M1	69	28	38	21	57.6	67.8	35.6

continued....

Appendix II continued...

17RK/+

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
36193	-	73	27	22	27.0	40.2	44.9
1392	106	84	16	24	16.0	32.3	60.0
6393	102	67	06	21	8.0	28.7	77.8
17M1	118	69	14	29	16.9	38.4	67.4
17M2	118	72	10	17	12.2	27.3	63.0
17M3	118	76	21	19	21.7	34.5	47.5

continued....

Appendix H continued...

20RK/20RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
20M1	95	73	52	12	41.6	46.7	18.8
20M2	95	67	57	22	46.0	54.1	27.9
1253/M1	105	60	40	06	40.0	43.4	13.0
1253/M2	105	71	31	18	30.4	40.8	36.7
1253/M3	105	71	38	13	34.9	41.8	25.5
1253/M4	105	70	33	03	32.0	34.0	8.3

continued....

Appendix H continued...

20RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
20F1/FN/ MG/M2/M1	61	62	38	22	38.0	49.2	36.7
20F1/FN/ MG/M2/M2	61	67	21	16	23.9	35.6	43.2
20F1/FN/ MG/M2/M3	63	58	42	22	42.0	52.5	34.4
FU/ML/ F1/20M1/M1	63	65	35	17	35.0	44.4	32.7

continued....

Appendix II continued...

22RK/22RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
22M1	150	56	41	13	42.3	49.1	24.1
22M2	150	74	32	11	30.2	36.8	25.6
22F2/22F3/ 22M3/M/M1	161	65	35	10	35.0	40.9	22.2
22F2/ 22F3/22M3/M/M2	161	106	31	20	22.6	32.5	39.2
22F3/ 22F3/22M3/M/M1	141	91	34	08	27.2	31.6	19.1
22F3/ 22F3/22M3/M/M2	141	90	36	18	28.8	37.5	33.3

continued....

Appendix II continued...

22RK/C57BL

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
22F1/MC/M1	74	228	41	73	15.2	33.3	64.0
22F1/MC/M2	74	132	21	30	13.7	27.9	58.8
22F2/MC/M1	69	134	45	63	25.1	44.6	58.3
22F1/MC/M3	87	114	17	71	13.0	43.6	80.7
22F1/MC/M4	90	129	23	61	15.1	39.4	72.6
22F2/ME/M1	128	85	15	33	15.0	36.1	68.8

continued....

Appendix H continued...

12RK/24RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
24F8/12M2/M2	60	62	38	14	38.0	45.6	26.9
24F8/12M2/M3	60	40	27	08	40.3	46.7	22.9
24F8/12M4/M3	196	54	29	18	34.9	46.5	38.3
24F8/12M4/M4	200	53	27	20	33.8	47.0	42.5
24F8/12M4/M1	77	96	29	14	23.2	30.9	32.6
24F8/12M4/M2	77	94	31	18	24.8	34.3	36.7

1RK/24RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
24F8/15/M3	72	64	36	17	36.0	45.3	32.1
24F8/15/M1	59	132	54	26	29.0	37.7	32.5
24F9/79/M2	68	63	37	19	37.0	47.1	33.9
24F9/70/M1	68	65	35	22	35.0	46.7	38.6
24F8/15/M2	72	34	21	07	38.2	45.2	25.0
24F8/15/M4	109	82	18	16	18.0	29.3	47.1
24F8/15/M5	109	67	33	23	33.0	45.5	41.1

continued....

Appendix H continued...

22RK/24RK

Mouse no.	Age (days)	IM (n)	NIIM (n)	HetIIM (n)	NIIM (%)	NIIM+HetIIM (%)	HetIIM (%)
22F3/24F6/ 21/M5/M1	61	81	09	30	10.0	32.5	76.9
22F3/24F6/ 21/M5/M2	61	71	04	56	5.3	45.8	93.3
22F3/24F6/ 21/M5/M3	61	87	02	45	2.3	35.1	95.7
22F3/24F6/ 21/M5/M4	61	98	02	43	2.0	31.5	95.6
22F2/24F6/ 21/M2/M1	62	88	12	44	12.0	38.9	78.6

Appendix I

Data on oogenesis

C3HFeJ

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C3HF3/C3HM1/F2	164	1	6	6	4	1	3
C3HF2/C3HM1/F	159	4	6	4	2	0	2
C3HFeJF1	93	7	14	13	3	1	2
C3HFeJF2	93	2	24	20	18	0	6
C3HFeJF3	93	3	13	12	8	0	6
C3HFeJF4	93	2	28	24	19	2	7
C3HFeJF5	93	1	18	17	13	1	5

continued..

Refer List of Abbreviations (page xv) for the meaning of abbreviations.

Appendix I continued..

C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
61	180	-	25	25	-	5	12
58	194	-	33	33	-	12	7
74	195	1	24	24	-	12	11
68	189	5	16	16	-	-	-
67	172	2	10	10	-	1	3
70	172	1	18	18	-	-	-
43	341	0	4	4	-	-	-
C57BL	78	2	28	28	24	10	1
C57BL	78	2	20	20	16	7	2
FY/ME	69	2	26	26	12	3	0
FZ/ME/1	70	1	25	25	22	1	0
FZ/ME/2	69	3	24	24	17	3	4

continued..

Appendix I continued...

C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
FUMLF1/							
FUMLM1/F1	61	4	22	22	18	5	0
FUMLF2/							
FUMLM1/F1	61	5	35	35	24	8	2
FB/FNMGM1/F	67	9	44	44	28	16	1
FUMLF2/							
FUMLM1/F2	67	1	20	20	17	8	0
FUMLF2/							
FUMLM1/F3	67	0	12	12	12	7	0
C57BLF1	65	1	43	41	41	8	3
C57BLF2	70	0	30	30	30	8	6
C57BLF3	71	2	19	18	16	9	3

continued..

Appendix I continued...

1RK/1RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
1F1	155	7	20	20	20	16	0
1F9	156	1	19	19	-	-	-
2/13	152	2	20	20	10	1	0
8726/F1	100	7	14	14	-	-	-
8726/F2	103	6	16	16	-	-	-
8726/F3	104	0	25	25	-	-	-
8742/F1	96	6	17	15	-	-	-
8742/F2	97	5	25	24	-	-	-
8742/F3	98	5	9	9	-	-	-
8742/F1	105	1	19	18	-	-	-
8742/F2	108	6	26	24	-	-	-
8742/F3	111	4	11	11	-	-	-
8866/F1	81	3	33	32	24	4	16
8866/F2	82	11	33	33	29	2	11

continued..

Appendix I continued...

1RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
56/3/1	-	8	24	24	-	-	-
57/1/1	76	8	22	22	-	1	0
57/1/2	76	2	22	22	-	-	-
57/1/3	77	0	12	12	-	1	0
57/1/4	79	3	11	11	-	-	-
C57/1/1	103	2	23	23	-	1	0
C57/1/2	105	8	23	23	-	2	0
C57/1/3	105	0	30	30	-	-	-
C57/1/4	109	9	31	31	-	0	2
4/C57/1	108	5	16	16	-	1	0
4/C57/2	110	11	32	32	-	4	0
4/C57/3	110	11	16	16	-	2	0
4/C57/4	111	0	12	12	-	3	0
4/C57/5	115	3	15	15	-	-	-
4/C57/6	115	4	12	12	-	-	-
4/C57/7	116	6	9	9	-	-	-

continued...

Appendix I continued...

1RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
33/1/1	97	6	20	29	-	6	2
33/1/2	98	12	23	23	-	2	4
33/1/3	98	4	16	16	-	3	3
2/32/1	-	-	16	16	-	-	-
2/32/3	-	-	21	21	-	-	-
2/32/2	96	-	34	34	-	-	-
2/32/4	97	-	31	31	-	-	-
2/32/5	97	6	24	24	-	2	0
2/32/6	97	5	18	18	-	4	0
1F7/M1/F1	100	5	21	21	18	14	0
FNMGF2/							
1F515M1/F1	63	10	41	41	36	22	0
1F7/M1/F2	104	7	19	19	14	13	0
1F7/M1/F3	104	6	24	24	20	11	0
33/1/1	132	15	18	18	12	-	-
2/32/2	133	4	14	14	13	-	-
63/1/1	107	13	30	30	-	-	-
61/1/1	95	2	31	31	21	-	-

continued..

Appendix I continued...

1RK/C3HFeJ

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C3HF6/1M1/F7	108	3	36	29	10	9	3
C3HF4/1M1/F1	159	2	19	18	16	9	4
C3HF6/1M1/F2	123	13	18	18	14	3	4
C3HF5/1M2/F2	130	11	20	19	9	4	2
C3HF6/1M1/F1	123	5	22	19	13	5	2
C3HF5/1M2/F1	130	10	10	8	3	1	1
C3HF6/1M1/F6	124	2	28	26	22	17	2
C3HF5/1M2/F3	140	11	20	21	19	7	3
C3HF5/1M1/F3	114	5	31	28	24	6	3
C3HF5/1M1/F2	115	10	21	18	14	5	1
C3HF5/1M2/F2	142	2	25	23	18	4	2
C3HF5/1M1/F1	119	2	19	19	14	2	1

continued..

Appendix I continued...

12RK/12RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
12F1	178	2	14	14	10	8	1
12F2	178	-	16	16	14	7	8
12F1/12M1/F2	127	1	5	5	4	-	-
12F1/12M1/F1	127	6	16	16	9	3	6
12F1/12M1/F3	141	0	11	10	9	1	1
12F1/12M1/F4	141	0	12	11	10	1	1
12F2/12M2/F	109	1	28	28	22	2	1
8680/F1	127	3	28	28	-	-	-
8680/F1	110	3	8	5	5	-	-

continued..

Appendix I continued...

12RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C57F1/12A/F1	63	6	35	35	28	-	-
C57F1/12A/F2	63	7	35	35	-	-	-
F1/12A/F3	122	4	24	24	-	-	-
C57BLF1/12FA/F1	105	2	19	19	-	-	-
C57BLF1/12FA/F2	105	2	26	26	-	-	-
C57BLF1/12FA/F3	106	4	26	26	-	-	-
C57BLF1/12FA/F4	105	5	17	17	-	-	-
FC/12M1/F2	120	3	35	31	23	12	3
FC/12M1/F3	120	1	24	22	14	7	1
FC/12M1/F4	122	2	31	18	15	7	1
FC/12M1/F5	125	6	20	17	14	5	4
FR/12M2/F2	117	12	20	20	17	10	0

continued..

Appendix I continued...

12RK/C3HFeJ

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C3HF11/12M1/F3	138	1	24	21	15	2	6
C3HF10/12M1/F1	143	2	11	0	8	0	4
C3HF10/12M1/F2	144	3	31	28	20	7	6
C3HF10/12M1/F	120	10	25	20	14	6	6
C3HF10/12M1/F3	126	8	23	22	18	6	9
C3HF10/12M1/F2	126	1	16	16	13	5	7
C3HF11/12M1/F4	156	0	15	14	10	3	5
C3HF10/12M1/F1	138	4	18	15	10	2	3
C3HF11/12M1/F1	157	8	20	19	13	1	8
C3HF12/12M1/F2	150	2	14	12	8	6	0

continued..

Appendix I continued...

24RK/24RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
1634/F1	104	4	12	12	-	-	-
1634/F2	105	5	20	17	-	-	-
1634/F3	106	2	23	23	-	-	-
1634/F1	123	2	25	25	23	8	2
1670/F1	111	0	10	9	9	5	4
1634/F2	124	0	28	25	23	14	4
1634/F3	145	5	13	11	9	7	0
1670/F2	115	8	23	20	18	4	5

continued..

Appendix I continued...

24RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
10/34/1	103	11	28	18	-	-	-
10/34/2	104	11	23	23	-	2	8
C57/6/2	104	-	29	29	-	8	4
C57/6/1	104	-	34	34	-	5	0
33/6/1	97	5	33	33	-	5	0
33/6/2	97	3	34	34	-	2	1
33/6/3	99	-	25	25	-	1	0
5/C57/1	96	0	23	23	-	14	0
5/C57/2	96	0	25	25	-	8	0
5/C57/3	97	0	19	19	-	-	-
5/C57/4	98	0	18	18	-	2	0
33/6/4	100	-	23	23	-	-	-
5/34/1	109	2	22	22	-	8	3

continued..

Appendix I continued...

24RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
37/6/1	103	3	21	21	-	12	0
37/6/2	105	12	41	41	-	13	0
37/6/3	110	6	31	31	-	11	5
37/6/4	111	2	20	20	-	-	-
37/6/5	111	6	23	23	-	14	3
8/32/1	111	8	20	20	-	8	1
8/32/2	115	4	20	20	13	3	3
8/32/3	115	6	25	25	21	13	2
8/32/4	116	3	39	39	25	12	4
24F1/C57BL/F1	92	-	17	17	-	11	0
8/32/1	135	-	28	28	-	-	-

continued..

Appendix I continued...

24RK/C3HFeJ

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C3HF8/24M1/F4	143	6	19	19	15	8	3
C3HF8/24M1/F6	144	4	33	29	17	5	5
C3HF8/24M1/F5	144	7	26	25	20	11	2
C3HF7/24M1/F3	169	1	24	24	19	11	8
C3HF8/24M1/F2	169	2	21	18	12	5	0
C3HF12/24M2/F2	63	9	40	40	22	3	0
C3HF12/24M2/F2	66	5	36	36	26	6	6
C3HF12/24M2/F3	66	8	29	26	12	3	0

continued..

Appendix I continued...

11RK/11RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
11F5/11M3/F1	116	1	34	34	19	18	0
11F5/11M3/F2	116	6	21	21	12	10	0
11F5/11M3/F3	118	1	12	12	12	9	0
11F5/11M3/F4	118	0	20	20	15	10	0

11RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
FR/11M1/F1	114	2	28	28	26	0	0
FR/11M1/F2	114	8	27	27	25	0	0
FP/11M2/F1	119	3	17	36	10	18	0
FP/11M2/F2	119	7	35	31	19	9	0
FP/11M2/F3	123	1	35	33	32	29	1
FV/11M2/F1	125	4	34	34	25	4	0

continued..

Appendix I continued...

14RK/14RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
14F1	126	3	52	52	40	2	0
14F2	127	3	42	42	40	1	0
14F5	127	2	31	31	22	-	-
8720/F1	105	6	23	23	-	-	
8720/F2	108	6	41	33	-	-	-
8720/F3	117	9	42	37	30	17	5
8720/F4	124	6	31	31	27	19	2

continued..

Appendix I continued...

14RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
66/14M2/F1	119	10	51	51	47	15	11
66/14M2/F2	120	0	34	34	-	20	6
14F2/MJ/F1	120	4	37	37	33	29	0
14F2/MJ/F2	120	4	27	27	20	10	4
14F1/MJ/F1	127	18	41	36	28	10	1

continued..

Appendix I continued...

20RK/20RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
1253/F1	215	1	10	7	-	-	-
1253/F2	201	1	13	11	11	6	2
1253/F3	205	1	15	14	14	7	1
1253/F4	206	1	14	14	14	11	1

20RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
C57BL/20M1/F2	82	10	56	51	15	7	0
C57BL/20M1/F1	82	6	25	24	17	11	0
C57BL/20M1/F3	83	6	32	28	8	11	0
C57BL/20M1/F4	83	8	35	31	16	5	0
C57BL/20M1/F[illegible]0	84	2	31	31	21	15	0

continued..

Appendix I continued...

22RK/22RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
22F4	171	-	18	18	9	2	3
22F2/22M3/F1	91	4	13	13	-	-	-
22F2/22M3/F1	90	5	24	22	-	-	-
22F2/22M3/F2	91	7	12	11	-	-	-
8496/F1	85	4	20	19	14	2	6
8496/F2	85	3	20	20	17	1	5
8497/F1	87	4	27	21	17	0	8
8497/F2	87	1	15	13	10	1	7
8497/F3	89	1	9	9	6	0	4
8497/F4	90	4	25	25	18	1	8

continued..

Appendix I continued....

22RK/C57BL

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
22F1/MC/F1	76	4	31	31	26	8	3
22F1/MC/F2	118	2	20	20	-	-	-
22F2/MC/F1	113	4	22	22	-	-	-
22F2/MC/F2	113	5	21	21	-	-	-
22F2/MC/F3	114	3	28	28	-	-	-
22F2/MC/F4	114	1	20	20	-	-	-
FY/22M3/F1	104	2	19	18	12	5	4
22F2/ME/F1	115	3	16	15	10	3	0
FY/22M3/F2	108	6	23	23	16	9	0
FE/22M3/F1	135	5	15	15	12	4	3
22F1/MD/F1	117	2	6	6	-	-	-
22F1/MD/F2	117	8	21	21	11	6	1
MZ/22M1/F1	116	10	16	26	15	13	1
MZ/22M1/F2	116	10	18	18	14	8	5

continued..

Appendix I continued...

1RK/12RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
1F7/12RK/F2	121	7	27	26	15	5	2
1F8/12RK/F1	119	5	31	28	21	9	3
1F8/12RK/F2	119	7	21	18	14	5	1
1F8/12RK/F3	120	4	30	24	14	8	0
1F8/12RK/F4	120	3	19	18	9	5	0

12RK/24RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
24F8/12M2/F2	63	4	29	27	18	18	0
24F8/12M2/F3	64	6	18	16	11	3	1

continued..

Appendix I continued...

1RK/24RK

Mouse no.	Age (days)	MV (n)	MO (n)	OC (n)	DC (n)	IM (n)	IIM (n)
24F9/79/F1	124	6	34	34	29	20	0
24F9/79/F2	124	2	24	24	20	11	0
24F9/79/F3	124	4	30	30	27	17	0
24F8/15/F1	133	2	18	18	16	11	0

Appendix J

Litter size of male mice

1RK/1RK

Mouse no.	Genotype of female partner	Number of pups
3	C57BL	7
3	C57BL	1
3	C57BL	6
3	C57BL	2
3	C57BL	4
1	C57BL	11
1	C57BL	9
1	C57BL	10
1	C57BL	9
1	C57BL	7
14	C57BL	2
13	C57BL	7
12	C57BL	2
11	C57BL	11

Continued...

Appendix J continued...

1RK/1RK

Mouse no.	Genotype of female partner	Number of pups
3	1RK/1RK	1
3	1RK/1RK	8
1	1RK/1RK	2
1	1RK/1RK	6
13	1RK/1RK	2
14	1RK/1RK	8
14	1RK/1RK	4
15	1RK/1RK	5
15	1RK/1RK	2
25	1RK/1RK	5

Continued...

Appendix J continued...

1RK/C3HFeJ

Mouse no.	Genotype of female partner	Number of pups
C3HF4/1M1/M1	1RK/C3H	4
C3HF4/1M1/M1	1RK/C3H	9
C3HF5/1M2/M2	1RK/C3H	5
C3HF5/1M2/M2	1RK/C3H	6
C3HF5/1M2/M2	1RK/C3H	6
C3HF5/1M2/M2	1RK/C3H	7
C3HF6/1M1/M1	1RK/C3H	5
C3HF6/1M1/M1	1RK/C3H	8
C3HF6/1M1/M1	1RK/C3H	8
C3HF6/1M1/M1	1RK/C3H	7
C3HF6/1M1/M1	1RK/C3H	6
C3HF5/1M1/M2	1RK/C3H	11
C3HF5/1M1/M1	1RK/C3H	7
C3HF5/1M1/M1	1RK/C3H	8
C3HF5/1M1/M1	1RK/C3H	8

Continued...

Appendix J continued...

12RK/12RK

Mouse no.	Genotype of female partner	Number of pups
12A	C57BL	5
12M1	C57BL	11
12A	C57BL	5
12M2	C57BL	8
12M4	C57BL	5
12RK	1RK/1RK	6
12RK	1RK/1RK	11
12M2	24RK/24RK	9
12F3/12RK/M2	24Rk/24RK	8
12F3/12RK/M2	24RK/24RK	9
12M4	24RK/24RK	11

Continued...

Appendix J continued...

12RK/C3HFeJ

Mouse no.	Genotype of female partner	Number of pups
C3HF10/12M1/M1	12RK/C3H	5
C3HF10/12M1/M1	12RK/C3H	7
C3HF10/12M1/M1	12RK/C3H	4
C3HF10/12M1/M1	12RK/C3H	6
C3HF10/12M1/M1	12RK/C3H	6
C3HF10/12M1/M1	12RK/C3H	7
C3HF11/12M1/M1	12RK/C3H	7
C3HF11/12M1/M1	12RK/C3H	7
C3HF11/12M1/M1	12RK/C3H	6
C3HF11/12M1/M2	12RK/C3H	7
C3HF11/12M1/M2	12RK/C3H	9
C3HF11/12M1/M2	12RK/C3H	5
C3HF11/12M1/M2	12RK/C3H	10
C3HF12/12M1/M1	12RK/C3H	5
C3HF12/12M1/M1	12RK/C3H	5
C3HF12/12M1/M1	12RK/C3H	9

Continued...

Appendix J continued...

24RK/24RK

Mouse no.	Genotype of female partner	Number of pups
6	C57BL	8
6	C57BL	5
6	C57BL	7
6	C57BL	3
6	C57BL	6
7	C57BL	5
9	C57BL	5
29	C57BL	6
19	C57BL	6
19	C57BL	6
19	C57BL	4
19	C57BL	3
20	C57BL	5
24F4/21/M1	C57BL	8
24F6/21/M5	C57BL	8

Continued...

Appendix J continued...

24RK/24RK

Mouse no.	Genotype of female partner	Number of pups
6	24RK/24RK	5
6	24RK/24RK	8
7	24RK/24RK	6
7	24RK/24RK	6
20	24RK/24RK	4
20	24RK/24RK	2
21	24RK/24RK	3
21	24RK/24RK	5
24F6/21/M1	22RK/22RK	2
24F6/21/M1	22RK/22RK	4
24F6/21/M1	22RK/22RK	4
24F6/21/M2	22RK/22RK	3
24F6/21/M5	22RK/22RK	9

Continued...

Appendix J continued...

24RK/C3HFeJ

Mouse no.	Genotype of female partner	Number of pups
C3HF7/24M1/M1	24RK/C3H	7
C3HF7/24M1/M1	24RK/C3H	3
C3HF7/24M1/M1	24RK/C3H	4
C3HF7/24M1/M2	24RK/C3H	5
C3HF7/24M1/M2	24RK/C3H	4
C3HF7/24M1/M2	24RK/C3H	9
C3HF8/24M1/M1	24RK/C3H	8
C3HF8/24M1/M1	24RK/C3H	4
C3HF8/24M1/M1	24RK/C3H	4
C3HF8/24M1/M1	24RK/C3H	7
C3HF8/24M1/M1	24RK/C3H	2
C3HF7/24M1/M3	24RK/C3H	2
C3HF7/24M1/M3	24RK/C3H	6

Appendix K

Litter size of female mice

1RK/1RK

Mouse no.	Genotype of male partner	Number of pups
2	C57BL	8
2	C57BL	9
4	C57BL	12
18	C57BL	11
27	C57BL	9
27	C57BL	9
1F7	C57BL	9
1F8	12RK/12RK	11
1F5	12RK/12RK	10
1F5/15/F3	12RK/12RK	8
1F5/15/F1	12RK/12RK	8

Continued...

Appendix K continued....

1RK/C57BL

Mouse no.	Genotype of male partner	Number of pups
FD/15/F2	C57BL	6
FD/15/F2	C57BL	9
FD/15/F3	C57BL	7
FD/15/F3	C57BL	7
1F7/MI/F	C57BL	3
FD/15/F4	C57BL	8
1F7/MI/F1	C57BL	3

Continued...

Appendix K continued....

1RK/C3H

Mouse no.	Genotype of male partner	Number of pups
C3HF4/1M1/F1	1RK/C3H	4
C3HF4/1M1/F1	1RK/C3H	9
C3HF5/1M2/F1	1RK/C3H	5
C3HF5/1M2/F2	1RK/C3H	6
C3HF5/1M2/F2	1RK/C3H	6
C3HF5/1M2/F3	1RK/C3H	7
C3HF6/1M1/F1	1RK/C3H	5
C3HF6/1M1/F2	1RK/C3H	8
C3HF6/1M1/F3	1RK/C3H	8
C3HF6/1M1/F5	1RK/C3H	7
C3HF6/1M1/F4	1RK/C3H	6
C3HF5/1M1/F4	1RK/C3H	11
C3HF5/1M1/F1	1RK/C3H	7
C3HF5/1M1/F2	1RK/C3H	8
C3HF5/1M1/F3	1RK/C3H	8

Continued...

Appendix K continued....

12RK/12RK

Mouse no.	Genotype of male partner	Number of pups
12F3/12RK/F1	24RK/24RK	8
12F3/12RK/F1	24RK/24RK	5

12RK/C57BL

Mouse no.	Genotype of male partner	Number of pups
FC/12M1/F1	C57BL	4
FC/12M1/F2	C57BL	8
FC/12M1/F2	C57BL	0
FC/12M1/F3	C57BL	6
FC/12M1/F3	C57BL	8
FR/12M2/F1	C57BL	7
FR/12M2/F1	C57BL	8

Continued...

Appendix K continued....

12RK/C3HFeJ

Mouse no.	Genotype of male partner	Number of pups
C3HF10/12M1/F1	12RK/C3H	5
C3HF10/12M1/F1	12RK/C3H	7
C3HF11/12M1/F1	12RK/C3H	7
C3HF11/12M1/F1	12RK/C3H	6
C3HF11/12M1/F2	12RK/C3H	7
C3HF11/12M1/F2	12RK/C3H	10
C3HF11/12M1/F3	12RK/C3H	7
C3HF11/12M1/F4	12RK/C3H	9
C3HF11/12M1/F4	12RK/C3H	5
C3HF10/12M1/F2	12RK/C3H	6
C3HF10/12M1/F2	12RK/C3H	6
C3HF10/12M1/F2	24RK/C3H	7
C3HF12/12M1/F2	12RK/C3H	5
C3HF12/12M1/F3	12RK/C3H	5
C3HF12/12M1/F1	12RK/C3H	9
C3HF10/12M1/F3	24RK/C3H	4

Continued...

Appendix K continued....

24RK/24RK

Mouse no.	Genotype of male partner	Number of pups
5	24RK/24RK	5
5	24RK/24RK	8
5	24RK/24RK	3
5	24RK/24RK	9
8	24RK/24RK	5
8	24RK/24RK	6
8	24RK/24RK	8
8	24RK/24RK	7
30	24RK/24RK	4
30	24RK/24RK	2
6	24RK/24RK	3
31	24RK/24RK	5
31	24RK/24RK	5
76	C57BL	5
24F2	C57BL	7
24F1	C57BL	9
24F10	C57BL	8
24F9	C57BL	9
24F9	1RK/1RK	6
24F8	1RK/1RK	10
24F8	12RK/12RK	9
24F8	12RK/12RK	11

Continued...

Appendix K continued....

24RK/C57BL

Mouse no.	Genotype of male partner	Number of pups
24F10/MI/F1	C57BL	0
24F10/MI/F1	C57BL	0
24F10/MI/F2	C57BL	0
24F10/MI/F2	C57BL	0
24F2/MI/F1	C57BL	4
24F2/MI/F1	C57BL	0
24F2/MI/F2	C57BL	0

Continued...

Appendix K continued....

24RK/C3HFeJ

Mouse no.	Genotype of male partner	Number of pups
C3HF7/24M1/F1	24RK/C3H	7
C3HF7/24M1/F1	24RK/C3H	6
C3HF7/24M1/F2	24RK/C3H	7
C3HF7/24M1/F2	24RK/C3H	2
C3HF7/24M1/F3	24RK/C3H	5
C3HF7/24M1/F3	24RK/C3H	4
C3HF8/24M1/F1	24RK/C3H	8
C3HF8/24M1/F1	24RK/C3H	7
C3HF8/24M1/F2	24RK/C3H	4
C3HF8/24M1/F2	24RK/C3H	2
C3HF8/24M1/F3	24RK/C3H	4
C3HF7/24M1/F4	24RK/C3H	9

Continued...

Appendix K continued....

11RK/11RK

Mouse no.	Genotype of male partner	Number of pups
11F3	C57BL	3
11F2	C57BL	8
11F1	C57BL	8

11RK/C57

Mouse no.	Genotype of male partner	Number of pups
11F2/ME/F1	C57BL	9
11F2/ME/F1	C57BL	4
11F2/ME/F1	C57BL	4
11F2/ME/F4	C57BL	0
11F2/ME/F3	C57BL	9
11F2/ME/F3	C57BL	0
11F2/ME/F2	C57BL	6
11F2/ME/F2	C57BL	5
11F2/ME/F3	14RK/14RK	5

Continued...

Appendix K continued....

14RK/14RK

Mouse no.	Genotype of male partner	Number of pups
14F1	C57BL	9
14F2	C57BL	6

14RK/C57

Mouse no.	Genotype of male partner	Number of pups
14F1/MJ/F1	C57BL	7
14F1/MJ/F1	C57BL	7
14F1/MJ/F2	C57BL	7
14F1/MJ/F2	C57BL	7

Continued...

Appendix K continued....

20RK/20RK

Mouse no.	Genotype of male partner	Number of pups
20F1	C57BL	6
20F1	C57BL	6
20F2	C57BL	4
20F2	C57BL	4

Continued...

Appendix K continued....

20RK/C57BL

Mouse no.	Genotype of male partner	Number of pups
20F1/FN/MG/M2/F1	14RK/14RK	0
20F1/FN/MG/M2/F1	14RK/14RK	0
20F1/FN/MG/M2/F2	14RK/14RK	0
20F1/FN/MG/M2/F2	14RK/14RK	2
20F1/FN/MG/M2/F3	14RK/14RK	0
20F2/FN/MG/M2/F1	14RK/14RK	6
20F2/FN/MG/M2/F1	14RK/14RK	6
20F2/FN/MG/M2/F3	14RK/14RK	0
20F2/FN/MG/M2/F3	14RK/14RK	5
20F2/FN/MG/M3/F4	14RK/14RK	0
20F2/FN/MG/M2/F2	14RK/14RK	7
20F2/FN/MG/M2/F3	C57BL	0
20F2/FN/MG/M2/F4	C57BL	[illegible]
20F2/FN/MG/M2/F4	C57BL	4

Continued...

Appendix K continued....

22RK/22RK

Mouse no.	Genotype of male partner	Number of pups
22F1	C57BL	6
22F1	C57BL	10
22F1	C57BL	7
22F2	C57BL	9
22F2	C57BL	10
22F2	24RK/24RK	3
22F3	24RK/24RK	9
22F6	24RK/24RK	4

Continued...

Appendix K continued....

22RK/C57BL

Mouse no.	Genotype of male partner	Number of pups
22F2/ME/F2	C57BL	6
22F2/ME/F2	C57BL	1
22F2/ME/F1	C57BL	1
22F2/ME/F1	C57BL	7
22F2/ME/F4	C57BL	1
22F2/ME/F4	C57BL	1
22F2/ME/F4	C57BL	0
22F2/ME/F3	C57BL	7
22F2/ME/F3	C57BL	0

Continued...

Appendix K continued....

1RK/12RK

Mouse no.	Genotype of male partner	Number of pups
15/1F3/12F3/12M2/F1	C57BL	1
15/1F3/12F3/12M2/F3	C57BL	3
15/1F3/12F3/12M2/F4	C57BL	5
15/1F3/12F3/12M2/F3	C57BL	4
15/1F3/12F3/12M2/F2	14RK/14RK	0
15/1F3/12F3/12M2/F2	14RK/14RK	0
15/1F3/12F3/12M2/F1	C57BL	4

12RK/24RK

Mouse no.	Genotype of male partner	Number of pups
24F8/12M4/F1	14RK/14RK	3
24F8/12M4/F3	14RK/14RK	6
24F8/12M4/F2	14RK/14RK	0
24F8/12M4/F4	14RK/14RK	5
24F8/12M4/F4	14RK/14RK	3
24F8/12M4/F3	C57BL	3

Continued...

Appendix K continued....

1RK/24RK

Mouse no.	Genotype of male partner	Number of pups
24F8/15/F2	14RK/14RK	0
24F8/15/F1	14RK/14RK	2
24F8/15/F1	14RK/14RK	0

Appendix L

Age and litter size of of female 24RK/24RK, 24RK/C57 and 24RK/C3H

24RK/24RK

Mouse no.	Age (days)	Genotype of male	Litter size
8	104	24RK/24RK	6
8	128	24RK/24RK	8
31	293	24RK/24RK	5
5	191	C57BL	9
8	197	C57BL	7
24F2	106	C57BL	7
24F10	110	C57BL	8
24F9	168	C57BL	9
24F8	110	1RK/1RK	10
24F9	166	1RK/1RK	6
24F8	154	12RK/12RK	9
24F8	198	12RK/12RK	11

Continued....

Appendix L continued....

24RK/C57BL

Mouse no.	Age (days)	Genotype of male	Litter size
24F10/MI/F1	103	C57BL	0
24F10/MI/F1	128	C57BL	0
24F10/MI/F2	103	C57BL	0
24F10/MI/F2	128	C57BL	0
24F2/MI/F1	107	C57BL	4
24F2/MI/F1	158	C57BL	0
24F2/MI/F2	107	C57BL	0

Continued...

Appendix L continued....

24RK/C3HFeJ

Mouse no.	Age (days)	Genotype of male	Litter size
C3HF7/24M1/F1	55	24RK/C3H	7
C3HF7/24M1/F1	102	24RK/C3H	6
C3HF7/24M1/F1	75	24RK/C3H	7
C3HF7/24M1/F2	55	24RK/C3H	7
C3HF7/24M1/F2	123	24RK/C3H	2
C3HF7/24M1/F3	55	24RK/C3H	5
C3HF7/24M1/F3	102	24RK/C3H	4
C3HF7/24M1/F4		24RK/C3H	9
C3HF8/24M1/F1	54	24RK/C3H	8
C3HF8/24M1/F2	54	24RK/C3H	4
C3HF8/24M1/F2	75	24RK/C3H	2
C3HF8/24M1/F3	54	24RK/C3H	4

Appendix M

Isotonic sodium citrate for spermatocytes

2.2 grams of tri-sodium citrate is dissolved in 100 ml deionised water.

Fixative for fibroblasts and oocytes and spermatocytes in melosis.

3 parts methanol and 1 part acetic acid

2 X SSC

0.3M sodium chloride containing 0.03M tri-sodium citrate.

Phosphate buffer for Giemsa staining.

3.4 grams.....KH_2PO_4
50% sodium hydroxide

Dissolve 3.4 grams of KH_2PO_4 in 1 litre deionised water and titrate with 50% sodium hydroxide to pH 6.8.

Paraformaldehyde

3.4 grams.....sucrose
4 grams.....paraformaldehyde
Sodium hydroxide (1N)

3.4 grams of sucrose was dissolved in about 70 ml of deionised water. 4 grams of paraformaldehyde was added to this solution and the solution was then made up to 100 ml with deionised water. The solution was heated slowly to 60-80°C while stirring. About 6 drops of 1N sodium hydroxide was added to the solution. Stirring was continued until the solution was clear. pH of the solution was adjusted to between 9 and 10 with 1N sodium hydroxide. The solution was then filtered and used fresh.

Preparation of coated slides to spread cells for electron microscopy

Clean slides were greased by passing the slide between two fingers to fill the small irregularities on the slide. Natural grease from the fingers fills the irregularities. The slides were then coated with 1% Formvar in Ethylene dichloride. After drying the slides were coated with 0.01% Cytochrome C.

Photoflo for rinsing fixed cells for electron microscopy

A 0.4% solution of photoflo is prepared and the pH adjusted to 8.0 with 1N sodium hydroxide.

Filtering and storage of fetal calf serum

Fetal calf serum was filtered through a MILLEX-GS 0.22um Filter unit and stored at -20°C in 6 ml aliquots.

Working solution of HC 110

HC 110 stock solution diluted 1:7 in water at 68°C.

Working solution of rapid fix for developing films

Add 946 ml of solution A to 1.9 litres of water. Mix well. Add 104 ml of solution B with rapid agitation. Add approximately 850 ml water to bring final volume to 3.8 litres.

Composition of complete tissue culture medium

RPMI 1640	425 ml
Fetal Bovine Serum	75 ml
TC Pencillin-Streptomycin*	0.3 ml

*Pencillin G potassium (100,000 units) and Streptomycin sulfate (100,000 mcg) in 10 ml.

Trypsinization of monolayer

The culture medium was removed and the monolayer rinsed with 1 ml of freshly prepared 1X Trypsin EDTA solution in Hanks balanced salt solution without calcium and magnesium. (The 10X stock solution of Trypsin EDTA solution was prepared by dissolving 5 grams of Trypsin (1:250) and 2.0 gms of EDTA in 20 ml Hanks balanced salt solution.) The monolayer was then treated with 1 ml of Trypsin EDTA solution at 37°C. When detachment of the monolayer was apparent under an inverted microscope, 4 ml of culture medium was added to stop the trypsinization.

Trypsin solution for staining procedure

0.5 ml of Bacto trypsin which has an activity equivalent to a 5% solution of trypsin 1:250 in 50 ml of 1X Hanks balanced salt solution (withoiut calcium and magnesium).

Wright stain

0.3 grams of Wright stain is dissolved in 100 ml methanol and aged in the dark. 1 ml of filtered stain is added to 4 ml of borate buffer to prepare the working solution of Wright stain.

Borate buffer

Equal amounts of $Na_2B_4O_7.10H_2O$ (1.84/500 ml) and Na_2SO_4 (3.551/250 ml) diluted 1:4.

Appendix N

Suppliers of chemicals, reagents and equipment.

Acetic Acid, Glacial (Reagent A.C.S.)	Fisher Scientific.
Bacto Trypsin	DIFCO Laboratories, Detroit, USA.
Barium hydroxide (Analar)	BDH Chemicals Ltd; Poole England.
Colcemid	GIBCO Diagnostics, New York, USA.
Ethylene dichloride	Marivac Ltd., Halifax, N.S. Canada.
Falcon flask (25 cm^2)	Becton Dickinson & Co., USA.
Fetal Bovine Serum	GIBCO Laboratories, Ohio, USA.
Formvar	Marivac Ltd., Halifax, N.S., Canada.
Hanks Balanced Salt Solution	GIBCO Laboratories, New York, USA.
HC-110	Kodak Canada Inc., Toronto, Canada.
Hydrochloric acid	BDH Chemicals. Canada.
Kodak Ektamatic SC paper	Kodak Canada Inc., Toronto, Canada.
Kodak Ektamatic S30 Stabilizer	Eastman Kodak Co., New York, USA.
Kodak S II Activator	Eastman Kodak Co., New York, USA.
Kodak technical pan Film 2415	Eastman Kodak Co., New York, USA.
Kodak rapid fix Solution A	Eastman Kodak Co., New York, USA.
Kodak rapid fix Solution B	Eastman Kodak Co., New York, USA.
Methanol (assay 99.5%)	Fisher Scientific.
Micro Concavity Slides	Becton Dickinson & Co., USA.
MILLEX-GS 0.22 um Filter Unit	Milliopore Corporation, USA.

Narco Model 3636 Carbondioxide Control Master	Oregon, USA.
Paraformaldehyde	BDH Chemicals, Toronto, Canada.
Photo-Flo 200 Solution	Kodak Canada Inc., Toronto, Canada.
Potassium Chloride	Sigma Chem. Co. St.Louis. MO. USA.
Potassium dihydrogen orthophosphate	BDH Chemicals, Canada.
RPMI 1640	GIBCO Laboratories, Ohio, USA.
Silver Nitrate (Analar)	BHD Chemicals. Canada.
Sodium chloride	J.T. Baker Co. Phillipsburg, N.J. USA.
Sodium sulphate	BDH Chemical, Toronto, Canada.
Sodium tetraborate	BDH Chemical, Toronto, Canada.
Sucrose (Analar)	BDH Chemicals, Canada.
TC Hanks Solution	DIFCO Laboratories, Michigan, USA.
TC Pencillin-Streptomycin	DIFCO laboratories, Michigan, USA.
tri-Sodium citrate	BDH laboratory, Poole, England
Trypsin EDTA	Grand Island Biological Co., New York, USA.
Wright stain	Sigma Chem. Co., St. Louis Mo. USA.