CHROMOSOME SEGREGATION AND MEIOTIC DEFECTS 
IN CARRIERS OF CHROMOSOMAL ABNORMALITIES

by

GORDON KIRKPATRICK

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ABSTRACT

Male carriers of chromosomal abnormalities (CA) are more frequent in the infertile population. Furthermore, these men have higher levels of sperm aneuploidy due to the aberrant segregation of the chromosomes involved in the abnormality. It is thought that the presence of a CA may also influence the segregation of other chromosomes, in a process known as in interchromosomal effect (ICE). It is believed that the behaviour of the CA during meiosis may account for the infertility observed in this population. We studied chromosome segregation, ICE and meiotic defects in a variety of CA.

In carriers of CA, we used FISH on spermatozoa to determine the segregation patterns of chromosomes involved in the abnormality. With the exception of the carriers of mosaic aneuploidy, we found significantly increased frequencies of unbalanced chromosome complements. With respect to the translocations and inversion, we observed frequencies of unbalanced chromosome complements comparable with similar rearrangements in the literature. We report the first examination of chromosome segregation in a CCR, in which we found a lower than expected level of sperm aneuploidy. In the two carriers of sSMC, we found the marker present in less than the theoretically predicted 50%. As well, we determined the frequency of abnormal segregation on uninvolved chromosomes to assess the presence of an ICE. We observed ICE in six of twelve carriers, which were confined largely to the acrocentric chromosomes 13 and 21. We also compared the frequency of chromosome imbalance in CA carriers with infertile, but karyotypically normal, men where we found higher levels of sperm aneuploidy than CA carriers or controls.

We used immunofluorescent techniques to observe the synopsis and recombination of homologous chromosomes in three carriers of chromosomal abnormalities, as well as seven infertile men and five fertile men. We observed reduced global recombination in two of the carriers of CA and in three of the infertile men. Increased synaptic errors were observed in all carriers of CA and in four of the infertile men. We noted an increased proportion of cells lacking sex chromosome recombination in all of the CA carriers and in four of the infertile men. We combined immunofluorescent techniques with FISH to study chromosome-specific recombination patterns on chromosomes 13, 18 and 21 and compared those results with
levels of aneuploidy in the sperm. However, we observed no relationship between chromosome-specific recombination and sperm aneuploidy. We studied the recombination and sex chromosome association, of the involved chromosomes, in the three carriers of CA, and observed decreased recombination on the involved chromosomes and frequent association between the chromosome abnormality and the sex chromosomes. Lastly, we report the use of a novel technique for the examination of meiotic cells derived from the ejaculate. We compared spermatocytes, derived from the ejaculate, with previously reported testicular derived spermatocytes, in one individual, and found no difference in the frequency of global or chromosome-specific recombination, synaptic errors or proportion of cells at various stages of prophase.
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LIST OF ABBREVIATIONS

Rules established by the Human Genome Organization (HUGO) for naming genes are followed in this thesis. Human genes are reported as all capital letters, while those in mice have only the first letter capitalized. Italicized letters indicate the gene or RNA, while non-italicized letters indicate the protein.

<table>
<thead>
<tr>
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<th>Description</th>
</tr>
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<tr>
<td>γH2AX</td>
<td>Phosporylated H2AX</td>
</tr>
<tr>
<td>2n</td>
<td>Diploid</td>
</tr>
<tr>
<td>1n</td>
<td>Haploid</td>
</tr>
<tr>
<td>Ad</td>
<td>Dark type A (spermatogonia)</td>
</tr>
<tr>
<td>Ap</td>
<td>Pale type A (spermatogonia)</td>
</tr>
<tr>
<td>ADB</td>
<td>Antibody diluting buffer</td>
</tr>
<tr>
<td>AIS</td>
<td>Androgen insensitivity syndrome</td>
</tr>
<tr>
<td>AMCA</td>
<td>Aminomethyl coumarin acetic acid</td>
</tr>
<tr>
<td>ART</td>
<td>Assisted reproductive technologies</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>ATR</td>
<td>Ataxia telangiectasia and Rad3 related</td>
</tr>
<tr>
<td>AZF</td>
<td>Azoospermic factor</td>
</tr>
<tr>
<td>BPY2</td>
<td>Basic protein Y2</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast cancer 1, early onset</td>
</tr>
<tr>
<td>c</td>
<td>Centromere</td>
</tr>
<tr>
<td>CA</td>
<td>Chromosomal abnormalities</td>
</tr>
<tr>
<td>CBAVD</td>
<td>Congenital absence of the vas deferens</td>
</tr>
<tr>
<td>CCR</td>
<td>Complex chromosomal rearrangement</td>
</tr>
<tr>
<td>CEN</td>
<td>Centromeric probe</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic fibrosis trans-membrane conductance receptor</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CO</td>
<td>Crossover</td>
</tr>
<tr>
<td>CREST</td>
<td>Calcinosis/Raynaud’s phenomenon/esophageal/dysmotility/sclerodactyly/telangiectasia</td>
</tr>
<tr>
<td>DAPI</td>
<td>4,6-diamidino-2 phenylindole</td>
</tr>
<tr>
<td>DAZ</td>
<td>Deleted in azoospermia</td>
</tr>
<tr>
<td>der</td>
<td>Derivative chromosome</td>
</tr>
<tr>
<td>DMC1</td>
<td>DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSB</td>
<td>Double-strand break</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>ES</td>
<td>Ejaculate sample</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescent in-situ hybridization</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>Fkbp6</td>
<td>FK506 binding protein</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GCA</td>
<td>Germ cell arrest</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>H2AX</td>
<td>H2A histone family, member X</td>
</tr>
<tr>
<td>HJ</td>
<td>Holliday junction</td>
</tr>
<tr>
<td>Hypo</td>
<td>Hypospermatogenesis</td>
</tr>
<tr>
<td>ICE</td>
<td>Interchromosomal effect</td>
</tr>
<tr>
<td>ICSI</td>
<td>Intracytoplasmic sperm injection</td>
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</tbody>
</table>
IgG    Immunoglobulin G
inv    Inversion
IVF    In vitro fertilization
LH    Luteinizing hormone
LSI    Locus-specific identifier
MI    Meiosis I
MII    Meiosis II
MA    Maturation arrest
MESA    Microsurgical epididymal sperm aspiration
min    Minute
MLH1    Mut-L homolog 1
MLH3    Mut-L homolog 3
Mre11a    Meiotic recombination 11 homolog A
mRNA    Messenger ribonucleic acid
MSCI    Meiotic sex chromosome inactivation
MSH4    MutS homolog 4
MSH5    MutS homolog 5
MSUC    Meiotic silencing of unsynapsed chromatin
MYC    Myelocytomatosis viral oncogene homolog
NCA    Numerical chromosomal abnormalities
NCO    Non crossover
NDJ    Non disjunction
no    Number
NP-40    Nonyl phenoxypolyethoxylethanol-40
NOA    Non-obstructive azoospermia
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>NOR</td>
<td>Nucleolar organizing region</td>
</tr>
<tr>
<td>OA</td>
<td>Obstructive-azoospermia</td>
</tr>
<tr>
<td>OAT</td>
<td>Oligoasthenoteratozoospermia</td>
</tr>
<tr>
<td>p</td>
<td>Short chromosome arm</td>
</tr>
<tr>
<td>PAR</td>
<td>Pseudo autosomal region</td>
</tr>
<tr>
<td>PBD</td>
<td>Phosphate-buffered detergent</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PESA</td>
<td>Percutaneous sperm aspiration</td>
</tr>
<tr>
<td>PGC</td>
<td>Primordial germ cell</td>
</tr>
<tr>
<td>PMA</td>
<td>Partial maturation arrest</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethylsulphonyl fluoride</td>
</tr>
<tr>
<td>PSCR</td>
<td>Post-meiotic sex chromosome repression</td>
</tr>
<tr>
<td>PZD</td>
<td>Partial zona dissection</td>
</tr>
<tr>
<td>q</td>
<td>Long chromosome arm</td>
</tr>
<tr>
<td>RAD51</td>
<td>RAD51 homolog (RecA homolog E. coli)</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNA Pol II</td>
<td>Ribonuclei acid polymerase II</td>
</tr>
<tr>
<td>ROB</td>
<td>Robertsonian translocation</td>
</tr>
<tr>
<td>RPA</td>
<td>Replication protein A</td>
</tr>
<tr>
<td>RT</td>
<td>Reciprocal translocation</td>
</tr>
<tr>
<td>SAC</td>
<td>Spindle assembly checkpoint</td>
</tr>
<tr>
<td>SB</td>
<td>Sex body</td>
</tr>
<tr>
<td>SC</td>
<td>Synaptonemal complex</td>
</tr>
<tr>
<td>SCC</td>
<td>Saline sodium citrate</td>
</tr>
<tr>
<td>SCOS</td>
<td>Sertoli cell only syndrome</td>
</tr>
</tbody>
</table>
SDSA Synthesis dependant strand annealing
SEI Single end invasion
SPO11 SPO11 meiotic protein covalently bound to DSB homolog
SRY Sex determining region Y
SSC Spermatogonial stem cell
sSMC Small supernumerary marker chromosome
SUZI Subzonal sperm injection
SYCP1 Synaptonemal complex protein 1
SYCP2 Synaptonemal complex protein 2
SYCP3 Synaptonemal complex protein 3
TESE Testicular sperm extraction
TRITC Tetramethyl rhodamine isothiocyanate
TTY2 Testis transcript Y2
TS Testicular sample
WHO World health organization
XAT X-autosomal translocation
XIST X-inactive specific transcript
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I would like to express deep appreciation to my supervisor Dr. Sai Ma for providing me with the opportunity to research in her laboratory and for her continuous support, guidance and training. I am also grateful to the members of my supervisory committee, Dr. David Huntsman, Dr. Petrice Eydoux and Dr. Dan Rurak, for their support and constructive suggestions. I am thankful to have had the friendship and assistance of the many members of Dr. Sai Ma’s lab, who have contributed both directly and indirectly to the research presented in this thesis. In particular, I would like to thank Steven Tang, Edgar Chan Wong, Kyle Ferguson, Agata Minor, Sina Sakian, Andrew Wilson and Kevin Ma.

This project would not have been possible without the support of Dr. Victor Chow from the Department of Urology, who performed the testicular biopsies required for the research presented in this thesis.

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Most importantly, I would like to thank my parents for their continued support and their endless love and encouragement.
CO-AUTHORSHIP STATEMENT

CHAPTER II:

The experiments were conceived of by Dr. Sai Ma. The majority of the experiments and data analysis were performed by Gordon Kirkpatrick. Kyle Ferguson assisted with the analysis of segregation for three cases. With the permission of Dr. Sai Ma, previously published data (nine control men) have been included in this thesis. The analysis of the additional control case was performed by Gordon Kirkpatrick. All figures were prepared by Gordon Kirkpatrick and the manuscript was written by Gordon Kirkpatrick and Dr. Sai Ma.

CHAPTER III:

The experiments were conceived of by Dr. Sai Ma. The analysis of interchromosomal effects in all carriers was performed by Gordon Kirkpatrick. With the permission of Dr. Sai Ma, previously published data (nine control men) have been included in this thesis. The analysis of the additional control case was performed by Gordon Kirkpatrick. Analysis of OAT men was performed by Haijun Gao and Steven Tang. Data analysis and figure preparation was performed by Gordon Kirkpatrick. The manuscript was written by Kyle Ferguson, Gordon Kirkpatrick and Dr. Sai Ma.

CHAPTER IV:

The experiments were conceived of by Dr. Sai Ma and Kyle Ferguson. Dr. Victor Chow performed all testicular biopsies. All experiments, data analysis and figure preparation were performed by Gordon Kirkpatrick. The manuscript was written by Gordon Kirkpatrick and Dr. Sai Ma.
CHAPTER 1: INTRODUCTION

The genetic content of humans is divided into structural units of DNA and protein known as chromosomes. Large-scale changes, in the organization or structure of the chromosomes, are known as chromosomal abnormalities. Chromosomal abnormalities can be balanced, in which there is no net loss or gain of DNA, or unbalanced, in which there is a net change. The most common risks, associated with individuals that are carriers of balanced chromosomal abnormalities, are related to sexual reproduction. The process of reproduction begins with the development of gametes, cells containing half the normal chromosome complement, which will go on to fertilization, where the fusion of two gametes will create a cell with a normal adult chromosome complement. The cell division meiosis, a process specific to gametogenesis, involves the segregation of homologous chromosomes, reducing the chromosome complement by half, ensuring that following fertilization the adult chromosome complement will be normal. This thesis will address the role of meiosis, in infertility and the development of chromosomally unbalanced sperm, in individuals with chromosomal abnormalities. To begin, I will discuss the spermatogenic process in general, then describe several common pathologies of infertility, then describe the division of meiosis specifically and finally discuss chromosomal abnormalities in detail.

1.1 Spermatogenesis

Spermatogenesis, which begins in the seminiferous tubules of the testes, is the process whereby a self-renewing population of spermatogonial stem cells develops into mature spermatozoa (Sherwood, 2004). The structure of the testes begins to form during embryo development with the arrival of primordial germ cells (PGCs), which have migrated into the extra-embryonic mesoderm. PGCs then begin to divide and develop an invasive phenotype, which allows them to migrate through the embryo to the genital ridge (Sutton, 2000). Once at the genital ridge, expression of the sex determining region Y (Sry) gene, on the Y chromosome, signals differentiation into testes (Koopman et al., 1991). Finally, environmental signals direct the differentiation of PGCs into spermatogonial stem cells (SSCs) (Sutton, 2000). Each cycle of spermatogenesis takes
approximately 64 days to compete (Heller and Clermont, 1963) and can be subdivided into three stages: 1) mitotic division of spermatogonia 2) meiotic divisions and 3) differentiation into mature spermatozoa (spermiogenesis).

In order to maintain spermatogenesis continuously throughout life, a population of spermatogenic stem cells constantly replenishes itself through mitotic division. The seminiferous tubule consists of the germinal epithelium and the pretubular tissue or lamina propria. The germinal epithelium consists of cells in various stages of development ranging from least differentiated cells, at the outer edge, to spermatozoa, being released into the inner lumen of the tube, all located within invaginations of Sertoli cells.

Cells at the outermost layer of the germinal epithelium have three classifications: dark type A (Ad), pale type A (Ap) and type B spermatogonia (Clermont, 1966) (Figure 1.1). The products of Ad type spermatogonia mitosis are more undifferentiated Ad type cells, which function to replenish the pool, but also Ap cells. After further mitotic divisions, these Ap cells differentiate further into type B cells. These type B cells differentiate again into primary spermatocytes, which will go on to enter meiosis. Primary spermatocytes begin the first meiotic division (MI) where they undergo a reductional division, in which homologous chromosomes segregate, reducing the number of chromosomes by half. The second meiotic division (MII) involves the segregation of sister chromatids further reducing the genomic content by half. The final stage of spermatogenesis, spermiogenesis, consists of the specialization of spermatids into highly specialized, functional spermatozoa. Included in this process is the development of the acrosome cap from the golgi body, the repackaging of DNA, and the development of the flagellum (Holstein et al., 2003). The mature spermatozoa are released from between the Sertoli cells into the lumen of the seminiferous tubule, in a process known as spermiation, where they are transported to the epididymis. Spermatozoa spend approximately two weeks in the epididymis where they develop motility (Jones, 1999).
Figure 1.1 Spermatogenesis within the seminiferous tubule.

Spermatogenesis begins with the mitotic differentiation of the spermatogonial stem cells followed by meiosis I (MI) and meiosis II (MII) in which the chromosome content is reduced from diploid (2n) to haploid (1n).

1.1.1 Regulation of spermatogenesis

The other primary function of the testes is the secretion of testosterone and other neuroendocrine factors by the Leydig cells. These hormones act on neighboring Leydig cells, blood vessels and the cells of the lamina propria to maintain Sertoli cells, and the cells of the pretubular tissue (Holsten et al., 2003). Furthermore, they influence the contractility of the peristaltic movements, which transport spermatozoa prior to the development of sperm motility. These functions are regulated throughout life by two
hormone products of the anterior pituitary: lutenizing hormone (LH) and follicle stimulating hormone (FSH). Both LH and FSH are regulated at the level of the anterior pituitary by gonadotrophin releasing hormone (GnRH), which is released by the hypothalamus. LH acts to increase testosterone production through stimulation of the Leydig cells. FSH acts on the Sertoli cells to stimulate and maintain spermatogenesis (Sherwood, 2004). Testicular function is regulated by two negative feedback loops: 1) GnRH release by the hypothalamus is suppressed by testosterone and 2) FSH secretion is suppressed at the level of the anterior pituitary by the release of inhibin from the Sertoli cells.

1.2 Male Infertility

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse. Infertility is known to affect approximately 15% of couples. The cause of infertility is defined as either: female factor (35%), male factor (30%), combined factor (20%), or unexplained infertility (15%). Of those with male factor infertility, the cause is further divided into: 1) sexual disorders, 2) obstruction of the reproductive tract, 3) immunological dysfunction or 4) abnormal sperm production. This thesis will primarily concern the later, the disrupted or aberrant production of sperm.

1.2.1 Sperm parameters

The determination of a patient’s semen parameters can be a useful tool in elucidating the nature of the patient’s infertility as well as determining prudent treatment options. Semen parameters consist of three primary characteristics as defined by the World Health Organization (WHO 1999): sperm concentration, motility and morphology (Table 1.1). Concentration is reported as the number of sperm per milliliter seminal fluid. Motility is classified as either: rapid progressive (>25 um/s), slow progressive, non progressive (<5 um/s) or immotility. Defects in morphology are classified as pertaining to either: 1) head defects, 2) midpiece and neck defects, 3) tail defects, including multiple tails, or 4) the presence of vacuoles or cytoplasmic droplets in the midpiece (WHO, 1999).
Based on semen parameters, patients are classified as either: oligozoospermia (low sperm count, <20x10^6/ml), azoospermia (absence of sperm), asthenozoospermia (low sperm motility, <50% normal motility), or teratozoospermia (abnormal sperm morphology, <30% normal morphology). The combination of all three sperm defects is referred to as oligoasthenoteratozoospermia (OAT). In cases of azoospermia, the distinction is further classified as obstructive azoospermia (OA) or non obstructive azoospermia (NOA). Obstructive azoospermia can have a number of causes including: 1) congenital bilateral absence of vas deferens, 2) vasectomy, 3) obstruction due to infective/inflammatory diseases 4) non-infective blockage and 5) ejaculatory problems.

### Table 1.1 World Health Organization (WHO) diagnoses of semen parameters (WHO, 1999)

<table>
<thead>
<tr>
<th>Type of male infertility</th>
<th>Concentration (10^6/ml)</th>
<th>Motility (%)</th>
<th>Normal morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligozoospermia</td>
<td>&lt;20</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>Normal</td>
<td>&lt;50%</td>
<td>Normal</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>Normal</td>
<td>Normal</td>
<td>&lt;30%</td>
</tr>
<tr>
<td>Oligoastenoteratozoospermia (OAT)</td>
<td>&lt;20</td>
<td>&lt;50%</td>
<td>&lt;30%</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>No sperm in the ejaculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspermia</td>
<td>No ejaculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal sperm parameters</td>
<td>≥20</td>
<td>≥50%</td>
<td>≥30%</td>
</tr>
</tbody>
</table>

#### 1.2.2 Testicular histology

In cases where an evaluation of sperm parameters is uninformative, more information can be gleaned from a histological evaluation of the testes. Spermatogenesis is commonly classified as either: normal spermatogenesis, Hypospermatogenesis (HS), germ cell arrest (GCA), or Sertoli-cell only syndrome. Hypospermatogenesis is diagnosed when all stages of spermatogenesis are observed but at reduced levels. GCA, also
referred to as meiotic arrest, is diagnosed when complete arrest is observed at one stage of spermatogenesis. Complete absence of spermatogonia in the testes is defined as Sertoli cell-only Syndrome (SCOS). This condition may be present from birth (congenital Sertoli cell-only Syndrome) or may be induced due to exposure to a variety of noxes including X-radiation, (acquired Sertoli cell-only Syndrome) (Holstein et al., 2003).

1.2.3 Genetic causes of infertility

As previously mentioned defects in spermatogenesis are the most common cause of male infertility. While all causes of infertility most likely have some indirect genetic component (Shah et al., 2003), a significant component of male infertility can be directly attributed to genetic causes. Of these genetic causes, the most common are Y chromosome microdeletions, cystic fibrosis, androgen receptor gene mutations, meiotic defects and finally, chromosomal abnormalities, which will be discussed in greater detail in section 1.4.

1.2.3.1 Y chromosome microdeletions

The Y chromosome contains genes crucial for proper spermatogenesis, and indeed, microdeletions on this chromosome are the most common molecular causes of male factor infertility (Krausz et al., 2003). The most common of these deletions are Y chromosome long arm microdeletions at the locus Yq11, known since 1976 to be involved in spermatogenesis (Tiepolo and Zuffardi, 1976). Three distinct, non-overlapping regions, referred to as azoospermic factors (AZF a, b and c) have been defined as spermatogenic loci (Vogt et al., 1996). More recently, a fourth (AZF d) locus has been proposed to exist between AZF b and c (Kent-First et al., 1999). While deletions in these regions cause varying degrees of infertility, their exact function in spermatogenesis remains unclear. The majority of observed deletions involve both AZF b and c loci, with fewer involving the two genes at the AZF a loci (Kuroda-Kawaguchi et al., 2001). These microdeletions are believed to be caused by intrachromosomal homologous recombination involving identical repeat sequences organized in palindromic blocks of DNA (Kuroda-Kawaguchi et al., 2001). The most frequent deletion, AZF c, which accounts for approximately 60% of cases, removes 8 gene families including: a) the deleted in azozospermia (DAZ) family (Reijo et al., 1995), b)
testes transcript Y2 (TTY2) and c) basic protein Y2 (BPY2) and results in azoospermia or severe oligospermia (Lahn and Page, 1997). AZF a deletions usually result in the absence of germ cells (SCOS) (Vogt et al., 1996). The candidate genes in the AZF a region are death box on the Y (DBY) and USP9Y (Foresta et al., 2000). Deletions involving AZF a alone, account for only 5% of cases (Krausz et al., 2003) while deletions involving AZF b and another deletion account for 35% of cases. Complete deletions of AZF b as well as AZF b and c lead to SCOS or pre-meiotic spermatogenic arrest. However, as there is often a range of phenotypes (from azoospermia to oligospermia) observed in cases with identical deletions, this suggests the influence of other genes.

1.2.3.2 Cystic Fibrosis transmembrane conductance regulator (CFTR) mutations

An additional source of infertility arises in patients with the autosomal recessive disorder cystic fibrosis (CF). Cystic Fibrosis involves a deletion in the cystic fibrosis transmembrane regulator (CFTR) gene. The CFTR gene is located in the q31.2 band of chromosome 7. 80% of CF patients have a deletion of 3 base pairs resulting in the loss of a phenyalanine at position 508. The life expectancy of CF patients is in the mid 30’s with clinical manifestations including progressive lung disease and pancreatic malfunction (Gazvani and Lewis-Jones, 2006). In addition to these symptoms, over 95% of CF men have obstructive azoospermia, arising from a congenital bilateral absence of the vas deferens (De Braekeleer and Ferec, 1996). While the disease is recessive, carriers of only one mutation in the CFTR are often found to be infertile, and genetic screening of these men when seeking assisted reproduction is encouraged as mutations in the CFTR gene can be transmitted to offspring (Gazvani and Lewis-Jones, 2006).

1.2.3.3 Meiotic disorders

Recently we have developed an increased understanding of the role of meiotic disturbances in infertility. Such a disturbance in meiosis can result from mutations in meiotic genes (Barlow and Hulten 1996, Hassold 1996, Grootegoed 1998) or from influence of an abnormal testicular environment (Speed and Chandley 1990, Rives et al., 1998, Mroz et al., 1999). While the importance of meiotic genes in relation to fertility has been extensively displayed in studies of knockout mice (reviewed in Hunt and Hassold 2002), little direct evidence for the importance of these meiotic mutations in human
infertility has been shown. Screening for deletions in meiotic genes, in infertile men, has had some limited success, with studies showing some evidence for SCP3 (Synaptonemal complex protein 3) mutations (Miyamoto et al., 2003), but none for mutations in Fkbp6 (FK506 binding protein) (Westerveld et al., 2005), or in Spo11 (SPO11 meiotic protein) (Christiansen et al., 2005). A recent study has determined that a deletion in AURAC (Aurora Kinase C), a gene involved in spindle assembly at meiosis, results in large-headed, polyployd and mutli-flagellar spermatozoa and infertility (Dieterich et al., 2007).

1.2.3.4 Other genetic causes of infertility

Numerous other syndromes, that have a genetic basis, also have an impact on fertility. Prader-Willi and Angelman syndromes, caused by deletions in an imprinting region in 15q on the paternal and maternal derived chromosomes respectively, have severely reduced fertility. Kallmann syndrome, Noonan syndrome, and Denys-Drash syndrome, all exhibit reduced fertility, and are all associated with deletions in single genes (Shah et al., 2003).

1.3 Meiosis

Meiosis is a specialized process of cellular division for producing gametes. This occurs in the ovaries and testes of women and men. Meiosis involves the replication of the normal diploid (2N) complement of DNA in the cell, followed by two successive rounds of division, meiosis I (M1) and meiosis II (M2) resulting in haploid (N) cells. The first meiotic division M1 involves the segregation of homologous chromosomes (known as a reductional division); this reduces the genetic complement from diploid to haploid status. There are two critical steps in this process. The first involves the synapses of homologous chromosomes, in which the homologues pair, and a protein structure known as the synaptonemal complex (SC) forms along the chromosomes length. Secondly, meiotic recombination occurs between these homologous chromosomes, allowing the exchange of genetic material. The second meiotic division (M2) is characterized by the segregation of sister chromatids, in a manner similar to mitosis.

Both M1 and M2 are subdived into four stages: prophase, metaphase, anaphase and telophase. Prophase of M1, perhaps the most critical stage of meiosis, can itself be
divided into five stages, based on the progression of the SC: leptotene, zygotene, pachytene, diplotene and diakinesis (reviewed in Champion and Hawley, 2002, Figure 1.2). During lepotene, homologous chromosomes condense and associate, during which time axial elements of the SC begin to form between the chromosomes. During the second stage, zygotene, the lateral elements of the SC form from the axial elements and synopsis occurs at points where transverse elements form between homologous chromosomes. The pachytene stage consists of the completion of homologous synapse. The final two stages, diplotene and diakinesis, are characterized by desynapsis, leaving chromosomes connected only at the chiasmata, where meiotic recombination has previously occurred.

1.3.1 Synapsis

Prior to synapsis of the homologous chromosomes, these chromosomes must find and then pair with each other based on sequence homology. This initial process is one of the least understood aspects of meiosis. However, a key step is believed to occur at the leptotene-zygotene transition, involving the clustering of chromosome telomeres at the nuclear envelope (Page and Hawley, 2003). The SC, first described in 1956 (Moses, 1956), is the protein structure, unique to synapsis, which facilitates the synapsis of chromosomes at meiosis, a process crucial for stabilizing the interaction between homologous chromosomes. Synapsis is characterized by the formation of the synaptonemal complex, which consists of three components: two axial/lateral elements and a transverse element (Figure 1.2). The two lateral elements, which have identified components in mammals: are the synaptonemal complex proteins 2 (SCP2) and 3 (SCP3) and are formed along the length of the chromosomes (Heyting et al., 1985). A DNA binding domain has been identified in these proteins; it facilitates their adhesion to the chromosomes (Offenberg et al., 1998). The transverse element includes a component SCP3, which has been shown to have a coiled–coil protein structure with two globular domains. This structure allows for the formation of parallel SCP1 dimers with N-terminal globular domains interacting with each other, and C-terminal domains interacting with the lateral elements (Page and Hawley, 2003).
Figure 1.2 Stages of prophase in meiosis 1.

During leptotene, chromosomes pair and axial elements of the synaptonemal complex (SC) form; during zygotene, the chromosomes synapse with the formation of the transverse elements of the SC; at the pachytene stage, synapsis is completed, followed by the completion of meiotic recombination which is characterized by the localization of the DNA mismatch repair protein MLH1; finally, during diplotene/diakinesis the chromosomes desynapse and begin to segregate, forming chiasmata at sites of recombination.

While the SC is highly conserved in eukaryotes, the temporal aspect of synapsis is believed to vary remarkably between species (Brown et al., 2005). Homologous chromosomes have been shown to pair via a number of distinct mechanisms. These can generally be divided into Double Stranded Break (DSB) dependant and independent mechanisms. DSB dependant mechanisms usually involve a homology search based on

1.3.2 Recombination

Recombination generates reciprocal exchanges of genetic material between two homologous chromosomes during prophase 1. This process is known to have two important functions: 1) the generation of genetic diversity and 2) the formation of chiasmata, the structural product of recombination crucial for proper chromosome segregation at M1.

The dominant theory of the mechanism of homologous recombination, proposed by Szostak \textit{et al.}, (1983), begins with the generation of double strand breaks (DSBs); these breaks produce 3’ single stranded DNA overhangs (Zenvirth \textit{et al.}, 2003) (Figure 1.3). It is estimated that the number of DSBs generated is vastly greater than the number of eventual crossovers; this suggests that many DSB are repaired without homologous recombination. In cases of successful homologous recombination, these DSBs are repaired across homologous chromosomes resulting in recombinant products. DSBs in DNA are created by the nuclease Spo11 (Keeney \textit{et al.}, 1997), while the 3’ overhang is created by a yet unknown exonuclease (Figure 1.3, step 1). These overhangs may progress to become single end invasions (SIEs) (Hunter and Kleckner, 2001). These SIEs will in turn conduct a homology search, catalyzed by the proteins RAD51 and DMC1 (Shinohara and Shinohara, 2004). This process, known generally as single end invasion, forms a displacement loop (D-loop) that anneals with the single-stranded overhang of the homologous break (step 2). Further DNA synthesis fills the single strand gaps at the exchanges, resulting in the formation of Holliday junctions (HJ) (step 3). Cleavage in two different orientations resolves the HJ resulting in the formation of either crossover (CO) or non-crossover (NCO) products (step 4). Evidence has shown that several components of the mismatch repair system are involved in the resolution of HJ (Hoffmann and Borts, 2004). Specifically, the heterodimer formed by mutS homologs MSH4 and MSH5 has
been implicated in this mismatch repair, in addition to other functions including: chromosome pairing (de Vries et al., 1999, Kneitz et al., 2000), crossover interference (Zavlevsky et al., 1999) and the stability of the Holliday junction (Snowden et al., 2004). Similarly, the heterodimer formed between mutL homologues MLH3 and MLH1 has been shown to be associated with crossover resolution and regulation. Indeed, MLH1 foci correlate to chiasmata, and are used in numerous studies to determine the number and distribution of crossovers (Hassold et al., 2004). The generation of crossovers links the two homologous chromosomes through the chiasmata; this structure ensures that the chromosomes are oriented correctly at the meiotic spindle, a process crucial for segregation.

Recently, studies have provided evidence suggesting the need for an alternate theory of recombination or at least an augmented one (Cromie and Smith 2007). These studies suggest that CO and NCO products do not arise from the same HJ intermediate. In fact, the pathway for the repair of these two distinct products branches immediately after DSB formation and proceeds through alternate intermediates (Borner, 2004, Terasawa et al., 2007). These studies suggest that the HJ model of recombination is only accurate in describing the generation of CO products. It is suggested that the NCOs occur by a process known as synthesis-dependant strand-annealing (SDSA). The mechanism of SDSA involves strand invasion, followed by a strand “pullout” that does not involve a HJ (Cromie and Smith 2007).

Recombination has been studied using a number of methods including gene-linkage analysis, chiasmata analysis and immunofluorescent analysis. Gene linkage analysis is an indirect method of determining the frequency of recombination. In this process, alleles, at specified locations on the same chromosome, are examined in a family. When two alleles, originating from separate grandparents, occur consecutively, a crossover can be inferred (Lynn et al., 2004). While studies have used this technique to relate reduced recombination to production of chromosomally abnormal conceptions (Warren et al. 1987), a major limitations of this technique is that recombination can only be examined on individual chromosomal regions at a time. A direct method for the analysis of recombination is through chiasmata analysis. Cells observed, during
diakinesis of prophase I, exhibit points of association between the homologous chromosomes, known as chiasmata, which represent the location of crossovers. Techniques, examining chiasmata during diakinesis, provided the first information on the frequency and distribution of crossovers in humans (Hulten, 1974). Chiasmata analysis on infertile men has also provided some evidence that defective recombination may be associated with male infertility (Chaganti et al., 1979; Chaganti et al., 1980; Micic et al., 1982). However, chiasmata analysis has been limited by the short duration of diakinesis and therefore the small number of cells that can be studied in a given patient. Furthermore, diakinesis spreads are challenging to analyze. It is difficult to determine the identity of chromosomes and the specific locations in which crossovers occur. The identification of proteins involved during meiosis, has led to the development of immunocytogenetic techniques for studying recombination. Antibodies against MLH1, a late-mismatch repair protein, have been used to observe and enumerate the sites of recombination (Barlow and Hulten, 1998). Immunocytogenetic techniques have advantages over previous techniques, in that they allow researchers to address questions that were previously difficult to answer, such as the range of inter-individual variation in recombination rates (Barlow and Hulten, 1998; Lynn et al., 2002; Hassold et al., 2004; Sun et al., 2005b).

1.3.3 Distribution of crossovers

Studies that have examined the normal distribution of crossovers in humans, have indicated that crossovers are distributed non-randomly across the chromosomes. Cytological studies have revealed several trends in the distribution of meiotic recombination in humans, that suggest that crossover position is influenced by a number of factors including: 1) nature of the chromosome involved, 2) position of other chromosomes, 3) effect of centromeres and telomeres, 4) presence of specific sequences of DNA.

Firstly, it has been noted in both genders that in general all chromosomes show at least one crossover and that these crossovers rarely occur near centromeres (Tease, 2004). Laurie and Hulten (1985) have pointed out several trends in human males that attempt to describe the variation in recombination along the length of a chromosome. These include:
1) single chiasmate chromosome arms showed subterminal or medial placement while double chiasmate chromosome arms showed a distal-proximal placement, 2) small arms showed single chiasma, 3) large acrocentrics (13, 14, 15) had two recombination sites while small arcocentrics (21, 22) had a single site with 21 having a terminal site and 22 having a medial site. The structure of the SC between the chromosomes has also been implicated in determining sites of recombination. Chromosomes with incomplete synopsis or complete gaps in synopsis have been shown to have more distally located site of recombination (Sun et al., 2005). Laurie and Hulten (1985) also noted an interference force, which causes crossovers to never appear close to other crossovers. This force seems to be even more powerful than the repulsive nature of centromeres, as can be observed in large acrocentrics where one crossover is often forced to be located proximal to the centromere in order to remain distal to the other crossover.

As previously noted there are relatively low rates of recombination in the pericentric region of the chromosome. This observation has been confirmed in numerous studies using a variety of techniques (Beadle, 1932; Jackson et al., 1996; Lynn et al., 2002). Explanations for the lack of recombination at the centromere have included interference from centromeric activity and the presence of highly condensed heterochromatin (Lynn et al., 2004; Choo, 1998). In contrast, crossovers have been commonly observed near the structure at the distal end of the chromosome, the telomere, albeit only in males (Laurie and Hulten, 1998). Studies of the frequency of meiotic exchanges have shown that certain “hot” (or cold) sequences of DNA exhibit increased (or decreased) rates of recombination. While certain regions, such as the centromeres, are very large, the vast majority of these hot/cold spots are very small. In males, sperm typing studies have shown that certain sequences, including the MHC region (Jeffreys et al., 2001), the B-globin gene and the Pseudo autosomal region (PAR) (May et al., 2002) of the Y chromosome, have unique characteristics. Within the MHC region, Jeffreys et al. (2001) noted several small (1-2kb) hot spots, interspersed non randomly with cold regions. Equally, in their analysis of the PAR, May et al. (2002) noted that recombination was limited to small regions of violent activity. While yeast show hot spots in regions of defined transcriptional activity (Wu and Lichten, 1994) or specific sequence patterns no
such pattern for recombination hot spots in humans has been found (reviewed in Kauppi et al., 2004).

1.3.4 Sex differences in meiosis

Gross differences in meiosis between genders are largely temporal in nature. In females meiosis begins during fetal development but is halted during M1, finishing M1 only upon ovulation. Cells are arrested once again in M2, this time only resuming meiosis upon fertilization of that oocyte with a sperm (Hunt and Hassold, 2002). In males, meiosis begins at puberty and continues uninterrupted. Furthermore, there is a stark difference in the male and female response to meiotic disturbance. Abnormalities in spermatogenesis generally result in meiotic arrest and apoptosis, while oogenesis is generally allowed to continue. This difference is responsible for the same meiotic abnormality causing infertility in males while allowing fertility in females (Paoloni-Giacobino et al., 2000).

Molecular sex differences in meiosis are largely due to differences in recombination. Sex differences in recombination have been noted not only in humans but also in a large number of model organisms, including fruit flies (Morgan 1912), zebrafish (Singer et al., 2002) and wallabies (Zenger et al., 2002). The first linkage map of the human genome (Donis-Keller et al., 1987) provided the first direct evidence that recombination was increased in females. Studies using direct measures of recombination have provided further evidence of this effect showing that the number of crossovers in men ranged from 46.1-52.8 (Barlow and Hulten 1998, Lynn et al., 2002), while in females the number ranged from 70.3-95.0 (Barlow and Hulten 1998, Tease et al., 2002). Recently, studies have suggested that this difference in frequency of recombination between genders is attributable to the considerable increase in length of the synaptonemal complex in females (Tease and Hulten 2004, Lynn et al., 2002). As well as crossover number, there is evidence that a larger variation in numbers of crossovers exists in females (Lenzi et al., 2005). Recent evidence showing that recombination foci occur earlier in females has also suggested a temporal difference in the regulation of recombination (Tease et al., 2006).
In terms of crossover placement, as previously noted, some gender differences have been discovered. In general, findings suggest that females show higher rates of recombination near the centromere, while males have higher rates closer to the telomere (Kong et al., 2002, Matise et al., 2003). This gender difference may be due to differences in synaptic initiation. In males, synopsis is initiated at subtelomeric sites (Brown et al., 2005), whereas in females, initiation occurs more frequently at interstitial sites (Bojko et al., 1983).

In addition to gender comparisons, work has been done to determine the degree of interindividual variation in rates of recombination. The largest of these studies, conducted by Hulten and colleagues (Laurie and Hulten 1985a, Laurie and Hulten 1985b) found that, in spermatocytes, the frequency of exchanges ranged from 44-56 with differences being found only on the larger chromosomes. The authors suggested that this reflected the fact that smaller chromosomes are often limited to a single exchange, and so have less capacity for variation. There has been significant interest in determining the presence of an age effect on recombination, however, numerous studies have shown no such effect (Broman 1998, Kong et al., 2002, Lange et al., 1975, Laurie and Hulten 1985).

1.3.5 Meiotic silencing of the sex chromosomes

Human males have two distinctly different sex chromosomes. However, these two chromosomes do share a small region of homology, termed the pseudo-autosomal region (PAR). It is within this region that the recombination, that is critical for the proper segregation of the sex chromosomes, occurs. During meiosis, the two sex chromosomes pair and form a structure known as the XY or sex body (SB) (reviewed in Handel, 2004). In the formation of the sex body, the chromosomes become inactivated in a process referred to as meiotic sex chromosome inactivation (MSCI). MSCI consists of the remodeling of the sex chromosomes into heterochromatin and the formation of a unique sub nuclear domain characterized by a complete lack of RNA synthesis and the accumulation of proteins unique to this sub domain. Unlike mitotic inactivation, MSCI has been shown to be \textit{Xist} (X-inactive specific transcript) independent (Turner, 2006), but instead requires a different mechanism, including the sequestration of epigenetic
marks such as histone H2AX. This histone has been shown to be indispensable for sex chromosome condensation (Fernandez-Capetillo et al., 2003).

During the leptotene stage of prophase 1, in response to the formation of DSB necessary for recombination, the H2AX (histone family, member X) is phosphorylated, forming $\gamma$H2AX, on all chromosomes (Mahadevaiah et al., 2001). During the zygotene/pachytene transition, BRCA1 (breast cancer 1) protein, localized to the sex chromosome, recruits the ATR (ataxia telangiectasia and Rad3 relative) which in turn leads to a second round of H2AX phosphorylation, this time specific to the sex chromosomes (Turner et al., 2004). MSCI is established with the recruitment of additional chromatin modifications including: histone H3 and H4 deacetylation (Khalil et al., 2004) and ubiquitylation of H2A (Baarends et al., 1999). BRCA1, $\gamma$H2AX, and ATR are lost during the diplotene stage of prophase 1, however, the other histone modifications remain, maintaining inactivation through the first and second meiotic segregations.

### 1.3.6 Checkpoints in meiosis

Meiosis is a highly regulated process that is monitored by well defined checkpoints, where errors in meiosis will cause initiation of apoptosis, and cell death. Two distinct meiotic checkpoints have been suggested to date: the pachytene checkpoint and the spindle checkpoint. The first meiotic checkpoint suggested, is one that occurs during the pachytene stage of meiosis (Odorisio et al., 1998, Roeder and Bailis 2000). The evidence for such a checkpoint comes from observed meiotic arrest in mice with various mutations for proteins involved in recombination and synapsis (Judis et al., 2004). Mittwoch and Mahadevaiah (1992) showed that apoptosis can be prevented if the asynapsis is repaired prior to checkpoint, affirming asynapsis as a cause opposed to a consequence of cell death. The next checkpoint occurs later in meiosis at metaphase 1, and acts to ensure that the chromosomes are aligned properly at the metaphase plate. It is believed that cells in which there is insufficient tension within the kinetochores of the spindle apparatus will trigger the checkpoint, and prevent the metaphase-anaphase transition (Eaker et al., 2001). It is believed that this checkpoint may be analogous to the well characterized spindle assembly checkpoint that operates during mitosis (Zhou et al., 2002).
Mounting evidence now suggests that meiotic checkpoints operate differently in males and females, with female checkpoints being more permissive to errors (Hunt and Hassold, 2002). In gene knock out studies on mice, the results of a single mutation were markedly different between sexes. Separate experiments, deleting either Scp3 (Yuan et al., 2000) or Fkbp6 (Crackower et al., 2003), resulted in spermatogenic arrest in male mice, while females remained fertile.

1.3.7 Non disjunction and aneuploidy

Aneuploidy describes a state where the chromosome number of an individual is not a multiple of the haploid set. In other words, the individual has an extra or missing chromosome, resulting in either trisomy for that chromosome (if extra) or monosomy (if missing). Aneuploidy is the most common chromosome abnormality, present in 5% of recognized human pregnancies (Hassold and Hunt 2001). It is the leading cause of miscarriage and congenital birth defects. In order for aneuploidy to occur, there must be an error in the segregation of chromosomes during meiosis; this is referred to as a non disjunction (NDJ) event. As meiosis involves two sequential segregations there are two points at which such a NDJ may occur (Figure 1.4). Indeed, errors in segregation are observed in both M1 and M2 with slightly different results. However, most observed NDJ events occur during M1 (Hassold et al., 1996). While all chromosomes can theoretically non-disjoin only a subset of chromosome aneuploidies are ever observed. It is not clear if this is due to a bias in the mechanism of segregation, in that certain chromosomes are less likely to non disjoin, or if certain chromosome aneuploidies are merely less conducive to germ cell survival. Of all the chromosomes that are commonly found to be aneuploid only a few are viable well into pregnancy. This includes: trisomy 13 (patau syndrome), trisomy 18 (Edwards syndrome), trisomy 21 (downs syndrome) and a variety of sex chromosome aneuploidies including Klinefelter’s (XXY) and Turner X and XYY. The vast majority of aneuploidies have been shown to be maternally derived. However, the exceptions are sex chromosome aneuploidies, in which paternal errors are implicated in a significant number of cases. As such, it has been suggested that the sex chromosomes may be uniquely vulnerable to non-disjunction during spermatogenesis.
Equally, it is possible that spermatogenesis is merely more permissive of sex chromosome aneuploidies than of autosomal aneuploidies (Hassold et al., 2004)

Figure 1.3 Production of aneuploid gametes through non-disjunction at meiosis.

Non-disjunction at Meiosis I (MI) produces nullisomic and disomic gametes while non-disjunction at Meiosis II (MII) produces normal, disomic and nullisomic gametes.

Recent studies over the last 15 years have highlighted the importance of errors in recombination in the genesis of aneuploidy. This is most prevalent in instances where there is an absence of recombination on a chromosome. In normal recombination, when there is at least one homologous recombination event per chromosome, after the SC disappears, the bivalent is held together by the chiasmata. If the chromosomes fail to recombine then the bivalents will no longer be attached and will become univalents. The likelihood of segregation disturbance and resulting non-disjunction is greatly increased as
univalents segregate in an unpredictable manner (Hassold and Hunt 2001). Indeed, several studies have shown a relationship between reduced recombination and non-disjunction (Gonsalves et al., 2004; Ma et al., 2006; Ferguson et al., 2007). Trisomies have been shown to be more likely to occur if there has been no recombination in that chromosome. For example, the vast majority of XXY cases involve the absence of a crossover at the pseudo autosomal region on the sex chromosomes. In addition to the absence of recombination, alterations in the localization of recombination are linked to non-disjunction. Studies have shown that recombination, too close or too far from the centromere, is linked to non-disjunction (Lynn et al., 2004).

Studies have noted certain patterns of chromosomal differences in non-disjunction. Chromosome 21 has been found to disjoin most often at maternal M1, while chromosome 18 trisomy is mostly maternal M2 derived. As mentioned previously, XXY is unusual in being equally paternally and maternally derived (Hassold and Hunt, 2001). To some, this pattern observed in acrocentrics suggests a common mechanism of non-disjunction (Hassold and Hunt, 2001). However, there remains some variety in the patterns of non-disjunction. This suggests to some that other mechanisms could be involved including: spindle assembly errors, or sister chromatid cohesion (Hassold and Hunt, 2001). Authors have suggested that the motor proteins associated with the centromere, that are involved in the active movement of chromosomes along the spindle fibers, may be a point of vulnerability, and that errors in the assembly or action of these proteins may account for errors in disjunction (Hodges et al., 2002).

1.4 Chromosome Abnormalities

Chromosomal abnormalities are closely linked to male infertility with a ten-fold increase in incidence of infertility over the general population (Johnson, 1998). Chromosomal abnormalities can be of two general varieties: structural or numerical. Structural chromosomal abnormalities, involve a large-scale rearrangement of the architecture of one or more chromosomes. Included in this category are translocations, involving the exchange of regions chromosome between chromosomes, and inversions, involving the movement of a region of a chromosome within that chromosome.
Numerical chromosomal abnormalities involve the loss or addition of one or more chromosomes or chromosome regions from the normal chromosome complement. The magnitude of numerical abnormalities can range from the presence of an additional whole chromosome, or trismoy, to the presence of small supernumerary marker chromosomes. Chromosomal abnormalities disrupt fertility through two primary mechanisms. Firstly, through the production of chromosomally abnormal sperm, which result in pre and post implantation losses if involved in fertilization. Secondly, the aberrant behaviour of the abnormality is believed to activate meiotic checkpoints resulting in direct disruption of spermatogenesis. However, the variable effect on fertility, observed in carriers of chromosomal abnormalities, remains largely unexplained.

1.4.1 Structural chromosomal abnormalities

Structural rearrangements are known to occur de novo in 20% of cases while the remainder are believed to be inherited (Jacobs, 1992). In general, the phenotypic impact of a structural abnormality is dependant on the presence of any gain or loss of DNA. Rearrangements exhibiting no such loss are said to be “balanced” rearrangements, while “unbalanced” rearrangements are those in which there is a net gain or loss of a region of chromosome(s). Even balanced rearrangements can result in phenotypic consequences, if the breakpoints, the locations where the moved regions of chromosomes are excised from, are located within critical coding or regulatory sequences. Generally, unbalanced rearrangements, involving gene rich DNA, are associated with mental retardation and physical deformities, while the impact of balanced rearrangements is limited to abnormal semen parameters (Van Assche et al., 1996).

1.4.1.1 Translocations

Translocations exist in two main forms: reciprocal translocations (RT) and Robertsonian translocations (ROB). ROBs involve the fusion of the long, q arms of two acrocentric (13, 14, 15, 21, 22) chromosomes at the centromere (Figure 1.5 A). ROBs occur in 0.086 % of newborns (Benet et al., 2005), with approximately half being transmitted from a parent, and half occurring de novo. The translocation results in a single dicentric chromosome (45, XY karyotype) and the subsequent loss of the p arms. The p-arms of all acrocentric chromosomes contain RNA (nucleolar organizing regions
[NOR]) genes. Studies have shown that not all 10 NOR are active (Varley, 1977) and so these regions are, to some degree, redundant and as such these rearrangements are still considered “balanced”. While there are ten pair-wise combinations of acrocentric chromosomes, the most common are by far the t(13;14) and t(14;21) comprising 75.6% and 9.9% of all Robertsonian translocations (Page et al., 1996). It is believed that ROBs form through recombination between homologous regions on non-homologous chromosomes. All acrocentrics contain repeat satellite DNA sequences around the centromere, which may provide the areas of homology required for homologous recombination. However, the predominance of the rob(13;14) and rob(14;21), which occur at specific and predictable breakpoints, suggest that these chromosomes may have specific inverted homologous sequences which encourage aberrant crossovers (Bandyopadhyay et al., 2001a, 2001b).
Figure 1.4 Formation and meiotic behavior of Robertsonian translocations (ROB).

(A) ROBs form through the fusion of two acrocentric chromosomes at the centromere. (B) During meiosis, ROBs form a trivalent structure allowing syanpses between the translocated and normal chromosomes. (C) The most common segregation patterns observed are Alternate which result in a balanced or normal chromosome complement or Adjacent (D,E) resulting in either nullisomic or disomic products.
Reciprocal translocations are the most common form of chromosomal rearrangement, occurring with a population incidence ranging from 0.092% (Benet et al., 2005) to 0.14% (Nielsen and Wohlert 1991). RT involve reciprocal exchanges of regions of DNA between any of the chromosomes (Figure 1.6 A). Double stranded breaks form in two chromosomes and the regions distal to the centromere switch. The resulting translocated chromosome, known as a derivative (der), contains a centric fragment (containing the centromere) and a translocated fragment. Unlike ROBs, which are restricted to exchanges in and around the centromere, RTs can have breakpoints at any point along the chromosome. In addition, unlike ROBs, there are no recurring RTs, with the exception of a remarkably common t(11:22) translocation. It is believed that, due to the exchanges of DNA and the active nature of the chromosomes during meiosis, that most de novo translocations occur during this period. As the majority of de novo rearrangements are paternally derived, it is believed that spermatogenesis may be a particularly hazardous time for the production of rearrangements (Shaffer and Lupski, 2000). Numerous theories have been suggested to explain the formation of RTs including: 1) faulty DSB repair in spermatozoa, where repair mechanisms are inactive, (Genesca et al., 1992); 2) presence of hairpin DNA structures at the breakpoints (Kogo et al., 2007); 3) proximity of the chromosomes (Nikiforova et al., 2000), and presence of recombination hotspots (Ashley, 2006).
Figure 1.5 Formation and meiotic behavior of reciprocal translocations (RT).

(A) RTs form through the reciprocal exchange of chromosome regions between two chromosomes. (B) During meiosis, RTs form a quadrivalent structure allowing synapton between the translocated and normal chromosomes. (C) The most common segregation patterns observed are Alternate which result in a balanced or normal chromosome complement, Adjacent 1 (D) resulting in either partial nullisomic or partial disomic products. Less common are Adjacent 2 (E) and 3:1 (F) segregation modes.

Translocations are associated with infertility, with the incidence of translocations ten times higher in the infertile population. However, the exact mechanism whereby translocations disrupt fertility is unclear. Indeed, identical translocations have been found
to have drastically different effects of fertility, and so the genetic background of the rearrangement may playa crucial role. Translocations are believed to influence fertility by two mechanisms: 1) interference with the spermatogenic process, 2) the production of sperm with unbalanced chromosome complements in the sperm. During meiosis, the rearranged chromosomes and their respective homologous pairs must adopt unique structures in order to pair all homologous regions. In the cases of reciprocal and Robertsonian translocations, the chromosomes adopt structures known as a quadrivalent (Figure 1.6 B) and trivalent (Figure 1.5 B) respectively. Translocations can disrupt spermatogenesis through a variety of mechanisms, generally related to the presence of the quadrivalent/trivalent. The formation of the quadrivalent/trivalent is expected to be more complex than the normal pairing and synapse of homologous chromosomes. As a result, there may be spatial or temporal constraints, during meiosis, whereby if the quadrivalent/trivalent forms too slowly the cell arrests (Forejt, 1982). In addition, it is known that the regions around the breakpoints of the translocation do not synapse completely and that in some carriers the rearrangement will associate with the heterochromatically silenced sex vesicle. There has been speculation this association could interfere with the expression of genes on both the rearrangement and the sex chromosomes thereby initiation meiotic arrest. The possible effects of the association of rearrangements with the sex body are discussed further in section 1.4.3.3.

As mentioned, translocation carriers can produce chromosomally unbalanced sperm, principally via the aberrant segregation of the translocated chromosomes, and their untranslocated homologous chromosomes. The production of unbalanced sperm can result in reduced fertility through pre- and post-implantation losses, or abnormal pregnancy outcomes following fertilization with those sperm (Morel et al., 2004). The effect of an unbalanced chromosome complement is largely dependant on the degree of imbalance of dosage sensitive genetic material. The physical size of the unbalanced region, as well as the gene density of the region are the primary determinants of phenotypic consiquence. Large losses or additions have such a deleterious effect on development that they result in abortion. Smaller imbalances may be compatible with intrauterine survival, with phenotypic effects becoming evident at some point later in life, of which mental defects are the most common (Raynham et al., 1996).
During meiosis, following the breakdown of the nuclear envelope, spindles form at each pole and move toward the equator where they attach to the centromeres. The quadrivalent/trivalent formed between the translocated chromosome(s) and their homologues can segregate at meiosis in a number possible ways (Figures 1.5 and 1.6 C, D, E and F) (reviewed in Benet et al., 2005) depending on the attachment of spindles from each pole.

RT segregations can be described as either 2:2, 3:1 of 4:0 depending on the number of chromosomes given to each daughter cell. Balanced chromosome complements result from alternate segregations (2:2), which produce sperm containing either the untranslocated chromosomes, or the balanced translocation (Figure 1.6 C). Two other 2:2 segregations, adjacent 1 (where the chromosomes with non-homologous centromeres segregate together) and adjacent 2 (where the chromosomes with homologous centromeres segregate together), produce unbalanced chromosome complements with deletions and duplications (partial aneuploidy) of regions of translocated chromosomes (Figure 1.6 D, E). Less common are 3:1 and 4:0 segregations, which produce even more grossly unbalanced chromosome complements (Figure 1.6 F).

The meiotic behavior of the quadrivalent will determine the number of sperm present, as well as the ratio of balanced/unbalanced karyotypes (Oliver-Bonet et al., 2004). Several characteristics of the quadrivalent have been suggested to effect the segregation: these include, 1) localization of breakpoints of the translocation in G light (gene rich) or G dark (gene poor) bands (Ashley, 1988); 2) frequency of exchanges in the interstitial region (Hulten et al., 1985); 3) length of chromosomal rearrangements (Jalbert and Sele, 1979), and 4) the presence of short fragments, small acrocentrics, as well as aggregated heterochromatin, which may all prevent the formation of chiasmata at meiosis, and therefore effect the proper segregation of chromosomes.

A high degree of variability, in the production of chromosomally abnormal sperm, has been observed in RT carriers. RTs have been shown to produce a range in the frequency of unbalanced sperm complements from 23-81%. The physical orientation of the chromosomes within the quadrivalent, as determined by the relative size of the
translocated and centric fragments, may explain the variability in the frequency of segregation modes. Indeed, Jalbert et al., (1980, 1988), provides a number of rules that are helpful in predicting the distribution of segregation modes. Firstly, if the translocated segments are small then an adjacent-1 segregation is more likely to occur. Conversely, if the centric fragment is small then adjacent-2 is more likely to occur. If one of the chromosomes in the quadrivalent is very small, then 3:1 segregations are more likely.

In ROB carriers, compared with RT carriers, the number of possible segregations is reduced. Six possible 2:1 segregations exist including alternate segregations, producing balanced or normal complements, as well as adjacent segregations producing two types of nullisomic and two types of disomic gamete (Figure 1.5 C, D, E). In contrast to RT carriers, unbalanced sperm from ROB carriers are essentially completely aneuploid. Prevalence for alternate segregations has been observed and is attributed to the cis-configuration at meiosis, in which the two normal chromosomes line up opposite the translocated chromosome. However, ROB carriers show a marked variety in the proportion of unbalanced sperm being produced, with described values ranging from 3.4% - 40% unbalanced sperm.

A subset of translocations with particularly interesting characteristics are X-autosomal translocations (XAT). Due to the unique qualities of the X chromosome, these rearrangements have equally unique implications. In female carriers of XAT, the normal process of X inactivation, in which one of the X chromosomes is transcriptionally silenced, is disturbed. Most commonly, the translocated segment on the der(autosome) (originating from the X chromosome) is not inactivated causing dosage imbalance on the X chromosomes. In males, fertility is often compromised, presumably due to interference with the proper formation of the sex body. In these carriers it is believed that a quadrivalent will form, consisting of the der(autosome), the normal autosome, the der(X) and the Y chromosome (Quack et al., 1988). Sperm studies of XAT have noted a preference of 3:1 segregations possibly due to the small size of the Y chromosome within the quadrivalent (Perrin et al., 2008).
1.4.1.2 Inversions

Inversions are an intrachromosomal rearrangement involving an 180° rotation of a segment of the chromosome within the plane of the chromosome. Two varieties of inversion are commonly described: pericentric and paracentric inversions. Pericentric inversions occur when the inverted segment of the chromosome includes the centromere (Figure 1.7 A), while paracentric inversions are those in which the centromere is not within the inverted segment (Figure 1.8 A). Pericentric inversions are vastly more common, with estimates of their incidence in newborns ranging between 1-2% (de la Chapelle et al. 1974). However, a large number of these inversions are recurrent inversions in heterochromatic regions of chromosomes 1, 2, 9, 16 and Y, and are considered polymorphisms (Colls et al., 1997). Paracentric inversions occur much less frequently with a proposed incidence of between 0.002 and 0.049% of newborns (Fryns et al., 1986). Despite a reorientation of the genes on the chromosome, inversions are usually without phenotypic consequence. The exceptions are when specific genes are disrupted due to physical disturbance at the breakpoints, or due to changes in gene regulation due to movement of centers of regulation or imprinting (Saito-Ohara F et al., 2002) as well as certain X chromosome inversions which have been shown to cause gonadal insufficiency.
Figure 1.6 Formation and meiotic behavior of pericentric inversions.

(A) Pericentric inversions form through the rotation of a region of the chromosome, containing the centromere, within the plane of the chromosome. (B) During meiosis, pericentric inversions form an inversion loop structure allowing syanpse between the inverted and normal chromosome. (C) If a recombination event occurs outside the inverted region, the segregation products will be normal or balanced. (D) If a recombination event occurs within the inverted region, half of the products are termed recombinant, and will be partially nullisomic or disomic.
Figure 1.7 Formation and meiotic behavior of paracentric inversions.

(A) Paracentric inversions form through the rotation of a region of the chromosome, not containing the centromere, within the plane of the chromosome. (B) During meiosis, paracentric inversions form an inversion loop structure allowing syanpse between the inverted and normal chromosome. (C) If a recombination event occurs outside the inverted region, the segregation products will be normal or balanced. (D) If a recombination event occurs within the inverted region, a dicentric and acentric recombinant product will form. The acentric product, lacking a centromere will be lost and the dicentric fragment will either be lost or torn apart producing partially nullisomic or disomic products.

In order to pair the homologous regions of the inverted and normal chromosomes during meiosis, they adopt a structure known as an inversion loop (Figures 1.7 B, 1.8 B).
In this structure, the inverted chromosome loops back upon itself, allowing the homologous partner to synapse along the maximum amount of its length. The complex twisting of the chromosomes to form the inversion loop does not always occur, with authors observing homosynapse of the distal regions with either asyanpsis or heterosyapsis of the inverted region (Gabriel-Robez and Rumpler, 1994).

In carriers of inversions, infertility and the production of abnormal sperm can both be linked to the presence of the inversion loop. It is believed that the creation of the inversion loop could be sufficiently time consuming that it imposes pressure on other meiotic machinery sufficient to impede progression of meiosis (Forejt, 1982). In addition, PCR based studies have shown a reduction in recombination within the inversion loop, a process which, in general, has been shown to have a detrimental effect on meiosis (Brown et al., 1998a). Alternatively, if recombination does occur within the inverted region, the sperm produced will be chromosomally unbalanced, further contributing to infertility. If crossovers occur within the inverted sequence, then half of the resulting sperm will have an unbalanced chromosome complement.

In pericentric inversions, a crossover in the inverted region leads to the production of two complimentary recombinant chromosomes. One recombinant chromosome has an extra copy of the distal region of the long arm and is lacking the distal region of the short arm, while the other recombinant is the reverse (Figure 1.7 D). Viability of the resulting conceptus of a recombinant sperm, depends on the size and genetic content of the distal fragments. So while larger inversions have an increased likelihood of crossover they have smaller distal regions and so a greater likelihood of a viable recombinant.

In paracentric inversions, a crossover in the inverted region will result in the formation of two normal sister chromatids (those that were not involved in the crossover), and two abnormal sister chromatids (Figure 1.8 C, D). It is generally assumed that the recombinant products of paracentric inversions are non viable. One chromatid will have no centromere and so will most likely be lost during meiosis. The other chromatid, will be dicentric, and will be pulled in opposite directions by opposing spindle fibers,
resulting in either exclusion from both daughter cells or rupture producing a chromosomes with duplications or deletions.

The incidence of unbalanced sperm varies largely in relation to the length of the inverted segment, as this length determines the likelihood of a recombination event within the interstitial segment. Studies have found levels of unbalanced sperm as low as 0%, when the segment is very short (Balkan et al., 1983), and 30%, when segment was long (Navarro et al., 1993).

1.4.1.3 Complex chromosomal rearrangements

Complex chromosomal rearrangements (CCR) are rearrangements involving three or more chromosomes and three or more breakpoints (Pai et al., 1980). Such rearrangements are extremely rare, with fewer than 100 being reported in the literature. CCRs are observed more frequently in women, often presenting with recurrent miscarriage (Batista et al., 1994). Male carriers present with infertility and are often found to have reduced sperm parameters or sterility. CCRs fall into two distinct categories: familial, which usually contain between 3 and 4 breakpoints, and de novo, which generally have greater than 4 breakpoints (Coco et al., 2004). Unlike de novo CCRs, which usually are associated with abnormal phenotypes in the carrier, familial CCR carriers are often phenotypically normal, but are at risk for reproductive complications.

As with less complex rearrangements, it is believed that carriers of CCR produce a high frequency of chromosomally abnormal sperm due to the aberrant segregation of the rearranged chromosomes during meiosis. Meiotic studies of CCR have shown that these rearrangements adopt structures similar to the trivalents and quadrivalents observed in Robertsonian and reciprocal translocations. In Coco et al (2004), the authors describe a CCR involving three chromosomes and four breakpoints, which forms a penatavalent and a univalent. Reduced sperm parameters and increased frequencies of abnormal chromosome complements, in the sperm, are both believed to be due to the complexity of the configurations the rearranged chromosomes adopt during meiosis (Chandley, 1981). Due to the presence of these structures during meiosis, a variety of patterns of
segregation are possible. While the chromosomes can segregate, such that the sperm produced have a normal or balanced chromosome complement, numerous other segregation modes are possible. In the case of three-way translocation, there are twenty possible 3:3 segregations of which only two are normal/balanced. Numerous 2:4, 1:5 and 6:0 are also conceivable, all producing severely unbalanced gametes.

1.4.2 Numerical chromosomal abnormalities

1.4.2.1 Aneuploidy

Aneuploidy involves the loss or gain of an entire chromosome from the normal set of two (disomy), producing either trisomies (extra chromosome) or monosomies (missing chromosome). Most numerical chromosomal abnormalities involve a colossal change in gene dosage, on the chromosome involved, and will not survive to term, and as such, are only observed in spontaneous abortions (Jacobs and Hassold, 1995). Of numerical abnormalities involving autosomal chromosomes (non sex chromosomes) the only ones that survive are trisomy 13 (probability 2.8%), trisomy 18 (probability 5.4%), and trisomy 21 (probability 22.1%). Of these, only trisomy 21 usually survives to childbearing age. Vastly more common, are aneuploidies of the sex chromosomes. Indeed, the most common chromosomal abnormality is Klinefelter’s syndrome (XXY), which occurs in 0.1-0.2 % of males.

When considering only infertile men, the prevalence of aneuploidy is much higher, approximately 5% in cases of oligospermia to 10% in cases of azoospermia) (De Braekeleer and Dao, 1991). Men with Klinefelter’s often display primary testicular failure, hypotrophy and increased gonadotrophin levels and are usually sterile. Klinefelters cases in which there is some degree of fertility are often mosaic, a state where there is an incomplete expression of a chromosome abnormality. While mosaicism of Klinefelters (46XY/47XXY karyotype) is quite common, what remains contentious is if the sperm produced are only derived from the cells with normal karyotypes (XY) or if both cell types (XY/XXY) can produce mature sperm (Vidal et al., 1984). The ability of XXY cells to pass through meiosis and spermatogenesis, producing viable spermatids, has been called into question (Luciani et al., 1970). However, studies examining the
chromosome constitution of sperm from mosaic Klinefelter’s patients have found an increase in sex chromosome disomy, suggesting that XXY cells can indeed progress through meiosis and produce sperm (Estop et al., 1998; Foresta et al., 1998). It has been suggested that the severity of alterations to the sperm parameters may be related to the chromosome constitution of the gonadal cells, specifically the proportion of mosaic and non-mosaic cells (Arce et al., 1980).

The other commonly observed sex chromosome abnormality in males is a 47 XYY karyotype, occurring in 0.1% of the general male population (Jacobs et al., 1974). XYY men display markedly variable fertility (Speed, 1989). While an additional unsynapsed Y chromosome should theoretically activate checkpoints during meiosis, leading to cell death, a majority of men with XYY are fertile (Linden et al., 1996). In addition, studies in XYY carriers, examining the frequency of the presence of an additional sex chromosome in the sperm, have found predominantly normal sperm (Faed et al., 1976; Gabriel-Robez et al., 1996). Together, these observations have led to speculation that the additional Y chromosome may be lost prior to meiosis.

As previously mentioned, the only autosomal chromosomal abnormalities compatible with life are those of chromosomes 13, 18 and 21. Carriers of constitutive aneuploidies of these chromosomes are sterile, however, as with sex chromosome aneuploidies, patients exhibiting mosaicism may show some degree of fertility. Many other chromosome aneuploidies in addition to 13, 18 and 21, if mosaic, are compatible with life. Among mosaic carriers of autosomal trisomies, the phenotype can vary greatly, from a presentation similar to a homogeneous trisomy of the same chromosome to a normal phenotype with normal intelligence (Tucker et al., 2007). As with sex chromosome aneuploidies, there is speculation as to any increased frequency of aneuploidy in the sperm of these carriers. Studies have found an increase frequency of aneuploidy in sperm from mosaic carriers, though they point out that such aneuploid sperm may be the result of an abnormal testicular environment, caused by trisomic cells, as opposed to being the products of trisomic cells, which undergo meiosis (Perrin et al., 2008).
1.4.2.2 Supernumerary marker chromosomes

Small supernumerary marker chromosomes (sSMC) are small, structurally abnormal chromosomes, which cannot be described by banding cytogenetics alone. These marker chromosomes require molecular cytogenetic approaches to distinguish the chromosome of origin (Liehr, 2008). sSMCs can be either de novo or inherited, and occur in 0.043% of the general population.

While not strictly a form of rearrangement, sSMCs share many of the same characteristics as structural rearrangements. Similar to rearrangements, sSMCs can contain additional euchromatic material or not, and so can be effectively unbalanced or balanced. As with rearrangements, the risk of phenotypic consequence, to the carrier, is dependant on the degree of imbalance (euchromatic content) in the sSMC (Buckton et al., 1985). While sSMCs contain largely heterochromatic regions of DNA, derived from the centric regions of chromosomes, a recent study found that 73% of sSMCs contained some euchromatin, with the quantity ranging from 0.3-27.2 Mb (Baldwin et al., 2008). sSMC derived from chromosome 15 are the most common, accounting for approximately 30% of autosomal markers (Buckton et al., 1985), however, sSMCs comprising regions derived from all chromosomes but chromosome 5 have been observed.

sSMC fall into a number of subtypes dependant on their mode of origin, including inversion duplications, isochromosomes and ring chromosomes. One proposed mode of formation for inversion duplications, involves a U-type exchange resulting from an aberrant crossover, near the centromere, between two homologous chromosomes, producing a new dicentric inversion duplication chromosome with non-identical telomeres (Schreck et al., 1977). A similar mechanism has been suggested to account for isochromosome formation, with a break occurring within the centromeric DNA (Dewald, 1985). Unlike markers derived from acrocentric chromosomes, those derived from nonacrocentric chromosomes are most often found in ring structures (Liehr et al., 2006). The mechanism whereby these ring chromosomes form is poorly understood, though it is believed that they may form following the fusion of breaks occurring around the centromere.
sSMC are associated with both normal and abnormal phenotypes and much work has been done to understand the association between genotype and phenotype in carriers of sSMC (reviewed in Liehr et al., 2006). sSMC have been associated with a wide array of phenotypes including mental retardation and sterility, and approximately one third of sSMC are correlated with specific conditions, including Pallister Killian syndrome (i(12p)) and cat eye syndromes (dup(22)) (Liehr et al., 2006).

Both the mode of origin, as well as the genetic content of a sSMC, are thought to be important in determining the possible phenotypic consequences (Buckton et al., 1985). In general, the phenotypic consequences of a sSMC are more difficult to predict in carriers of de novo sSMC (Paoloni-Giacobino et al., 1998). When the sSMC is inherited, the phenotypic consequence in the subsequent generation is generally consistent with the parent (Brondum-Nielsen and Mikkelsen, 1995). However, there are some exceptions, where an inherited sSMC has had significant phenotypic effects not observed in the parent (Anderlid et al., 2001; Callen et al., 1992).

sSMCs are associated with infertility (Buckton et al., 1985) and increased aneuploidy in the gametes. sSMCs are less likely to be passed on to offspring from a male carrier as opposed to a female carrier, suggesting that meiotic cells carrying a marker may be more likely to activate checkpoints, also accounting for the reduced sperm parameters observed in some carriers (Webb, 1994; Steinbach et al., 1983). Meiotic studies of sSMC carriers have found varying behaviour of the marker, from an association of the marker with the sex body (Jaafar et al., 1993) to the marker existing predominantly as a univalent (Chandley and Edmond, 1971). The association of the marker with the sex body could lead to disruption of MSCI leading to meiotic arrest, and could explain the infertility observed in marker carriers.

Studies that have examined the segregation of sSMCs, determining their frequency in the sperm of carriers, have found a range from 50% (Mennicke et al., 1997) to 17% (Paetzold et al., 2006). Certain cases, in which the marker is found in less than the theoretically predicted 50% of sperm, suggest some degree of selection against the marker, either during meiosis or later in spermatogenesis. Alternatively, authors have
argued these findings could suggest some degree of tissue specific mosaicism, with respect to the sSMC, a finding that has been supported in some studies (Cotter et al., 2000).

1.4.3.2 Interchromosomal effect

The majority of sperm with abnormal chromosome complements, in carriers of chromosomal abnormalities, are derived from the abnormal segregation of the abnormal chromosomes. However, there is some evidence that the presence of a rearrangement or abnormality may alter the segregation of uninvolved chromosomes. The first evidence for such an effect came from a study in which it was found that a significantly higher percentage of parents, of Down syndrome children, had translocations not involving chromosome 21 (Lejune et al., 1969). This finding initiated speculation that the presence of translocation could have an interchromosomal effect (ICE). As a result, an increased aneuploidy in the sperm may be observed besides the unbalanced chromosomal abnormalities resulting from the chromosomal rearrangement. Chromosome translocations have been shown to affect meiotic segregation of uninvolved chromosome pairs in the mouse (Ford and Evans 1973) and in Drosophila (Grell et al., 1971). However, the presence of this interchromosomal effect (Lejune et al., 1969) in humans has been highly controversial with numerous studies finding both the presence (Blanco et al., 2000; Douet-Guilbert et al., 2005; Machev et al., 2005; Anton et al., 2004) and absence (Honda et al. 1999; Rives et al. 2003) of such an effect.

Confirming both the existence and magnitude of an ICE has been further complicated by the fact that male carriers of chromosomal rearrangements often display abnormal semen parameters, which in itself is an indicator of elevated rates of sperm aneuploidy. Furthermore, several studies have examined sperm aneuploidy in translocation carriers with normal semen parameters, and found no existence of an ICE (Pellestor et al., 2001; Oliver-Bonet et al., 2004; Douet-Guilbert et al., 2005). These results have led authors to suggest that, as ICE are observed primarily in carriers with altered sperm parameters, that the altered testicular environment may be the cause of the increase in disomy (Egozcue et al., 2000).
Part of the controversy surrounding ICE is due to the fact that the mechanism whereby it might originate is not fully understood. As previously mentioned, during meiosis, rearranged chromosomes must adopt unique configurations in order to pair homologous regions. Meiotic studies on infertile carriers of translocations have shown that the fidelity of synapsis is often compromised in the rearranged chromosomes (Gabriel-Robez et al., 1986, Chandley et al., 1986; Oliver-Bonet et al. 2005). These asynapsed regions of the translocation are transcriptionally silenced, leading to meiotic arrest and infertility in the carrier (Turner et al. 2005; Ferguson et al., 2008). However, these asynapsed regions have also been found to associate with the sex chromosomes during meiosis, and it has been suggested that this interaction may interfere with the disjunction of the sex chromosomes, as well as other autosomal chromosomes (Anton et al. 2004).

The presence of an ICE in relation to inversions was initially suggested after discovery of parental inversions in children with aneuploidy (Groupe de Cytogénéticiens Français, 1986). The mechanism contributing to ICE in inversion carriers may differ from that in translocation carriers. Studies on model organisms have shown that the presence of an inversion can alter recombination patterns on both the involved and non-involved chromosomes (Zetka and Rose, 1992) possibly increasing the risk of non-disjunction. As with carriers of rearrangements, the presence of ICE in relation to carriers of chromosomal abnormalities, including numerical abnormalities, remains contentious. ICE in relation to the presence of sSMC have been both supported (Martin et al., 1986) and refuted (Steinbach and Djalali, 1983).

1.4.3.3 Sex body association and infertility

As previously mentioned, studies have shown that numerical and structural chromosomal aberrations can impair meiosis. While translocations may impact fertility by increasing the likelihood of an unbalanced gamete, which will in turn result in a failed pregnancy, they may also impede spermatogenesis directly. It has been suggested that translocations impede spermatogenesis through impairment of the normal silencing of sex chromosomes in meiosis. Meiotic studies of translocation carriers have shown degrees of asynapsis around the breakpoints of the quadrivalent. However, it has been observed that
there is inter and intra individual variation in the degree of this asynapsis. Interestingly, it has been shown that the quadrivalent and other chromosomal abnormalities migrate to, and interact with, the sex body. It has been suggested that this general mechanism has arisen to allow the unsynapsed regions of the quadrivalent or abnormality to avoid detection by the pachytene checkpoint, which monitors the fidelity of synapse (Oliver-Bonet et al., 2005b). Indeed, they suggest that this mechanism may protect any unsynapsed chromatin from detection by meiotic checkpoints. They argue that the sex body provides a uniquely “safe” nuclear environment. However, they also point out that even in cases where evasion of the pachytene checkpoint is successful, the interaction with the sex body may be detected later by the spindle checkpoint. This observation has generated speculation that MSCI silencing spreads to the quadrivalent due to its interaction with the silenced SB, and that this is in part the cause of sterility in carriers (Gabriel-Robez et al., 1986).

Some authors have suggested that the mechanism of sterility involves the spreading of inactivation from the sex body to the quadrivalent, reducing expression of key genes in and around the breakpoint (Jaafar et al., 1993). Indeed, in 2007, using micro-array technology, Holmolka et al. (2007) showed reduced expression around the breakpoints in translocation carriers. Authors have pointed to several lines of evidence including: 1) the temporal progression from autosome invasion of the SB to heterochromatinization of the invading arm and 2) the strong relationship between SB inactivation and XY condensation. Both of these suggest that the mechanism is restricted to spreading of inactivation.

However, as early as 1972, Lifschytz and Lindsay suggested that interaction, between the quadrivalent and the SB, could interfere with SB inactivation. This would allow the expression of certain genes on the X chromosome, with a lethal effect on the cell. Interestingly, recently, Holmolka, in meiotic studies of translocation carriers, showed such an increased expression on the X chromosome suggesting that this interaction does indeed interfere with the normal silencing of the sex body.
1.4.3.4 Meiotic silencing of unsynapsed chromatin

Evidence has accumulated leading authors to suggest that MSCI may be part of a more general process, the meiotic silencing of unsynapsed chromatin (MSUC), which does not discriminate between autosomal, and sex chromosomes (Turner 2006). Studies on mice, have found hallmarks of MSCI, such as ATR, γH2AX and BRAC1, around the breakpoints of translocations (Barrends et al., 2005). Furthermore, sex chromosomes that are able to syanpse completely are able to escape inactivation. A study involving XYY mice found that if the YY bivalent forms there is no sequestration of silencing marks and the Y chromosome genes are expressed normally (Turner et al., 2006). If this model is accurate, then suppression of unsynapsed regions of the quadrivalent of a translocation would be present even in instances when there was no interaction between the quadrivalent and the SB. Exactly this was noted by Ferguson et al. (2008) when they found the presence of BRCA1 and gamma-H2AX (indicators of heterochromatic silencing), and the absence of PNA polymerase in unsynapsed regions of quadrivalents irrespective of their association with the SB.

While a considerable amount of evidence has been generated linking the unsynapsed chromatin in translocation carriers to alterations in MSCI, glaring holes in our understanding of the relationship between translocations and infertility remain. Only a subset of translocation carriers ever displays infertility, and relationships between characteristics of translocations and their resulting degree of infertility, remain tenuous. Indeed, translocation studies have found meiotic arrest occurring at a number of distinct stages (or not at all), even in cases of identical translocations (Vozdova et al., 2008). This suggests that multiple mechanisms may be involved in the determination of which developmental stage a chromosomal abnormality triggers arrest.

1.5 Hypotheses and Specific Objectives

Chromosomal abnormalities (CA) are known to be vastly more common in the infertile population. The treatment of male infertility has been greatly aided by the development of intracytoplasmic sperm injection (ICSI). ICSI involves the direct injection a single sperm into the cytoplasm of an oocyte, thereby allowing men with
severely compromised sperm parameters to achieve pregnancy (Palermo et al., 1992). However, there is concern that the process of ICSI, by bypassing many of the physiological steps in normal fertilization, would increase the frequency with which chromosomally abnormal sperm were involved in fertilization (Martin, 1996). Studies have shown that ICSI increases the risk introducing aneuploid conceptions (Lam et al., 2001). This concern, while present in the general infertile population, is more acute among carriers of chromosomally abnormalities. Indeed, studies have found an increase in the frequency of inherited chromosomal abnormalities following ICSI when compared with natural pregnancies (Lam et al., 2001; Bonduelle et al., 2002; Gjeris et al., 2008). The development of fluorescent in-situ hybridization (FISH) has allowed the study of chromosome aneuploidy in large numbers of sperm. Much work has been done to determine the risk, in carriers of chromosomal abnormalities, of producing unbalanced chromosome complements in the sperm. However, many specific abnormalities and indeed whole classes of abnormalities have yet to be examined.

Recent advances in immunocytogentic techniques have allowed the detailed study of meiosis in infertile men. These studies have illustrated that defects in both recombination and synapsis are associated with infertility in some individuals (Gonsalves et al., 2004, Ma et al., 2006a, Sun et al., 2007b). Carriers of CA display marked variation in the degree to which spermatogenesis is disrupted. Our understanding of the mechanism whereby CA disrupt the spermatogenic process is poorly understood. It is unclear if carriers of CA have similar meiotic abnormalities as infertile men. While a few studies have examined the meiotic behaviour of reciprocal translocations, it is unclear if these results are applicable to other carriers of CA.

We suspect that the behaviour of CA, during meiosis, can explain both the degree to which spermatogenesis is disrupted as well as the proportion of gametes with unbalanced chromosome complements. The association of the CA with itself and other chromosomes, particularly the sex body, may explain the presence of interchromosomal effects as well as the variation in segregation patterns of the abnormal chromosomes. Variation in defects in recombination and synapsis as well as association with the sex body may explain the variation in reduced sperm parameters observed in carriers of CA.
Thus, our hypotheses are that: (1) Specific characteristics of the CA are the
determinants for the segregation patterns will vary between carriers of different types of
CA, resulting in a range in the proportion of unbalanced chromosome complements,
dependent on the specific characteristics of the CA including chromosomes involved and
breakpoints of the rearrangement; (2) In carriers of CA, the presence of a CA will disrupt
the segregation of chromosomes not involved in the CA, resulting in an
interchromosomal effect observable as an increase in sperm disomy; (3) The meiotic
behaviour of the CA, such as sex body association, synapse and recombination, may
explain the frequency of abnormal chromosome complements in the sperm and also
whether spermatogenesis will be impaired or not.

The following specific objectives will be addressed by the work presented in this thesis:

Objective 1: To determine the frequency, of each possible segregation pattern in the
involved chromosome(s), in carriers of various chromosomal abnormalities.

Objective 2: To determine if the presence of a chromosomal abnormality disrupts the
segregation of chromosomes not involved in the abnormality, resulting in an increase in
sperm disomy for chromosomes not involved in the abnormality.

Objective 3: To determine if abnormalities in the progression through meiosis, fidelity of
synapese, frequency of recombination and association of CA with the sex body are more
prevalent in carriers of chromosomal abnormalities compared with fertile or infertile
men.

Objective 4: To determine if, in carriers of chromosomal abnormalities and infertile men,
there is a relationship between the frequency of crossovers on chromosomes 13, 18, 21, X
and Y and the presence of aneuploidy of those chromosomes.
1.6 References


CHAPTER II: CHROMOSOME SEGREGATION IN CARRIERS OF CHROMOSOMAL ABNORMALITIES

2.1 Introduction

Chromosomal abnormalities (CA) involve large-scale reorganizations of the chromosomes. CA can include both chromosome rearrangements; involving the movement of chromosome regions within or between chromosomes, as well as numerical abnormalities; involving the addition or subtraction of chromosomes or chromosome regions.

Rearrangements can take a variety of forms, the most common of which are reciprocal translocations (RT), which occur in 0.092% of the population, and involve the mutual exchange of chromosome regions between two chromosomes. Robertsonian translocations (ROB), occurring in 0.086% (de Braekeeler and Dao, 1991) of the population, involve the fusion of two acrocentric chromosomes. Inversions (inv) involve the repositioning of a region of a chromosome within the chromosome, such that it flips orientation. Inv, in which the centromere is within the inverted region, are known as pericentric inversions, and occur in 1-2% (de la Chapelle, 1974) of individuals, while paracentric inversions, in which the inverted region does not include the centromere, occur in 0.002-0.049% (Anton et al., 2005). Complex chromosomal rearrangements (CCR) are rearrangements involving three or more chromosomes and three or more breakpoints (Pai et al., 1980). Such rearrangements are extremely rare, with fewer than 100 being reported in the literature.

Numerical chromosomal abnormalities (NCA) vary in size between additional or missing whole chromosomes, known as aneuploidy, which are the most common form of CA, to additional small supernumerary marker chromosomes (sSMC), that occur in 0.043% of population and are so small that cannot be described by banding cytogenetics.

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1 A version of this chapter will be submitted for publication. Kirkpatrick, G. and Ma, S. Chromosomal Segregation in Carriers of Chromosomal Abnormalities.
alone (Liehr, 2004). The majority of de novo aneuploidies do not survive to term, with the exception of some (13, 18, 21 and XY), which survive with varying degrees of phenotypic consequence. Carriers of aneuploidy, in which the additional/missing chromosome is not present in every cell, are known as mosaic, and have phenotypic consequences that vary depending on the degree of mosaicism and the cell types in which the aneuploidy is present.

CA are associated with infertility, with an incidence of CA in the infertile population ten times higher than in the general population (Egozcue et al., 2000). In addition to reduced sperm parameters, carriers of CA are at an increased risk for the production of chromosomally abnormal sperm. Rearrangement carriers typically produce gametes with additional or missing regions of those chromosomes involved in the rearrangement, resulting from the mis-segregation of the rearranged chromosomes and their normal homologues. The mis-segregation of the rearranged chromosomes can be attributed to the abnormal behaviour of these chromosomes during meiosis (Chandley, 1981).

During meiosis I, the rearranged chromosomes, and their normal homologous chromosomes, must adopt unique structures in order to pair their homologous regions, prior to recombination and segregation. These structures vary depending on the nature of the rearrangement. RT form a cruciform quadrivalent structure between the two rearranged (derivative) chromosomes and their normal homologues, while ROB form a trivalent structure between the fusion product and the two normal homologous chromosomes. Inverted chromosomes, and their homologous partners, form an inversion loop, in which one chromosome loops back in a region of the chromosome, allowing the entire length of the chromosome to pair. CCR, which involve a chromosome exchange between three or more chromosomes, form structures similar to quadrivalents, though involving more chromosomes. The behavior of trisomies and sSMCs during meiosis is poorly understood. Studies have suggested, even in non-mosaic carriers of NCA, that the additional chromosome may in some cases be lost prior to or during meiosis (Evans et al., 1970, Chandley et al., 1976). Meiotic cells, in which the NCA is present, have shown varying patterns of behaviour. Studies of carriers, with additional whole chromosomes,
have revealed meiotic behaviours including: formation of a trivalent with homologous chromosomes, association of the additional chromosome with the sex body and presence of the additional chromosome as a univalent (Solari and Ray Valzacchi, 1997).

While these pairing structures allow the rearranged and normal chromosomes to pair, and presumably escape meiotic arrest, they do have consequences during the segregation of chromosomes. Under normal circumstances, homologous chromosomes will segregate, providing an equal and balanced contribution to each daughter cell. However, in carriers of CA, a number of segregation patterns are possible, including: normal, balanced (same as carrier) and unbalanced products. In carriers of RT and ROB, alternate segregations produce products with just normal chromosomes or just derivative chromosomes producing normal or balanced products respectively. All other segregations, involve some combination of normal and derivative chromosomes, thus producing unbalanced products. Inv carriers have one normal chromosome and one balanced derivative chromosome and segregation will generally produce normal or balanced products. Unbalanced products are produced only if a recombination event occurs within the inversion loop. If such an event does occur, half the products from that segregation will be unbalanced. CCR have a number of possible segregation patterns, in proportion to the complexity of the rearrangement, the vast majority of which produce products with unbalanced chromosome complements. The mechanisms that effect the segregation of NCA are largely unknown. It remains unclear if the percentage of sperm, from a carrier, that contain a NCA, will deviate from the theoretical 50% that might be expected should the NCA segregate independently.

In this study, we used fluorescent in situ hybridization (FISH) to examine the segregation patterns of the rearranged chromosomes in a variety of carriers CA. We examined 11 carriers of CA including three RTs, an X-autosome translocation (XAT), two ROBs, one of which contained a sSMC, a paracentric inv, a CCR involving chromosomes 1, 2 and 10 and an inv on chromosome 10, and a carrier of a sSMC alone. Finally, we examined the segregation of two mosaic carriers of aneuploidy, one of the X chromosome and one chromosome 21. Of the carriers examined, six represent rearrangements never before studied in this manner. Indeed, to our knowledge, this study
includes the first FISH examination of the meiotic segregation of any CCR. In addition, our study is the first to analyze the segregation of a carrier of both ROB and sSMC. This study provides data that will be crucial for our increased understanding of the meiotic behaviour of CA and will provide valuable information relevant to genetic counseling.

2.2 Materials and Methods

2.2.1 Clinical information

Carriers of CA were ascertained via fertility clinic workups for primary infertility. All patients had history of infertility ranging from 2 to 6 years. Of the carriers of chromosomal abnormalities, seven out of eleven carriers had severe OAT with sperm count arranging from a few hundred to 3.8 million per millileter, while one had only severe abnormal sperm morphology (<5%) with normal sperm count and motility (Table 2.1). Serum gonadotropins and testosterone were normal in all patients. CA were characterized based on clinical cytogenetic reports and included two Robersonian translocations [45,XY,rob(13;14)(q10;q10) (Figure 2.1 A) and 46,XY,rob(13;21) (p11.1;p11.1),+mar (Figure 2.1 B)], three reciprocal translocations [46,XY,t(4;15)(p12;p11.1) (Figure 2.2 A); 46,XY,t(6;21)(q16;q21) (Figure 2.2 B) and 46,XY,t(9;22)(p13.1;q13.2) (Figure 2.2 C)], an X:autosome translocation [46,XY,t(X;20)(p10;q10)] (Figure 2.2 D), a paracentric inversion, [46,XY,inv(5)(q22.1;q23.2)] (Figure 2.1 C), and a 46,XY,t(1;2;10)(1pter-1p35.1::10q26.13->1pter; 2pter->2q21.3::1p35.1->1pter; 10pter->10q11.23::10q24.33->10q11.23::2q21.2->2qter) complex chromosomal rearrangement (Figure 2.3 A). In addition, we examined a carrier of an sSMC alone (47,XY,+mar). Two carriers of mosaic aneuploidy [46,XY/47,XYY; 47,XY,+21/46,XY] were found via cytogenetic analysis. In both cases, 4% (2/50) of blood leukocytes showed an additional chromosome. For each chromosomal abnormality, the patterns of segregation, for each probe set used, were compared with a control individual. Two control men, both of proven fertility, were used for all control samples.
### Table 2.1 Clinical characteristics of patients’ sperm parameters

<table>
<thead>
<tr>
<th>Patient Description</th>
<th>Age</th>
<th>Density (x10^6/ml)</th>
<th>% Total Motility</th>
<th>% Normal forms (WHO criteria)</th>
</tr>
</thead>
<tbody>
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<td>3.2</td>
<td>6</td>
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<tr>
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<td>0</td>
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<td>4</td>
</tr>
<tr>
<td>46,XY,t(X;20)</td>
<td>37</td>
<td>0.1</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>45,XY,rob(13;14)</td>
<td>48</td>
<td>70</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>45,XY,rob(13;21)^a</td>
<td>40</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>46,XY,rob(13;21),+mar</td>
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<td>0.3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>46,XY,t(1;2;10)</td>
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<td>0.9</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>46,XY,inv(5)</td>
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<td>141</td>
<td>70</td>
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</tr>
<tr>
<td>47,XY,+mar</td>
<td>38</td>
<td>20</td>
<td>&gt;50</td>
<td>1</td>
</tr>
<tr>
<td>mos 47,XXY/46,XY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mos 47XY+21/46,XY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

^a Previously reported in Hatakeyama et al., 2005
Figure 2.1 Meiotic configurations, segregation modes and probe sets used in Robertsonian translocations and inversion.

(A, B) rob(13;14) and rob(13;21) form trivalent structures, shown here in the cis orientation, allowing for alternate (red) and adjacent (purple) segregation patterns. Locus specific probes for chromosomes 13 (green), 14 (red) and 21 (red) were used in analysis of segregation. (C) The inverted region of chromosome 5 is shown within the blue bracket. P-telomeric (green) and q-telomeric (red) were used to determine the recombinant frequency
Figure 2.2 Meiotic configurations, segregation modes and probe sets used in reciprocal and X: autosomal translocations.

(A, B, C, D) Translocations are believed to form a quadrivalent structure producing alternate (red), adjacent-1 (purple) and adjacent-2 (green) segregations. (A) The t(4;15) was analyzed with centromeric probes for chromosomes 4 (blue) and 15 (red) and chromosome 4 p-teleomeric probe (green). (B) The t(6;21) was analyzed with locus-specific probes for chromosomes 6 (blue) and 21 (red), and centromeric probe (green) for 6. (C) The t(9;22) was analyzed with centromeric probe for 9 (blue) and 22 (red) and q-telomeric probe for 22. (D) t(X;20) was analyzed with X/Y centromeric probes (blue), and chromosome 20 p-telomeric (green) and q-telomeric (red) probes.
Figure 2.3 Breakpoints and theoretical configuration of the t(1;2;10) complex chromosomal rearrangement.

(A) Normal and derivative chromosomes from the t(1;2;10) are shown with chromosome 1 in red, 2 in blue and 10 in green. The inverted region of chromosome 10 is shown in a lighter shade of green. (B) The theoretical heptivalent structure formed during meiosis including an inversion loop in between 10 and der10.

2.2.2 Marker characterization

Marker chromosome identification was performed in Liehr’s lab as described previously in Starke et al. (2003). Cultured metaphase cells (2-7 per probe set) from the 46,XY,rob(13;21),+mar carrier were evaluated with probe sets including subcentromeric M-FISH mix for chromosomes 14 (described in Starke et al., 2003) as well as cen 15, cen 14/22, and cen 22. The 47,XY,+mar carrier was evaluated with probe sets (10 metaphase cells each): 1) Centromeric M-Fish (described in Nietzel et al., 2001), 2) cen 13/21, cen
14/22 and cen 15 and 3) subcentromeric M-FISH for chromosomes 14 and 22 (described in Starke et al., 2003).

### 2.2.3 FISH hybridization and analysis

The methods of sperm preparation, probe hybridization, and FISH analysis have been previously described in Tang et al. (2004). Briefly, sperm were fixed onto glass slides (with 3:1 methanol:acetic acid) washed in 2x SSC (saline-sodium citrate solution) and incubated in dithiothreitol for 20 min. Slides were then washed in an alcohol series (70, 80, 90 and 100%). The probe set was applied to air-dried slides and co-denatured at 75°C in a thermocycler for 5 min. Slides were incubated for 24 hours at 37°C in a humid chamber. Following incubation, slides were washed in 73°C 0.4x saline sodium citrate (SSC)/0.3% NP-40 solution for 2 min with agitation for 3 sec, followed by a wash in 2x SSC/0.1% NP-40 for 30 sec. Slides were incubated in 4′,6-diamidino-2-phenylindole (DAPI) for one minute. Slides were then air-dried in the dark and then covered with antifade and a coverslip.

Segregation was analyzed with a unique probe set for each abnormality (Table 2.2). In general, segregation patterns of translocation carriers were determined by analysis using a probe set consisting of three probes, two of which hybridize on either side of the breakpoint on one chromosome and one hybridizing to a region on the other. In ROB cases, a probe on either side of the breakpoint was sufficient. The number of breakpoints, in the CCR, necessitated that the segregation be analyzed with three separate FISH probe sets, each corresponding to one of the involved chromosomes. With respect to the inversion carrier, the recombinant frequency was determined using probes located on either side of the inverted region. Numerical abnormalities were assessed with probes specific for the additional chromosome. As the marker chromosomes contained centromeric DNA from chromosomes 14 and 22, we used an α-satellite DNA probe for chromosomes 14/22 to determine the frequency of the marker chromosomes in sperm. The centromeres of chromosomes 14 and 22 are indistinguishable and cannot be differentiated with FISH probes (Figure 2.4 C, D). In order to confirm that increases in signal number were associated with the presence of the marker and not with disomy 14 or disomy 22 we performed Dual-colour FISH using non-centromeric probes for
chromosomes 14 and 22 in both the sSMC carrier and sSMC and RT carrier, as well as the RT carrier and a control case. Each probe set was also hybridized to a control individual and when possible 1000 sperm were scored.

Table 2.2 Probe sets used in analysis of segregation patterns in carriers of CA

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Chromosome</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Fluorophore)</td>
<td>(Fluorophore)</td>
<td>(Fluorophore)</td>
</tr>
<tr>
<td>46,XY,t(9;22)</td>
<td>22 α-sat (SO)</td>
<td>22 q-tel (SG)</td>
</tr>
<tr>
<td>46,XY,t(6;21)</td>
<td>6 α-sat (SG)</td>
<td>6 LSI (SA)</td>
</tr>
<tr>
<td>46,XY,t(4;15)</td>
<td>4 (SA)</td>
<td>4 p-tel (SG)</td>
</tr>
<tr>
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<td>X/Y α-sat (SA)</td>
<td>20 p-tel (SG)</td>
</tr>
<tr>
<td>45,XY,rob(13;14)</td>
<td>13 LSI (SG)</td>
<td>14 LSI (SO)</td>
</tr>
<tr>
<td>46,XY,rob(13;21),+mar</td>
<td>13 LSI (SG)</td>
<td>21 LSI (SO)</td>
</tr>
<tr>
<td></td>
<td>14/22 α-sat (SO)</td>
<td>18 α-sat (SA)</td>
</tr>
<tr>
<td></td>
<td>14 LSI (SG)</td>
<td>22 LSI (SO)</td>
</tr>
<tr>
<td>47,XY,+mar</td>
<td>14/22 α-sat (SO)</td>
<td>18 α-sat (SA)</td>
</tr>
<tr>
<td></td>
<td>14 LSI (SG)</td>
<td>22 LSI (SO)</td>
</tr>
<tr>
<td>46,XY,t(1;2;10)</td>
<td>1 p-tel (SG)</td>
<td>1 q-tel (SO)</td>
</tr>
<tr>
<td>46,XY,inv (5)</td>
<td>2 p-tel (SG)</td>
<td>2 q-tel (SO)</td>
</tr>
<tr>
<td>mos 47,XYY/46,XY</td>
<td>10 p-tel (SG)</td>
<td>10 q-tel (SO)</td>
</tr>
<tr>
<td>mos 47,XY,+21/46,XY</td>
<td>5 p-tel (SG)</td>
<td>5 q-tel (SO)</td>
</tr>
<tr>
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<td>5 LSI (SG)</td>
<td>5 LSI (SO)</td>
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<tr>
<td></td>
<td>13 LSI (SG)</td>
<td>21 LSI (SO)</td>
</tr>
</tbody>
</table>

All probes from Vysis Inc., (Downers Grove, IL) with the exception of * from Cytocell

![Figure 2.4 Probe sets used in carriers of sSMC for marker enumeration in sperm.](image)

(A, B) A probe set containing chromosome 14/22 centromeric probes, which also hybridized to the marker, were compared with a probe set containing locus-specific probes for chromosomes 14 and 22, which did not hybridize to the marker.
Only sperm with intact head and tail morphology, and within an area of the slide where consistent hybridization was evident, were scored. Two signals of the same colour were scored as two copies of the corresponding chromosome when they were comparable in brightness and size and were separated from each other by a distance longer than the diameter of each signal.

2.2.4 Statistical analysis

The frequencies of segregation patterns, for each rearrangement, were compared with controls using the Chi-square test. All statistical analyses were performed using the GraphPad Prism V5.0 program (GraphPad Software, San Diego, CA). P<0.05 was considered significant.

2.3 Results

2.3.1 Reciprocal translocations and X-autosome translocation

In the analysis of the RT and XAT, 4077 sperm were analyzed, with a range in hybridization efficiency from 94.7-98.5% (Table 2.3). The RT and XAT carriers showed a range in alternate segregation from 43.0%, in the t(6:21) to 68.8%, in the t(X:20). The t(4:15) and t(9:22) carriers showed a tendency towards Adjacent 1 segregations with 35.9% and 37.9% respectively compared with 2.3% and 8.5% respectively for Adjacent 2. In contrast, the t(6:21) and t(X:20) carriers showed almost no preference between the two Adjacent segregation modes. 3:1 segregation was found to be highest in the t(X:20) carrier.

2.3.2 Robertsonian translocations

In the segregation analysis of the rob(13;14), 10131 sperm were analyzed, with a hybridization efficiency of 99.66% (Table 2.3). Adjacent and 3:0 segregations were observed in 10.4% and 0.07 % respectively, while 89.4% of sperm displayed normal or balanced segregations (Alternate). The rob(13;14) showed significantly higher (P<0.0001) frequencies of adjacent segregation patterns. With respect to the 46,XY rob(13;21)+mar, meiotic segregation analysis on chromosomes 13 and 21 was performed on 2045 sperm (Table 2.3). The majority (91.2 %) of sperm had normal or balanced
chromosome constitutions, resulting from alternate meiotic segregations. Unbalanced chromosomes constitutions resulting from adjacent segregations, accounted for 8.36%.

2.3.3 Inversion

A total of 1214 sperm were analyzed in examining the segregation of the inversion 5, with a hybridization efficiency of 93.7% (Table 2.3). The recombinant frequency, indicative of sperm products of a meiotic cell in which a recombination event occurred within the inverted region, was determined to be 3.37%, a value significantly higher than the frequency observed in controls.
<table>
<thead>
<tr>
<th>Chromosome Abnormality</th>
<th>Chromosome(s) analyzed</th>
<th>No. of sperm</th>
<th>Normal /Balanced</th>
<th>Unbalanced</th>
<th>Adj. 1</th>
<th>Adj. 2</th>
<th>3:1/disomy/ nullisomy</th>
<th>other</th>
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<td>57.5</td>
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<td></td>
<td></td>
<td>Control</td>
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<td>0</td>
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<tr>
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<td>27.5</td>
<td>22.5</td>
<td>8.0</td>
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<td>Control</td>
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<td></td>
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<td>13/14</td>
<td>Carrier</td>
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<td>89.4</td>
<td>10.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>11.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Carrier</td>
<td>2045</td>
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<td>8.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2013</td>
<td>85.7</td>
<td>13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>4133</td>
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<td>-</td>
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<sup>a</sup> Additional data for 45,XY,t(13:21) not available.
### Table 2.3 continued

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<th>Chromosome Abnormality</th>
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<th>No. of sperm</th>
<th>Normal/ Balanced</th>
<th>Unbalanced</th>
<th>Adj. 1</th>
<th>Adj. 2</th>
<th>3:1/disomy/ nullisomy</th>
<th>Other</th>
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<td>47,XY,+mar</td>
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<td></td>
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*a* previously reported in Hatakeyama *et al.*, 2005

*b* The frequency of unbalanced segregation modes was significantly higher when compared with controls (Chi-squared, p<0.05)
2.3.4 Complex chromosomal rearrangement

A total of 3094 patient sperm and 3047 control sperm were analyzed, with hybridization efficiencies ranging from 92.7% to 98.3% and 94.7% to 97.8% respectively (Table 2.4). Chromosome 2 displayed the lowest frequency of balanced segregants with 75.7% while chromosome 1 displayed the highest frequency of normal or balanced segregants with 84.2%. With respect to chromosome 1, the frequency of additional p (indicative of an additional derivative 2 chromosome (der 2)) and q (der 1) signals were 4.8% and 5.2% respectively while the frequency of missing p and q signals were 3.3% and 2.2%. Chromosome 2 analysis revealed additional p (der 2) and q (der 10) signals in 5.9% and 9.1% and missing p and q signals in 2.4% and 4.7%. Analyses of chromosome 10 found 80.5% balanced or normal, while additional p (der 10) and q (der 1) signals were 5.8% and 6.5% and missing p and q signals were 2.0% and 3.4%. Segregants involving more than one additional p or q signal were found in only 0.88% and 0.7% of spermatozoa from chromosomes 2 and 10. Diploidy (or a doubling of the balanced complement) was observed in 0.18%, 0.98% and 0.8% of spermatozoa in chromosomes 1, 2 and 10 respectively.
<table>
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<th>FISH signal</th>
<th>sperm chromosome complement(s)</th>
<th>% (#)</th>
<th>#</th>
<th>sperm chromosome complement(s)</th>
<th>% (#)</th>
<th>#</th>
<th>sperm chromosome complement(s)</th>
<th>% (#)</th>
<th>#</th>
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<tr>
<td>OG</td>
<td>1/der1+der2</td>
<td>84.2</td>
<td>121</td>
<td>2/der10+der10</td>
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<td>10/der10+der10</td>
<td>80.55</td>
<td>1006</td>
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<td>1 + der1/der1+der2</td>
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<td>0</td>
<td>2 + der10/der10+der10</td>
<td>9.12</td>
<td>1</td>
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<td></td>
<td>+der1</td>
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<td></td>
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<td></td>
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<tr>
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<td>4.83</td>
<td>0</td>
<td>2 + der2/der10+der2+der2</td>
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<td>10 + der10/der10+der2+der2</td>
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<td>+der2</td>
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<tr>
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<td>4.71</td>
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<td>der10/der10/rec1/der10</td>
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<td>der10</td>
<td>2.45</td>
<td>1</td>
<td>der10/der10/rec1/der10</td>
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<td>OGGG</td>
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<tr>
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</tr>
</tbody>
</table>

Table 2.4 Segregation of chromosomes 1, 2 and 10 in sperm from t(1:2:10)
2.3.5 Small supernumerary marker chromosomes

FISH analysis, of cultured metaphase cells, characterized the marker chromosome in the 46,XY,rob(13;21),+mar as an invdup(14)(q11.1) (Figure 2.1 A). Furthermore, the analysis determined that the marker consists entirely of heterochromatic material due to the absence of signals for subcentromeric probes. The marker, present in the 47,XY,+mar case, was determined to be an inv dup(22)(q11.1) (Figure 2.1 B). As in the previous case, the marker was determined to consist entirely of heterochromatic material. The carriers’ karyotypes were determined to be 46,XY,rob(13;21)(p11.1;p11.1),+invdup(14)(q11.1) and 47,XY,+invdup (22)(q11.1).

We searched for the presence of the sSMC in the sperm of two carriers: a 46,XY rob(13;21),+mar (RB/mar), and a 47,XY,+mar (+mar) and compared these results with a 45,XY,rob(13;21) case (RB), and a fertile control (Table 2.5). We examined 2404 sperm cells from the RB/mar and 2009 sperm cells from the +mar with 14/22 centromere probe and compared these results with those from the RB (2063 sperm analyzed) and a fertile control (4685 sperm analyzed). Chromosomes 14 and 22 were analyzed individually in 1216 sperm in the RB/mar, 2279 in the +mar, 2078 in the RB and 4083 sperm the control. Analysis with the centromeric 14/22 probe revealed that, in the RB/mar, 11.3% of sperm had three signals, suggestive of the presence of the marker chromosome in sperm. In the +mar the frequency sperm with three signals was determined to be 11.2%. Both frequencies were significantly higher than the combined frequency of disomy for chromosomes 14 and 22 in their respective cases (RB/mar 1.56% (P<0.0001), +mar 0.61% (P<0.0001)). Additionally, in both cases, the three signal frequency was significantly higher than the three signal frequency in the control (0.1%, P<0.0001) and RB (0.14%, P<0.0001), as well as the combined frequency of chromosome 14/22 disomy for the RB (0.48, P<0.0001) and control (0.61, P<0.0001).
<table>
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<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
</tr>
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<tr>
<td>Dual colour</td>
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<tr>
<td>14/22 cen probe</td>
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<td>46,rob(13;21),+mar (n=1216)</td>
<td>45,rob(13;21) (n=2078)</td>
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</tr>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td>Normal (RG)</td>
<td>2246</td>
<td>98.6</td>
<td>1182</td>
<td>97.2</td>
</tr>
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<td>0.61</td>
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<td>0.83</td>
<td>14</td>
<td>1.15</td>
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</table>
2.3.6 Mosaic aneuploidy

In the sperm from two carriers of mosaic aneuploidy, one 47,XXY/46,XY case (Xmos) and one 47,XY,+21/46,XY case (21mos), we searched for the presence of an addition X and 21 chromosome respectively (Table 2.3). We scored 10084 and 5093 sperm in the Xmos and 21mos cases, with hybridization efficiencies of 93.2% and 98.4%. In the Xmos case, we found an additional X chromosome signal in 0.13% of sperm, a level not significant when compared with the control. In the 21mos case, we found an additional 21 signal in 0.29% of sperm, a level also not significantly higher than the control.

2.4 Discussion

2.4.1 Reciprocal translocations and X-autosome translocation

Our results indicated the frequency of gametes exhibiting unbalanced segregation modes amongst RTs, ranged from 43.0%-57.5%. These results are consistent with a review of 44 RTs, which found the frequency of unbalanced segregants to range from 44.1% to 81.4% with a mean of 59.5% (Benet et al., 2005). Our results showed a relatively stable frequency of Adj 1, ranging from 27.5% to 37.9%, while Adj 2 segregations ranged from 2.3% – 22.5%.

Based on the chromosomes involved and the positions of the breakpoints, predictions can be made as to the segregation outcomes of a quadrivalent (Jalbert et al., 1980). Firstly, the larger the centric fragment, in proportion to the translocated fragment, on the derivative chromosomes, the more likely it is to produce Adj 1 segregants. Conversely, the smaller the centric fragment in proportion to the translocated fragment, the more likely it is to produce Adj 2 segregants. The t(4;15) had the largest ratio of centric to translocated fragments on the derivative chromosomes (Figure 2.2 A), and of the RT carriers, it produced the fewest Adj 2 segregants (2.3%). Conversely, the t(6;21) had the lowest ratio of centric to translocated fragments on the derivative chromosomes (Figure 2.2 B) and produced the highest frequency of Adj 2 segregants (22.5%). Jalbert also suggests that quadrivalents, in which one of the chromosomes is very small, are at an increased likelihood of producing sperm with 3:1 segregations (Jalbert et al., 1980). In
two RTs studied, both containing one of the two smallest chromosomes, we noted 3:1 segregations in 9.1% and 8.0% of sperm in the t(9;22) and t(6;21) respectively. In contrast, we noted 3:1 segregations in 4.1% of sperm in the t(4;15).

With respect to the t(X;20), chromosome segregation analysis found the frequency of normal or balanced segregants to be 68.8%, while Adj 1 and Adj 2 segregations accounted for 7.3% and 6.3% respectively. When compared to the only previously analyzed t(X;autosome) (Perrin et al., 2008) we found an increased frequency of normal/balanced segregants and a decreased frequency of both Adj 1 and Adj 2 segregations. 3:1 segregations accounted for 14.1% of sperm analyzed. The preference for 3:1 segregation modes has also observed in other t(X;autosome) (Perrin et al., 2007). The presence of a small chromosome in the trivalent could account for the increase in 3:1 segregations as described by Jalbert et al. (1980). However, the absence of complete pairing between the sex chromosomes could also account for unusual segregation patterns observed.

2.4.2 Robertsonian translocations

In the two ROB cases reported here, we found balanced or normal segregations in 89.4% and 91.2% of sperm in the rob(13;14) and rob(13;21)+mar respectively. The unbalanced chromosome complements consisted primarily of Adjacent segregations accounting for 10.4% and 8.35% respectively. The rob(13;14) is the most common variety of ROB with an incidence of 0.097% in the general population (Anton et al., 2004). As such, numerous FISH studies have examined chromosome segregation in these carriers. In general, as with reciprocal translocations, it is though that the breakpoints and the chromosomes involved in the rearrangement are the primary determinants of the patterns of segregation in ROB. ROB, that involve the same chromosomes, generally have identical or similar breakpoints. In that respect, they provide an opportunity to evaluate the effect, of the genetic background of the individual, on the segregation of the rearrangement. Several studies of 7 t(13;14) carriers, have found a range in the frequency of balanced/normal segregations from 73.6-91% (Anton et al., 2004) suggesting that genetic or environment factors may play a significant role in the segregation of chromosomes, though the impact of multiple centres performing the studies can not be
discounted. In contrast to rob(13;14), the rob(13;21) is one of the rarest ROB, comprising only 2% of the ROB reported (Chen et al., 2007). Indeed, the one previous examination of chromosome segregation revealed a higher frequency, of unbalanced segregants, than we observed, at 11.61% (Hatakeyama et al., 2005).

In addition to environmental and genetic factors, the specific orientation, of the chromosomes within the trivalent, has been suggested to affect the patterns of segregation (Sybenga, 1975). A cis configuration, in which the normal chromosomes synapse to the same side of the translocated chromosome, is believed to promote alternates segregations (Luciani et al., 1984). Conversely, a trans configuration, in which the normal chromosomes synapse on opposite sides of the translocated chromosome, would result in a greater frequency of adjacent or unbalanced segregations (Luciani et al., 1984). In both ROB carriers presented here, lower levels of unbalanced segregation types could reflect a preference for the cis orientation at meiosis, and resulting alternate segregations.

2.4.3 Inversion

As previously mentioned, inversions produce unbalanced chromosomes only if a recombination event occurs within the inverted region. If such a recombination event occurred in the case presented here, the q arms of the recombining chromosomes below the recombination site, would fuse forming an acentric fragment (that would be lost) while the p arms, including the centromere and part of the q arms, would fuse forming a dicentric chromosome. The later would be pulled in opposing directions during anaphase causing the chromosome to break, and forming two unbalanced chromosomes.

A review of segregation studies on inversions found a large degree of heterogeneity among recombinant frequencies, with a range from 0.38-37.85% in pericentric inversions and 0.03-0.81 paracentric inversions. This variation in recombinant frequency has been attributed primarily to the size of the inversions, as generally, the incidence of recombination becomes more likely the larger the inverted region. The inversion described here displayed an uncharacteristically high recombinant frequency (3.37%) when compared with inversions of a similar size (~17 Mb) in Anton et al. (2005). It is possible that this uncharacteristic frequency of recombinants could be due to the unequal
distribution of recombination across the length of the chromosome. Human chromosomes display a non-random distribution of crossovers, with numerous factors such as nature of the chromosome involved, effect of centromeres and telomeres and the presence of specific “hot” or “cold” sequences of DNA influencing the distribution of crossovers.

2.4.4 Complex chromosomal rearrangement

Segregation studies on carriers of various rearrangements have been sources of valuable information to reproductive counseling. However, due to their rarity, FISH segregation studies have not been carried out on a carrier of a CCR, and thus the risk of producing abnormal gametes, in such a carrier, is still largely unknown. We present the first such study of a CCR, reporting on the meiotic segregation of the t(1:2:10).

Due to the number of possible segregants, it was not possible to assess all involved chromosomes simultaneously. As such, the segregation analysis of the CCR was completed separately on each of the involved chromosomes. The frequency of unbalanced chromosome complements, of the involved chromosomes, varied between 24.24% in chromosome 2 and 15.8% in chromosome 1. These unbalanced chromosome complements were comprised, almost exclusively, of either normal/balanced complements with the addition of one of the derivative chromosomes, or one of the derivative chromosomes alone, producing partial dismoy of nullisomy of one of the rearranged chromosomes. A complicating factor in the analysis of chromosome 10 is the presence of an inversion. Due to the probe sets used, recombinant products could not be distinguished from other unbalanced products (Table 2.5). Nevertheless, due to the size of the inversion, we would expect a significant number of the abnormal chromosome complements, observed with this probe combination, consist of recombinant products resulting from recombination events within the inverted region.

Assuming that the three involved chromosomes segregate independently, we can estimate a total frequency of abnormal chromosome complements, among the involved chromosomes, to be ~50%. This is compared with carriers of reciprocal translocations, which have been shown to have frequencies, of abnormal chromosome complements, ranging from 19.3-81.4% (with a mean between 54.5% and 59.5%) (Benet et al., 2005).
While our result is similar to that observed in reciprocal translocations, it is interesting, considering the larger magnitude of the CCR in relation to most reciprocal translocations, that the frequency of abnormal complements was not higher.

An explanation for the inconsistency between the magnitude of a rearrangement and the frequency of abnormal chromosome complements may be related to the relative impact of CCRs on spermatogenesis. It has been hypothesized that the infertility associated with CCRs is due to spermatogenic arrest that occurs as a result of the complex meiotic configurations that occur during meiosis (Lespinasse et al., 2004). Meiotic studies of reciprocal translocations have demonstrated compromised syapsis and silencing in the breakpoints of rearranged chromosomes, likely contributing to spermatogenic arrest (Ferguson et al., 2008). Indeed, a study by Coco et al. (2004) observed spermatogenic arrest at late pachytene in a CCR, possibly suggesting that spermatogenesis was disrupted as a result of rearranged chromosomes attempting to adopt these confirmations. It is conceivable that in spermatocytes in which meiotic segregation is severely compromised, such as CCRs, the likelihood of activating meiotic checkpoints and triggering cell death is higher. Indeed, CCRs are generally found to have lower sperm concentration, and thus a greater degree of disruption of spermatogenesis compared with less complex rearrangements. In other words, meiotic checkpoints may be more permissive of smaller rearrangements, with fewer involved chromosomes, allowing those with abnormal segregation patterns to pass, and therefore produce unbalanced gametes.

While no previous studies have examined the segregation patterns of a CCR, work has been done to elucidate the general reproductive risk associated with CCRs (reviewed in Patsalis, 2007). They report three factors that increase risk, for disruption of spermatogenesis, in 3-way translocation cases – involvement of an acrocentric chromosome, a breakpoint in close proximity to centromere, and a large difference in the sizes of the involved chromosomes. According to these criteria, the CCR reported here is at low risk, only satisfying the second criteria (on chromosome 10).
2.4.5 Small supernumerary marker chromosomes

We aimed to determine the patterns of meiotic segregation of the marker in two carriers sSMC, one alone and one in the presence of an ROB. FISH analysis on sperm from the RB/mar and +mar carriers determined the frequency of the marker in sperm to be 13.5% and 11.5% respectively. Previous studies have also examined the segregation of sSMC in sperm, finding 17.1% (Paetzold *et al.*, 2006) and 6.23% (Cotter *et al.*, 2000). There are a number of possible explanations for the presence of the marker in less than the theoretically predicted 50% of sperm. The marker chromosome could be lost prior to meiosis, during mitotic divisions in the germ line. Indeed, studies of carriers of a variety of numerical abnormalities have observed meiotic cells lacking the additional chromosome (Chandley *et al.*, 1976; Faed *et al.*, 1976; Speed *et al.*, 1991). It is also conceivable that markers can cause meiotic arrest, disrupting spermatogenesis only in cells containing the marker. If this were the case, it could account for both the reduced frequency of the marker in sperm as well as the reduced sperm concentration in the carrier. Indeed, a study of two carrier of sSMC, with no known history of infertility, found that frequency of sperm containing the marker was not significantly different that the theoretically expected 50% (Martin *et al.*, 1986).

We were also interested in the effect that the presence of a sSMC might have on another CA. We compared the segregation patterns of the RB/mar with a previously reported, identical t(13;21) (Hatakeyama *et al.*, 2005). We noted a reduced frequency of unbalanced segregations, relating to the ROB, in the RB/mar carrier when compared with the RB carrier. It is conceivable that the presence of the marker chromosome adds an additional burden to cells with an already abnormal chromosome constitution. Cells with an unbalanced chromosome complement may be at risk for activation of meiotic checkpoints and meiotic arrest, but clearly many escape detection and proceed through spermatogenesis. However, cells with unbalanced chromosome complement, that also contain a sSMC, may be more likely to activate meiotic checkpoints than cells with unbalanced complements alone, further disrupting spermatogenesis, but reducing the frequency of unbalanced chromosome complements in the sperm. Supporting this concept is the relationship between the severity of the patient’s chromosomal
abnormalities and the degree of their infertility. The mar carrier, the smallest of the chromosomal abnormalities we report, had a sperm count of 7.8 million sperm/ml while the RT carrier had 3.8 million sperm/ml. In contrast, the RT/mar carrier, the most severe chromosomal abnormality we report, had only 0.3 million sperm/ml and displayed the lowest motility at 10% total motility.

2.4.6 Mosaic aneuploidy

We examined two carriers of mosaic aneuploidy, for the presence of increased disomy, of the aneuploidy chromosome, in the sperm. With respect to the Xmos case, we found no increase in XX or XY disomy in the sperm. Similarly, in the 21mos case, we found no increase in disomy of chromosome 21 in the sperm.

A number of possible explanations can account for the lack of chromosomally abnormal sperm in carriers of mosaic aneuploidy. It is possible that the pool of germ cells, actively producing sperm, is exclusively chromosomally normal. In mosaic carriers, the germ cells may simply not be one of the tissues affected, or may have lost the additional chromosome in prior mitotic divisions. Furthermore, it is possible that aneuploid spermatocytes are actively undergoing meiosis, and producing aneuploid sperm, but that selective pressures against these sperm prevent them from being observed in the ejaculate.

Previous studies of XXY men, including both constitutive and mosaic carriers, have noted slight but significant increases in sex chromosome disomy in the sperm (Estop et al., 1998, Foresta, 1998, Kruse, 1998). However, the only marginal increase in disomy in mosaic carriers, as well as the absence of spermatogenesis in the majority of constitutive carriers (Hall et al., 2006), has led authors to suggest that, in mosaic carriers, only unaffected cells are able to progress through meiosis (Luciani et al., 1978, Vidal, 1984). Furthermore, if XXY cells were indeed undergoing meiosis, one would expect a sex ratio, among the euploid spermatozoa, of 2:1 for X:Y. However, most studies have found a sex ratio not markedly different from that observed in controls (Blanco et al., 2001; Morel et al., 2003) further suggesting that XY cells rather than XYY cells are predominantly contributing to spermatogenesis. It has also been suggested that XYY are
not able to go through meiosis, but that their presence alters the testicular environment such that it disrupts segregation in neighboring XY cells, thus accounting for the slight increase in sex chromosome disomy observed in mosaic XXY carriers (Rives et al., 2001). A study of a mosaic XYY carrier, with a similar degree of mosaicism (5%, 5/100) as that observed in our study, was found to have a small but significant increase in sex chromosome disomy (1.42%) (Rives et al., 2001). It is likely that a different distribution of mosaic cells between these two carriers can explain the differing levels of sex chromosome aneuploidy observed.

Previous studies of XYY men, have also provided insight into the behaviour of additional chromosomes during meiosis. Studies have found the frequency of meiotic cells containing the additional chromosome to range from none (Evans et al., 1970, Chandley et al., 1976) to as high as 58–100% (Solari and Rey Valzacchi, 1997; Blanco et al., 2001; Rives et al., 2005; Milazzo et al., 2006). In men with a mixture of chromosomally normal and abnormal meiotic cells, it may be that spermatogenesis is only successful, or is preferentially successful, in the chromosomally normal meiotic cells, thereby only producing chromosomally normal sperm. This hypothesis is supported by a number of studies on sperm aneuploidy, in which XYY men were found to have only slight increases abnormal chromosome complements (Mercier et al., 1996; Blanco et al., 1997; Chevret et al., 1997; Mennicke et al., 1997; Rives et al., 2003).

As autosomal aneuploidies have more severe phenotypic consequences than sex chromosome aneuploidies, the number of studies, on even mosaic autosomal trisomies, has been limited. However, studies have reported mosaicism in parents of constitutive autosomal trisomies (Beratis et al., 1972) suggesting that mosaic carriers may produce increased levels of disomic sperm. A recent study of a mosaic carrier of trisomy 18, found a significantly increased frequency of disomy 18 in the sperm (Perrin et al., 2008). However, similarly high levels of disomy were observed for chromosomes 13, 21 and the sex chromosomes, suggesting a generalized disruption of segregation, not necessarily due to the meiosis in trisomic 18 spermatocytes. In a meiotic study of a constitutive trisomy 21 carrier, the authors found the additional 21 as either a univalent or trivalent in a small minority of meiotic cells. The authors argued that in the majority of cells the additional
21 interacts with the sex body (Johannisson et al. 1983). The interaction of the additional chromosome with the sex body is suggested to cause meiotic arrest through disruption of sex chromosome inactivation and segregation (Johannisson et al. 1983).

The accumulated evidence suggests that a number of barriers exist to the development of functional meiotic cells with the presence of an additional chromosome. In cells that do progress, the meiotic behaviour of the additional chromosome predisposes the cell to activation of meiotic checkpoints and meiotic arrest. The cumulative effect of these barriers results in the production of sperm with only a small minority, if not an insignificant minority, of aneuploid chromosome complements.

We have analyzed the segregation patterns in a variety of chromosomal abnormalities. In general our results are consistent with previous studies of similar abnormalities. We have analyzed several previously unstudied abnormalities including three reciprocal translocations, one inversion, two marker chromosomes and a CCR. These results will provide valuable clinical information to carriers considering assisted reproduction. While assisted reproductive technologies, particularly ICSI, allow infertile carriers of chromosomal abnormalities to achieve pregnancy, there is concern that these carriers will produce a chromosomally abnormal conceptus. The results from this study will help determine the necessity, for measures such as preimplantation genetic diagnosis, a process that determines the chromosome constitution of embryos prior to implantation, in carriers of chromosomal abnormalities.
2.5 References


CHAPTER III: INTERCHROMOSOMAL EFFECTS IN CARRIERS OF CHROMOSOMAL ABNORMALITIES

3.1 Introduction

It has been established that the presence of a chromosomal abnormality (CA) increases the likelihood of meiotic disjunction errors involving the rearranged or abnormal chromosomes. However, it has been suggested that such CA can also influence the segregation of uninvolved chromosomes. As a result, increased aneuploidy in the sperm may be observed, for chromosomes not involved in the abnormality, known as an interchromosomal effect (ICE). An ICE was first described in humans by Lejeune (1963), and chromosome translocations have been shown to affect meiotic segregation of uninvolved chromosomes in mice (Ford and Evans, 1973) and in Drosophila (Grell, 1971). However, in humans, the existence of an ICE has remained a source of controversy with some studies observing such an effect (Blanco et al., 2000, Douet-Guilbert et al., 2005, Machev et al., 2005, Anton et al., 2004) while others have not (Honda et al., 1999; Rives et al., 2003).

Confirming both the existence and magnitude of an ICE has been further complicated by the fact that male carriers of CA often display abnormal semen parameters, which in itself is an indicator for elevated rates of sperm aneuploidy. Several studies on sperm from infertile men with normal 46,XY karyotypes have shown that men with abnormal semen parameters are at an increased risk of producing aneuploid sperm (Pang et al., 1999; Vegetti et al., 2000; Martin et al., 2003). This observed increase in sperm aneuploidy from infertile men has been further supported by the increase in de

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novo chromosomal abnormalities of paternal origin after intracytoplasmic sperm injection (ICSI) (Bonduelle et al., 2002; Van Opstal et al., 1997; Tang et al., 2004).

Several studies have examined sperm aneuploidy in translocation carriers with normal semen parameters and found no existence of an ICE (Pellestor et al., 2001; Oliver-Bonet et al., 2004; Douet-Guilbert et al., 2005), further suggesting that the presence of an ICE may be associated with abnormal semen parameters. However, meiotic studies on chromosomally normal men have suggested that defective recombination contributes to both the infertility and increased sperm aneuploidy in men with impaired spermatogenesis (Gonsalves et al., 2004; Ma et al., 2006; Ferguson et al., 2007). Conversely, meiotic studies on carriers of reciprocal translocations suggest that defective recombination is not a contributing factor to infertility in men with chromosomal rearrangements (Oliver-Bonet et al., 2005; Pigozzi et al., 2005). It is likely that the existence of an ICE in male carriers of chromosomal rearrangements, and the increased sperm aneuploidy in karyotypically normal men with abnormal semen parameters, may be two distinct, unrelated phenomena. Thus, the magnitude of sperm aneuploidy, and therefore the risk of a chromosomal abnormality after ICSI, is likely to differ between the two infertile groups.

In this study we used fluorescence in-situ hybridization (FISH) on decondensed sperm nuclei to examine the existence of an ICE in ejaculate samples from twelve CA carriers, seven of which represent previously unstudied structural chromosomal abnormalities. Included are seven translocations, two inversions, a carrier of a complex chromosomal rearrangement, a carrier of a marker chromosome, and two carriers of mosaic aneuploidy. In order to compare the magnitude of sperm aneuploidy in carriers of CA with that observed in chromosomally normal men with similar semen parameters, FISH was also performed on sperm from ten karyotypically normal men with severe oligoasthenoteratozoospermia (OAT). We studied the segregation of chromosomes 13, 18, 21, X and Y, as aneuploidies for these chromosomes are a major cause of spontaneous abortion and congenital malformations in livebirths.
3.2 Materials and Methods

3.2.1 Clinical information

The eleven carriers of chromosomal abnormalities and ten men with OAT were ascertained via fertility clinic workups for primary infertility. All patients had history of infertility ranging from 2 to 6 years. Of the carriers of chromosomal abnormalities, seven out of twelve carriers had severe OAT with sperm count arranging from a few hundreds to 4.2 millions per millimeter, while two had only severe abnormal sperm morphology (<5%) with normal sperm count and motility, and two had normal sperm parameters (Table 3.1). Serum gonadotropins and testosterone were normal in all patients. All patients were carriers of chromosomal rearrangements based on clinical cytogenetic reports including: three Robersonian translocations [45,XY,rob(13;14)(q10;q10), 45,XY,rob(13;21)(q10;q10) and 46,XY,rob(13;21)(p11.1;p11.1),+mar], three reciprocal translocations [46,XY,t(9;22)(p13.1;q13.2); 46,XY,t(4;15)(p12;p11.1) and 46,XY,t(6;21)(q16;q21)], an X:autosome translocation [46,XY,t(X;20)(p10;q10)], a 46,XY,t(1;2;10)(1qter->1p35.1::10q26.13->1-qter; 2pter->2q21.3::1p35.1->1pter; 10pter->10q11.23::10q24.33->10q11.23::2q21.2->2qter) complex chromosomal rearrangement, two inversions, [paracentric 46,XY,inv(5)(q22.1;q23.2) and pericentric 46,XY,inv(Y)], a carrier of a small supernumerary marker chromosome (sSMC) and two carriers of mosaic aneuploidy (47,XXY/46,XY; 47,XY,+21/46XY). In both carriers of mosaic aneuploidy the additional chromosome was found in 4% (2/50) of blood leukocytes. The 45,XY,rob(13;21) case was previously reported on in Hatakeyama et al. (2006). All OAT patients had normal karyotypes and displayed semen parameters with very low sperm count (<5 x 10^6/ml), low motility (<50%) and low normal morphology (<30%), according to WHO (1999) guidelines. Sperm aneuploidy and the ICSI outcome of patient OAT9 were reported previously (Tang et al., 2004). The mean age of the carriers of chromosomal abnormalities was 39.8 (range: 32-53), while the mean age of the OAT patients was 38.1 years (range: 29-50). Ten men of proven normal fertility (naturally fathered at least one child) with normal somatic karyotypes and normal semen analysis, were recruited to form the control group of this study. The mean age of the normal fertile
control donors was 31.6 years (range: 29-33). This study was approved by the UBC clinical ethical aboard prior to the initiation of the experiments.
### Table 3.1 Clinical characteristics of patients’ sperm parameters

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Sperm Parameters</th>
<th>Density (x10^6/ml)</th>
<th>% Total Motility</th>
<th>% Normal forms (WHO criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile carriers of chromosomal rearrangements (n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46,XY,t(9;22)</td>
<td>35</td>
<td>3.2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>46,XY,t(6;21)</td>
<td>53</td>
<td>2.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>46,XY,t(4;15)</td>
<td>42</td>
<td>4.2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>46,XY,t(X;20)</td>
<td>37</td>
<td>0.1</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>45,XY,rob(13;14)</td>
<td>48</td>
<td>70</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>46,XY,rob(13;21),+mar</td>
<td>32</td>
<td>0.3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>45,XY,rob(13;21)</td>
<td>38</td>
<td>3.8</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>46,XY,t(1;2;10)</td>
<td>36</td>
<td>0.9</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>46,XY,inv(5)</td>
<td>39</td>
<td>141</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>47,XXY/+mar</td>
<td>38</td>
<td>20</td>
<td>&gt;50</td>
<td>1</td>
</tr>
<tr>
<td>47,XXY/46,XY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>47,XY,+21/46,XY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>46,XY OAT men (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAT3</td>
<td>38</td>
<td>1.0</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>OAT7</td>
<td>36</td>
<td>4.0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>OAT9</td>
<td>41</td>
<td>0.8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>OAT10</td>
<td>38</td>
<td>3.6</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>OAT11</td>
<td>38</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>OAT13</td>
<td>29</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OAT14</td>
<td>50</td>
<td>2.7</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>OAT15</td>
<td>34</td>
<td>1.2</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>OAT16</td>
<td>35</td>
<td>0.3</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>OAT17</td>
<td>42</td>
<td>1.0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

#### 3.2.2 FISH hybridization and analysis

The methods of sperm preparation, probe hybridization, and FISH analysis were described previously (Tang et al., 2004). Briefly, sperm were fixed onto glass slides (with 3:1 methanol:acetic acid) washed in 2 x SSC (saline-sodium citrate solution) and incubated in dithiothreitol. Triple-colour FISH, with α-satellite DNA probes for chromosomes 18 (SpectrumAqua), X (SpectrumGreen) and Y (SpectrumOrange), and dual-colour FISH, with probes for chromosomes 13 (SpectrumGreen) and 21 (SpectrumOrange) (Vysis Inc., Downers Grove, IL) were used for both patient and control samples. Hybridization procedures followed the manufacturer’s protocols. Only sperm with intact head and tail morphology, and within an area of the slide where
consistent hybridization was evident, were scored. We attempted to score a minimum of
10,000 sperm for each probe set for each individual; however, in some men only several
hundred to thousands sperm could be found in the semen samples.

3.2.3 Statistical analysis

Data from chromosomally normal fertile men were pooled into a control group to
determine a baseline rate of sperm aneuploidy. Disomy rates for individual men from the
two infertile groups were compared to the pooled group of control men using the Chi-
square test. All statistical analyses were performed using the GraphPad Prism V5.0
program (GraphPad Software, San Diego, CA). P<0.05 was considered significant.

3.3 Results

Using dual-colour FISH for chromosomes 13 and 21, and triple-colour FISH for
chromosomes 18, X and Y, a total of 317,584 sperm were scored from the two infertile
groups and 182,387 sperm from ten normal control men. Hybridization efficiencies of
the FISH probes ranged from 98.74% to 99.95%. Out of the eleven carriers of
chromosomal abnormalities, seven men showed significantly increased disomy for at
least one chromosome when compared to the control group (Table 2). The carrier of the
t(1;2;10) complex chromosomal rearrangement showed significantly increased disomies
13 and 21 (p<0.001; Chi-square test); the carrier of the t(9;22) showed an increase in
disomy 21 (p<0.001; Chi-square test); the carrier of the t(6;21) translocation showed an
increase in disomy 13 (p<0.001; Chi-square test); the carrier of the t(X;20) showed an
increase in disomy 13 (p<0.001; Chi-square test); the carrier of the rob(13;14) showed an
increase in disomy 21 (p<0.001; Chi-square test); the carrier of the rob(13;21)+mar
showed an increase in XX or YY disomy (p<0.05; Chi-square test); and the +mar carrier
showed an increase in disomy 18, three men showed a significant increase in disomy 13,
and three men showed an increase in disomy 21 (Table 2). Of the ten men with severe
OAT, only two (OAT10; OAT16) had disomy rates that were not significantly increased when compared to the control group, while the other eight men showed increased disomy for at least one of the chromosomes studied.
Table 3.2 Disomy rates in sperm of control men and infertile men with normal and abnormal karyotypes.

<table>
<thead>
<tr>
<th>Group 1: Control men (n=10)</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>XX or YY</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>CON1</td>
<td>10311</td>
<td>0.12 (12)</td>
<td>0.03 (3)</td>
<td>0.07 (7)</td>
</tr>
<tr>
<td>CON4</td>
<td>10047</td>
<td>0.28 (28)</td>
<td>0.24 (24)</td>
<td>0.05 (5)</td>
</tr>
<tr>
<td>CON5</td>
<td>10019</td>
<td>0.26 (26)</td>
<td>0.30 (30)</td>
<td>0.03 (3)</td>
</tr>
<tr>
<td>CON6</td>
<td>10098</td>
<td>0.18 (18)</td>
<td>0.16 (16)</td>
<td>0.02 (2)</td>
</tr>
<tr>
<td>CON7</td>
<td>10265</td>
<td>0.13 (13)</td>
<td>0.25 (26)</td>
<td>0.18 (18)</td>
</tr>
<tr>
<td>CON8</td>
<td>10226</td>
<td>0.22 (22)</td>
<td>0.26 (27)</td>
<td>0</td>
</tr>
<tr>
<td>CON9</td>
<td>10015</td>
<td>0.05 (5)</td>
<td>0.44 (44)</td>
<td>0.08 (8)</td>
</tr>
<tr>
<td>CON10</td>
<td>10223</td>
<td>0.11 (11)</td>
<td>0.08 (8)</td>
<td>0.05 (5)</td>
</tr>
<tr>
<td>CON12</td>
<td>10171</td>
<td>0.71 (72)</td>
<td>0.05 (5)</td>
<td>0.08 (8)</td>
</tr>
<tr>
<td>CON13</td>
<td>10145</td>
<td>0.12 (12)</td>
<td>0.04 (4)</td>
<td>0.01 (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>91375</td>
<td>0.23 (207)</td>
<td>0.20 (183)</td>
<td>0.06 (56)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: infertile carriers of chromosomal rearrangements (n=12)</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,XY,t(9;22)</td>
<td>10303</td>
<td>0.12 (12)</td>
<td>0.13 (13)</td>
<td>0.05 (5)</td>
</tr>
<tr>
<td>46,XY,t(6;21)</td>
<td>10141</td>
<td>0.22 (22)</td>
<td>0.17 (17)</td>
<td>0.06 (6)</td>
</tr>
<tr>
<td>46,XY,t(4;15)</td>
<td>9996</td>
<td>0.19 (19)</td>
<td>0.15 (15)</td>
<td>0.04 (4)</td>
</tr>
<tr>
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<td>10032</td>
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<tr>
<td>46,XY,rob(13;21),+mar</td>
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<td>0.44 (10)</td>
<td>0.18 (4)</td>
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<td>45,XY,rob(13;21)</td>
<td>10170</td>
<td>0.03 (3)</td>
<td>0.11 (11)</td>
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<tr>
<td>46,XY,inv(5)</td>
<td>10013</td>
<td>0.14 (14)</td>
<td>0.03 (3)</td>
<td>0.01 (1)</td>
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<td>47,XY,+mar</td>
<td>2518</td>
<td>0.56 (14a)</td>
<td>0.40 (10)a</td>
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<td>10084</td>
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<td>-</td>
<td>0.04 (4)</td>
</tr>
<tr>
<td>47,XY,+21 /46XY</td>
<td>5087</td>
<td>0.08 (4)</td>
<td>0.06 (3)</td>
<td>0.04 (2)</td>
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</table>
Table 3.2 continued

<table>
<thead>
<tr>
<th>Group 3: 46,XY OAT men (n=10)</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
<th># of cells</th>
<th>Frequency of disomy [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XX or YY</td>
<td>XY</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>OAT3</td>
<td>10049</td>
<td>0.54 (54)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 (52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 (25)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OAT7</td>
<td>10123</td>
<td>0.24 (24)</td>
<td>0.30 (30)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 (17)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OAT9</td>
<td>10121</td>
<td>0.16 (16)</td>
<td>18.63 (1885)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 (51)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OAT10</td>
<td>10138</td>
<td>0.04 (4)</td>
<td>0.03 (3)</td>
<td>0.11 (11)</td>
</tr>
<tr>
<td>OAT11</td>
<td>10056</td>
<td>0.02 (2)</td>
<td>0.73 (73)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04 (4)</td>
</tr>
<tr>
<td>OAT13</td>
<td>10176</td>
<td>0.33 (34)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47 (48)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 (9)</td>
</tr>
<tr>
<td>OAT14</td>
<td>10551</td>
<td>0.18 (19)</td>
<td>0.49 (52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 (8)</td>
</tr>
<tr>
<td>OAT15</td>
<td>10177</td>
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<td>0.37 (38)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10 (10)</td>
</tr>
<tr>
<td>OAT16</td>
<td>10124</td>
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<td>0.28 (28)</td>
<td>0.02 (2)</td>
</tr>
<tr>
<td>OAT17</td>
<td>9985</td>
<td>1.82 (182)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 (84)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54 (54)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.001 when compared to the pooled control value for the same chromosome, Chi-square test

<sup>b</sup>p<0.05 when compared to the pooled control value for the same chromosome, Chi-square test
3.4 Discussion

The aim of this study was to determine if carriers of structural chromosomal abnormalities display evidence of an ICE and, if so, if this increase in sperm aneuploidy is comparable to that observed in men with similar semen parameters but who are chromosomally normal. Sperm aneuploidy was analyzed from nine proven fertile men, ten chromosomally normal men with severe OAT, and twelve carriers of structural abnormalities with variable degrees of abnormal sperm parameters, including seven translocations, two inversions, and a complex chromosomal rearrangement, two sSMC and two carriers of mosaic aneuploidy.

We observed significant inter-individual variation in disomy rates within the control group, which is consistent with other reports on normal men (Rubes et al., 2005). This variation in sperm aneuploidy may be related to the variation in the frequency of meiotic recombination that has been reported among normal men (Lynn et al., 2002; Sun et al., 2005a., Ferguson, et al., 2007). Inter-individual variation in disomy rates was observed for all chromosomes studied in the OAT group, as well as the group of chromosomal abnormality carriers. Aneuploidy for the sex chromosomes was most frequently increased in the OAT men, suggesting that the sex chromosomes may have an increased susceptibility for segregation error in spermatogenesis, or that sperm maturation is more tolerant of errors in segregation involving sex chromosomes compared to that of the autosomes. Several other studies have found that patients with less than 5 x 10^6/ml sperm concentrations have particularly higher rates chromosomal abnormalities in their sperm (Vegetti et al., 2000; Calogero et al., 2001; Rubio et al., 2001; Martin et al., 2003). While seven of the men with severe OAT in our study showed a significant increase in XY disomy, considerable heterogeneity existed between individuals, with the level of increased sex chromosome aneuploidy ranging from slight to extreme (i.e. up to a 100-fold increase compared to the controls). In contrast, only one of the carriers of CA showed a significant increase in XY disomy when compared to the control group. With respect to chromosomes 13 and 21, the OAT showed increased autosomal disomy in three of the ten men studied. In contrast, the CA group showed significant increases in disomy in six of the twelve carriers studied. Thus, it appears that
infertile carriers of CA can produce increased sperm aneuploidy, primarily of acrocentric chromosomes, but that these carriers produce lower magnitudes of sperm aneuploidy than chromosomally normal men with similarly impaired semen parameters.

Several recent meiotic studies on infertile 46,XY men have shown that a subset of this population display defective recombination, as well as abnormalities in the pairing of meiotic chromosomes (Gonsalves et al., 2004; Sun et al., 2007). In the present study we found that the chromosomally normal men with OAT produced elevated rates of sperm aneuploidy, which may be related to defects in meiotic recombination. In a previous study we analyzed meiotic recombination and sperm aneuploidy in infertile men with either severe OAT or non-obstructive azoospermia and found that a subset of these men displayed defective recombination, particularly involving the sex chromosomes (Ferguson et al., 2007). Furthermore, a high frequency of sex chromosomes lacking recombination was found to increase the risk of sex chromosome aneuploidy in the sperm. Thus, the elevated sex chromosomes aneuploidy that we observed in the sperm of our chromosomally normal OAT population may be the result of defective sex chromosome recombination during meiosis in these men.

In contrast to the studies on 46,XY infertile men, recent immunofluorescent studies on translocation carriers have suggested that, while recombination may be disturbed specifically on the rearranged chromosomes, recombination on other chromosomes appears to be unaffected (Oliver-Bonet et al., 2005; Pigozzi et al., 2005). Thus, the differences in sperm aneuploidy rates between infertile men with normal karyotypes and carriers of structural abnormalities is most likely due to mechanistic differences in both the cause of infertility, and the cause of meiotic non-disjunction. Nevertheless, an analysis of recombination in a t(Y;1) carrier showed reduced genome-wide recombination (Sun et al., 2005b), suggesting that some carriers of chromosomal abnormalities may display abnormal recombination and possibly an increased risk of nondisjunciton for chromosomes not involved in the rearrangement.

Part of the controversy surrounding the ICE is the fact that the mechanism whereby it might originate is not fully understood. While recombination defects may contribute to
infertility and the increased incidence of sperm aneuploidy in some chromosomally normal men, this mechanism does not appear to be a major factor in carriers of chromosomal rearrangements. Rather, meiotic studies on infertile carriers of translocations have shown that the fidelity of synapsis is often compromised in the rearranged chromosomes (Gabriel-Robez et al., 1986, Chandley et al., 1986; Oliver-Bonet et al., 2005). These asynapsed regions of the translocation are transcriptionally silenced, leading to meiotic arrest and infertility in the carrier (Turner et al., 2005). However, these asynapsed regions have also been found to associate with the sex chromosomes during meiosis, and it has been suggested that this interaction may interfere with the disjunction of the sex chromosomes, as well as other autosomal chromosomes (Anton et al., 2004). This translocation-XY association may explain the increased incidence of XY sperm disomy observed in other studies on carriers of chromosomal rearrangements (Anton et al., 2004, Douet-Guilbert et al., 2005, Machev et al., 2005).

We observed a significant increase in sex chromosome aneuploidy in only one carrier, which may suggest a low frequency of rearrangement-XY associations in the remaining men. Meiotic studies on infertile carriers of translocation have shown that the frequency of translocation-XY associations can vary widely between different abnormalities, which may explain the variability in sex chromosome aneuploidy among translocation carriers (Oliver-Bonet et al. 2005, Pigozzi et al. 2005, Ferguson et al., 2008). Nevertheless, there is relatively little information on the pairing and recombination of meiotic chromosomes in carriers of CA, and the hypothesized mechanisms for the origin of an ICE are largely speculative.

It has been suggested that the presence of an ICE in infertile carriers of chromosomal rearrangements may simply be related to the impaired semen parameters, which is known to be associated with elevated sperm aneuploidy. However, the results of this study suggest that meiotic non-disjunction is a more common occurrence in infertile men with a 46,XY karyotype than in those with abnormal karyotypes, despite their similar semen parameters. We observed evidence of an ICE in the six of the twelve carriers of chromosomal rearrangements. The magnitude of increased aneuploidy in the carriers of chromosomal rearrangements was smaller than that observed in the chromosomally normal men with severe OAT, and only one of the carriers of
chromosomal rearrangements showed an increase in sex chromosome aneuploidy. The difference in the magnitude and type of sperm aneuploidy between the two infertile groups is likely related to the different causes of infertility. Infertility in some 46,XY men with OAT may be related to defective meiotic recombination, leading to an increased risk of aneuploid sperm in these men (Ma et al., 2006, Ferguson et al., 2007). However, infertility in carriers of structural chromosomal abnormalities appears to be caused by asynapsis around the breakpoints, which may have only a minimal effect, if any, on the non-disjunction of chromosomes not involved in the rearrangement. Nevertheless, the existence or magnitude of an ICE may be dependent on the characteristics of the rearrangement, such as the chromosomes involved, sites of breakpoints and size of rearrangement. Thus, further studies are needed to determine if certain karyotypic abnormalities are most at risk of an ICE, and detailed meiotic studies on carriers of chromosomal rearrangements will be necessary in order to shed light on the mechanisms that may contribute to an ICE.
3.5 References


Pang MG, Hoegerman SF, Cuticchia AJ, Moon SY, Doncel GF, Acosta AA, Kearns WG (1999) Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridization in spermatozoa from nine patients with oligoasthenoteratozoospermia undergoing intracytoplasmic sperm injection. Hum Reprod 14, 1266-1273.


CHAPTER IV: MEIOTIC ERRORS IN CARRIERS OF CHROMOSOMAL ABNORMALITIES AND INFERTILE MEN

4.1 Introduction

Meiosis is a critical cell division during spermatogenesis, in which the genetic content of the germ cells is reduced by half. During the first meiotic division, the homologous chromosomes undergo two crucial processes: synapsis and recombination. Synapsis of the homologous chromosomes is facilitated by the formation of a proteinaceous structure between the homologues known as the synaptonemal complex (SC). It is within this structure that recombination, necessary for the generation of genetic diversity, occurs. In addition, chiasmata, formed at the sites of recombination, tether the homologous chromosomes facilitating proper segregation. Recent immunofluorescent techniques have allowed the detailed study of the process of pairing and recombination in males (Barlow and Hulten, 1998, Lynn et al., 2002; Ma et al., 2006a). Antibodies against synaptonemal complex 1 (SYCP1) (transverse elements) and SYCP3 (axial elements) allow the visualization of the synaptonemal complex, while antibodies against the Mut-L homologue 1 (MLH1) and CREST antiserum, allow the visualization of the location of crossovers and centromeres respectively.

Several studies, using these techniques, have provided insight into the role of meiotic errors in infertility. Infertile men, specifically those with non-obstructive azoospermia (NOA), have been shown to display reduced recombination and a higher incidence of synaptic errors when compared with controls (Gonsalves et al., 2004; Judis, 2004, Sun et al., 2004, 2007; Ma et al., 2006b; Ferguson et al., 2007). It is thought that defects, in recombination and synapsis, are caught by meiotic checkpoints, leading to spermatogenic arrest and reduced sperm concentration (Edelmann et al., 1996; Baker et

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1 A version of this chapter will be submitted for publication. Kirkpatrick, G. and Ma, S. Meiotic errors in carriers of chromosomal abnormalities and infertile men.
As meiosis results in the segregation of chromosomes into separate daughter cells, it has been thought that errors in meiosis may cause an increase in the non-disjunction of chromosomes, resulting in an increase in sperm aneuploidy. Indeed, abnormalities in both the frequency (Hassold et al., 1991; Shi et al., 2001; Reish et al., 2004) and distribution (Hasold et al., 1995; Lamb et al., 1996; Ferguson et al., 2009) of crossovers have been linked with increased non-disjunction and the production of aneuploid sperm. Furthermore, recent studies have shown that errors on specific chromosomes are linked to aneuploidy of those chromosomes in the sperm (Ma et al., 2006b; Ferguson et al., 2007; Sun et al., 2008).

Chromosomal abnormalities (CA) are associated with infertility, with the frequency of CA ten times higher in the male infertile population (de Braekeleer and Dao, 1991). The infertility in these carriers is thought to be due to the behaviour of the CA during meiosis. Carriers of chromosomal rearrangements are known to adopt unique structures in order to pair the homologous regions of the rearranged chromosomes during meiosis. Carriers of reciprocal and Robertsonian translocations have been shown to adopt cruciform structures, known as quadrivalents and trivalents respectively. Inversions form an inversion loop in which the normal or inverted chromosome loops back allowing the homologous regions to pair. However, despite improved synapsis between the rearranged and normal chromosomes, some degree of asynapsis is commonly observed around the breakpoints of the rearrangement. The behaviour of numerical abnormalities during meiosis is poorly understood. Furthermore, some studies have suggested that often, numerical abnormalities are lost prior to meiosis. Studies, that have observed the presence of a numerical abnormality during meiosis, have noted varied behaviour, including association of the numerical abnormality with homologous chromosomes and presence of the abnormality as a univalent.

Meiotic analysis with electron microscopy, has indicated a preferential association of CA with the sex body. This association has been observed in a variety of CA including: reciprocal translocations (Chandley et al. 1986; Gabriel-Robez et al. 1986; Luciani et al., 1987), Robertsonian translocations (Luciani et al., 1984; Rosenmann et al. 1985), and numerical abnormalities (Johannisson et al., 1983). Mouse studies have
further confirmed this interaction, and linked it to the disruption of spermatogenesis (Forejt and Gregorova, 1977; Forejt, 1979; Forejt et al., 1981). Interaction of the CA with the SB is hypothesized to disrupt spermatogenesis through disruption of the normal behaviour of the XY sex body. During meiosis, the X and Y chromosomes exhibit markedly different behavior from the autosomes. Unlike the autosomes, the sex chromosomes can only pair along two small pseudo autosomal regions (PAR). As a result of the large regions of asynapsis between the sex chromosomes, the sex chromosomes undergo transcriptional silencing, in a process known as meiotic sex chromosome inactivation (MSCI) and form a peripheral structure known as the XY body (Handel, 2004, Solari, 1974).

It is believed that the association of the rearrangement with the sex body occurs as a result of the unsynapsed regions on the CA. Indeed, studies have noted especially high sex body association in carriers with a high degree of asynapsis (Chandley et al., 1986). The association with the SB may allow silencing of the unsynapsed regions of the rearrangement in a similar manner to MSCI. Markers of transcriptional silencing have been observed around the breakpoints of rearrangements during meiosis. Jaafar et al (1989) suggested that spreading of inactivation from the sex body to the autosome could be a cause of spermatogenic arrest. Studies in mice, in which there is an interaction between the rearrangement and the sex body, have shown reduced expression in the rearrangement, however, they have also noted increased transcriptional activity on the X chromosome (Homolka et al., 2007). This and other evidence has lead to concern that the interaction of the rearrangement, with the abnormality, can result in disruption of MSCI leading to activation of meiotic checkpoints, meiotic arrest and reduced sperm parameters. A study by Oliver Bonet et al., (2005) compared two carriers of reciprocal translocations, one azoospermic and one normozoospermic, and found interactions between the translocation and the sex body only in the azoospermic carrier.

A few studies have utilized immunoflorescent techniques to examine the detailed meiotic behaviour of CA. However, these studies have been limited to carriers of reciprocal translocations. We report the first examination of genome wide synapsis and recombination in carriers of a Robertsonian translocation, a small supernumerary marker.
chromosome (sSMC) and an inversion. We compare these results with fertile controls and karyotypically normal infertile men. In addition, we combined FISH with immunofluorescent techniques to study synapsis and recombination, on chromosomes 13, 18, 21 and the sex chromosomes. FISH on sperm was used to determine the relationship between recombination and aneuploidy in specific chromosomes. In carriers of CA, we used FISH, following immunostaining, for the chromosomes involved in the abnormality, to study the meiotic behaviour of the CA. Lastly, in two of the carriers of CA, we report the use of a novel method of examining spermatocytes derived from the ejaculate. We compare ejaculate-derived spermatocytes with testicular-derived spermatocytes from one individual to determine the efficacy of this technique for future use.

4.2 Materials and Methods

4.2.1 Patient tissue collection and group classification

Ethical approval was obtained, prior to the initiation of the study, from the University of British Columbia Ethics Committee. We examined spermatocytes derived from testicular tissue and ejaculate samples. Testicular tissue was obtained from seven men undergoing fertility treatment, of which one was a carrier of a CA and five men, of proven fertility, undergoing a vasectomy reversal. Among the infertile men, the testicular tissue was used to retrieve sperm, to generate a pathology diagnosis, and a small piece was used for the meiotic analysis reported in this study. The fertile men undergoing vasectomy reversal had samples frozen in the event that the reversal failed. Once again a small piece was used in the study described here. In two carriers of CA and one infertile man, meiosis was examined in spermatocytes that were located in the ejaculate. In addition to a testicular histology report, infertile men were screened for cystic fibrosis mutations, Y-chromosome microdeletions and abnormal hormone profiles.

Spermatocytes were collected and analyzed from fifteen men seeking fertility treatment. These men were classified into four groups. Group I was the control group, consisting of five fertile men who had undergone a vasectomy after fathering a child naturally. The karyotypically normal infertile men were classified into groups based on their pathological diagnosis. Group II (obstructive azoospermia [OA]), included men
diagnosed with normal spermatogenesis, but with no sperm in the semen (n=3). In two of these men (OA4, OA6) the nature of the obstruction was not discovered, while OA3, was found to have congenital absence of the vas deferens as a result of being heterozygous for the dF508 CFTR mutation. Group III (non-obstructive azzospermia [NOA]) included men diagnosed with abnormal spermatogenesis (n=3), including: NOA1, diagnosed with early maturation arrest, due to a Y-deletion of the AZFc region, NOA2, diagnosed with hypospermatogenesis (a reduced number of germ cells showing normal maturation), NOA5, diagnosed with Sertoli cell only syndrome (a lack of germ cells) and NOA7 diagnosed with late maturation arrest at the spermatid stage. Group IV (chromosomal abnormalities [CA]), included men found to be carriers of chromosomal abnormalities based on clinical cytogenetic reports (n=3) including: CA1, a carrier of a small supernumerary marker chromosome [47,XY,inv dup(22)(q11.1)]; CA2, a carrier of a Robertsonian translocation [45,XY,rob(13;21)(q10;q10)]; and CA3, a carrier of an inversion in chromosome 1 [46,XY,inv(1)(p21q31)]. All patients had normal hormonal profiles.

4.2.2 Preparation of tissue

Immunofluorescent analysis required that patients agree to a testicular biopsy in order to retrieve a small amount of tissue. Tissue was processed using a modification of the one used by Barlow and Hulten (1998). In modified HTF (human tubal fluid), seminiferous tubules were teased apart into 3-5 mm segments. These segments were placed in Hypo-extraction buffer [30 mM Tris, 50 mM sucrose, 17 mM citric acid, 5 mM EDTA, 0.5 mM dithiothreitol (DTT) and 0.1 mM phenylmeth- ylsulphonyl flouride] and incubated for 50-60 min at 37°C. Following incubation, segments were transferred to 20ul of 100mM sucrose (pH 8.2) on a slide. Tubule segments were squeezed with fine forceps, and 10 ul of the germ cell/sucrose slurry was transferred to a slide with fixative (1% paraformaldehyde with 0.2% Triton X). Slides were then incubated for 24 hours in a humid chamber.

4.2.3 Fluorescent immunostaining

Following incubation, slides were air-dried for 30 min and washed twice in 0.4% Photoflow (Kodak 200 solution). Slides were then soaked in 1x ADB solution (1%
donkey serum, 0.3% bovine serum albumin, 0.005% Triton X, PBS; pH 7.2) at room temperature for 30 min. Primary antibody cocktail [rabbit antihuman MLH1 (Oncogene, San Diego, CA, USA), 1:37.5; SCP3 antimouse immunoglobulin (Ig)G1 (produced by P.Moens, York University), 1:300, SCP1 antimouse immunoglobulin (Ig)G1 (produced by P.Moens, York University), 1:300; CREST antisera, 1:25; 1x ADB] was applied to drained slides. Slides were covered with a coverslip and incubated in a humid chamber at 37°C for 24 hours. Slides were washed for 20 min in 1x ADB, and then washed again in 1x ADB for 48 hours at 4°C. Secondary antibody cocktail [Flourescein (FITC) Donkey antirabbit IgG (Jackson ImmunoResearch, West Grove PA, USA), 1:50; Rhodamine (TRITC) Goat antimouse IgG (Jackson ImmunoResearch), 1:100; (AMCA) Donkey antihuman IgG (Jackson ImmunoResearch), 1:50; 1xADB] was applied for 1 hour at 37°C in a humid chamber. Following incubation, slides underwent three successive washes in PBS for 10, 20 and 30 min with agitation every five minutes. Slides were then air-dried, and antifade was applied and covered with a coverslip. Slides were analyzed with a Zeiss Axioplan epifluorescent microscope. Images were captured using Cytovision v2.81 Image Analysis software (Applied Imaging International, San Jose, CA, USA).

Criteria for capturing pachytene cells required that: all chromosomes were present; that MLH1 labeling was clear; and that the sex chromosomes were observable. Cell coordinates were noted and image printouts were analyzed for numbers of recombination foci and the presence of synaptic abnormalities.

### 4.2.4 FISH on spermatocytes and spermatozoa

Following immunofluorescent analysis of MLH1 and SYCP3/SYCP1 on pachytene spermatocytes, fluorescent in-situ hybridization (FISH) was performed to identify chromosomes 13, 18 and 21. Coverslips were removed and slides were soaked in PBD, followed by washing in an alcohol series (70, 80, 90 and 100%). A probe mixture, of CEN 18 (Spectrum Aqua), LSI 13 (Spectrum Green) and LSI 21 (Spectrum Orange) (Vysis Inc., Downers Grove, IL, USA) was applied to air-dried slides and covered with a coverslip and sealed with rubber cement. Slides were co-denatured in a thermocycler at 75°C, for 5 min, and then placed in a humid chamber, at 37°C, for 24 hours. Slides were washed in 73°C 0.4x saline sodium citrate (SSC)/0.3% NP-40 solution for 2 min with
agitation for 3 sec, followed by a wash in 2x SSC/0.1% NP-40 for 30 sec. Slides were air-dried in the dark and then covered with antifade and a coverslip. Using the coordinates recorded during MLH1/SCP3 analysis, cells were found and the location and frequency of recombination were recorded for chromosomes 13, 18 and 21.

With respect to the CA group, in addition to analysis with chromosomes 13, 18 and 21, slides were hybridized with probe mixtures specific for the chromosomes involved in the CA and for the sex body. CA1 was hybridized with a probe mixture containing CEN 14/22 (Spectrum Red) and X/Y (spectrum Aqua). CA2 was hybridized with a probe mixture containing LSI 13 (Spectrum Green), LSI 21 (Spectrum Orange) and X/Y (Spectrum Aqua). CA3 was hybridized with a probe mixture containing 1q tel (Spectrum Orange), 1ptel (Spectrum Green) and X/Y (Spectrum Aqua).

For all men studied, spermatozoa were hybridized, on separate slides, with a probe mixture of X (Spectrum Green), Y (Spectrum Orange) and 18 (Spectrum Aqua) and chromosomes 13 (Spectrum Orange) and 21 (Spectrum Green) (Vysis Inc., Downers Grove, IL, USA) following the same decondensation, hybridization, and post-hybridization procedures described in the analysis of spermatocytes.

4.2.5 Tissue preparation for spermatocytes derived from the ejaculate

Ejaculate samples were washed in modified HTF and spun down. The pellet was re-suspended in 30ul Hypo-extraction buffer and incubated for 3 hours at 37°C. Following incubation, the 30ul of sample was dropped on slides previously covered in fixative (1% paraformaldehyde with 0.2% Triton X). Slides were then incubated for 24 hours in a humid chamber. Following incubation, ejaculate samples were treated identically to testicular samples.

4.2.6 Statistical analysis

Comparisons of means, between multiple groups, were made with the Kruskall-Wallace test with a Dunn’s post test. Comparisons of means between individual groups and comparisons of means, of recombination, between individuals were made using the Mann Whitney test. The Fishers exact test used for comparisons, between individuals and
between individuals and pooled controls, of the: proportion of cells at various stages of prophase; frequency of recombination in the XY body; frequency of recombination on individual autosomes; and proportion of cells with synaptic anomalies. In addition, individual values that were beyond the 95% confidence interval of the control group were considered significantly different from the controls. The Pearson’s correlation test was used to compare disomy rates with the frequency of recombination on individual chromosomes. Statistical analyses were performed using the GraphPad Prism V5.0 program (GraphPad Software, San Diego, CA). P<0.05 was considered significant.

4.3 Results

4.3.1 Progression through prophase 1

Prophase cells were scored as being in either: 1) leptotene, if they contained only small fragments of the SC, 2) zygotene, if synapsis of the chromosomes had begun but was not complete, 3) zygotene/pachytene, if the synapsis was complete but there were no recombination foci, or 4) pachytene, if synapsis and recombination foci were both present (Figure 4.1). While cells in the diplotene stage were observed, they were extremely rare and so were not scored. The mean frequencies of cells in leptotene, zygotene, zygotene/pachytene, and pachytene in the control group were 5.6%, 5.3%, 13.8% and 74.9% respectively (Table 4.1). The NOA, OA and CA groups did not differ from the control group (p>0.05, Kruskall-Wallace test). However, several individuals had a significantly reduced proportion of cells at pachytene stage (NOA1, NOA2, OA3, CA3) when compared to the control group. Individual men showed a significantly increased proportion of cells in: leptotene (NOA1, NOA7, OA3), zygotene (NOA1, NOA7, OA3, OA4, CA3), and zygotene/pachytene (NOA2, OA4, CA3) when compared with the control group (Table 4.1).
Figure 4.1 Progression of spermatocytes through prophase of meiosis I.

(A) Spermatocytes in Leptotene display small fragments of SC accompanied by between 23-46 CREST foci. (B) Zygotene stage spermatocytes display incomplete synapse. (C) Spermatocytes at the Zygotene/Pachytene transition are characterized by complete synapse but an absence of recombination foci. (D) Pachytene spermatocytes display complete synapse and recombination.
### Table 4.1 Analysis of progression through prophase

<table>
<thead>
<tr>
<th>Path.</th>
<th># of cells</th>
<th>Leptotene (%)</th>
<th>Zygotene (%)</th>
<th>Zygotene/Pachytene (%)</th>
<th>Pachytene (%)</th>
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<td><strong>Group 1: Control Men (n=5)</strong></td>
<td></td>
<td></td>
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<tr>
<td>C1</td>
<td>45 Norm.</td>
<td>224</td>
<td>7.6</td>
<td>4.8</td>
<td>11.2</td>
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<tr>
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<td>50 Norm.</td>
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<td>7.3</td>
<td>1.1</td>
<td>6.0</td>
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<td>C3</td>
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<td>230</td>
<td>6.4</td>
<td>8.7</td>
<td>18.2</td>
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<tr>
<td>C4</td>
<td>37 Norm.</td>
<td>321</td>
<td>1.2</td>
<td>6.8</td>
<td>19.6</td>
</tr>
<tr>
<td>C5</td>
<td>46 Norm.</td>
<td>326</td>
<td>9.3</td>
<td>17</td>
<td>10.4</td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
<td></td>
<td><strong>5.6±3.0</strong></td>
<td><strong>5.3±3.3</strong></td>
<td><strong>13.8±6.3</strong></td>
<td><strong>74.9±8.1</strong></td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td>(3-8.2)</td>
<td>(2.4-8.2)</td>
<td>(8.3-19.3)</td>
<td>(67.8-82)</td>
</tr>
<tr>
<td><strong>Infertile men (n=10)</strong></td>
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<tr>
<td><strong>Group 2: Non Obstructive Azoospermia (NOA) (n=4)</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>NOA1</td>
<td>44 MA</td>
<td>335</td>
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<td>NOA2</td>
<td>30 HS</td>
<td>126</td>
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</tr>
<tr>
<td>NOA5</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>362</td>
<td>9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.9</td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
<td></td>
<td><strong>8.5±0.8</strong></td>
<td><strong>11.1±3.9</strong></td>
<td><strong>15.4±5</strong></td>
<td><strong>64.9±4.6</strong></td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td>(8.2-8.8)</td>
<td>(9.6-12.6)</td>
<td>(13.5-17.3)</td>
<td>(63.1-66.7)</td>
</tr>
<tr>
<td><strong>Group 3: Obstructive Azoospermia (OA) (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OA3</td>
<td>34 Norm.</td>
<td>144</td>
<td>27.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OA4</td>
<td>33 Norm.</td>
<td>237</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OA6</td>
<td>32 Norm.</td>
<td>166</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
<td></td>
<td><strong>10.2±15.3</strong></td>
<td><strong>10±2.4</strong></td>
<td><strong>11.8±8.6</strong></td>
<td><strong>67.9±12</strong></td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td>(4.2-16.2)</td>
<td>(9.1-10.9)</td>
<td>(8.5-15.1)</td>
<td>(63.2-72.6)</td>
</tr>
<tr>
<td><strong>Group 3: Carriers of Chromosomal Abnormalities (CA) (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>38 -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA2</td>
<td>38 -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA3</td>
<td>31 MA</td>
<td>317</td>
<td>4.3</td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: HS: Hypospermatogenesis, MA: Maturation arrest, Norm.: Normal, SCOS: Sertoli cell only syndrome  
<sup>a</sup>p<0.05 when compared with the control group (significantly lower)  
<sup>b</sup>p<0.05 when compared with control group (significantly higher)  
Group mean were compared using Kruksall-Wallace with a Dunn’s post test; individual values were considered significant from the control group if they were beyond the 95% confidence interval of the control group.

### 4.3.2 Analysis of genome-wide recombination

We assessed global rates of recombination and noted the presence of synaptic errors by immunostaining cells for antibodies that mark sites of recombination (MLH1) and components of the SC (SYCP1, SYCP3) (Figure 4.2 A). A total of 475 pachytene nuclei were examined in the control group and the mean frequency of recombination was
49.8 with a range of 48.5-50.9 (Table 4.2). The recombination levels observed in our control men are within those reported by others (Lynn et al., 2002; Hassold et al., 2004; Sun et al., 2005; Ferguson et al., 2007). Mean rates of recombination did not vary between groups (Kruskall-Wallace test). However, individuals showed significantly higher (NOA2, NOA7) and significantly lower (OA4, OA6, OA3, CA1, CA2) rates of recombination when compared with controls (p<0.05, Mann-Whitney test).

The proportion of cells in which recombination occurred in the sex body ranged from 86%-94% in the control men and 89%-97% in the OA group (Table 4.2). The mean proportion of cells with a recombination event in the sex body was significantly lower in the NOA and CA groups when compared with the control group (p<0.05, Mann-Whitney test). In addition, individuals in the NOA and CA groups showed significantly reduced frequencies of sex body recombination (NOA1, NOA2, CA2) (p<0.05, Fisher test) when compared with pooled controls. There was no difference in the frequency of achiasmate autosomal chromosomes (chromosomes lacking a recombination event) between groups (Kruskall-Wallace test), or among any individual when compared with controls (Fisher test) (Table 4.2).
Figure 4.2 Immunofluorescent and FISH analysis of pachytene nuclei.

(A) Spermatocytes were immunolabelled to visualize the SC (red), MLH1 (green) and centromeres (blue). Pachytene nuclei displaying an absence of recombination in the sex body (B) and autosomal bivalents (D) were noted. (C) Synaptic errors including gaps and asynapsis were observed. FISH following immunostaining (F) allowed us to examine synapsis and recombination on chromosomes 13, 18 and 21 (E).
Table 4.2 Analysis of MLH1 foci and synaptic errors in fertile men, infertile men and carriers of chromosomal abnormalities

<table>
<thead>
<tr>
<th>Group</th>
<th># of cells</th>
<th>Mean recombination foci per cell</th>
<th>Range of recombination foci</th>
<th>Recombination in XY bivalent (%)</th>
<th>Proportion of cells lacking an MLH1 focus in an autosomal bivalent (%)</th>
<th>Proportion of cells with gaps (%)</th>
<th>Proportion of cells with unpaired regions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control Men (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>100</td>
<td>50.1±4.0</td>
<td>41-61</td>
<td>86.0</td>
<td>0</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C2</td>
<td>100</td>
<td>50.4±4.7</td>
<td>35-60</td>
<td>94.0</td>
<td>3.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>C3</td>
<td>75</td>
<td>48.5±4.7</td>
<td>33-58</td>
<td>92.0</td>
<td>5.3</td>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>C4</td>
<td>100</td>
<td>49.3±4.4</td>
<td>36-61</td>
<td>91.0</td>
<td>0</td>
<td>21.0</td>
<td>2.0</td>
</tr>
<tr>
<td>C5</td>
<td>100</td>
<td>50.9±3.6</td>
<td>42-61</td>
<td>88.0</td>
<td>0</td>
<td>10.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean±SD (95% CI)</td>
<td></td>
<td>49.8±0.9</td>
<td>49-50.6</td>
<td>90.2±3.2</td>
<td>1.7±2.4</td>
<td>8.9±7.5</td>
<td>1.4±1.1</td>
</tr>
<tr>
<td>Group 2: Non Obstructive Azoospermia (NOA) (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOA1</td>
<td>63</td>
<td>51.0±5.6</td>
<td>41-68</td>
<td>79.4±c</td>
<td>0</td>
<td>9.5</td>
<td>15.8c</td>
</tr>
<tr>
<td>NOA2</td>
<td>100</td>
<td>53.8±5.7b</td>
<td>41-67</td>
<td>75.8±c</td>
<td>1.0</td>
<td>2.0e</td>
<td>2.0</td>
</tr>
<tr>
<td>NOA5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NOA7</td>
<td>100</td>
<td>54.9±4.4b</td>
<td>44-65</td>
<td>84.0±e</td>
<td>0</td>
<td>22.0±e</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean±SD (95% CI)</td>
<td></td>
<td>53.2±2</td>
<td>79.7±4.1a</td>
<td>0.3±0.6</td>
<td>11.2±10.1</td>
<td>6.3±8.3</td>
<td></td>
</tr>
<tr>
<td>Group 3: Obstructive Azoospermia (OA) (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OA4</td>
<td>100</td>
<td>46.1±4.1a</td>
<td>37-64</td>
<td>89.0</td>
<td>1.0</td>
<td>13.0</td>
<td>4.0c</td>
</tr>
<tr>
<td>OA6</td>
<td>113</td>
<td>47.4±4.4a</td>
<td>35-62</td>
<td>89.4</td>
<td>1.8</td>
<td>5.3</td>
<td>0.9</td>
</tr>
<tr>
<td>OA3</td>
<td>100</td>
<td>48.8±3.4a</td>
<td>40-57</td>
<td>97.0±e</td>
<td>1.0</td>
<td>17.0c</td>
<td>0e</td>
</tr>
<tr>
<td>Mean±SD (95% CI)</td>
<td></td>
<td>47.4±1.4</td>
<td>91.8±4.5</td>
<td>1.3±0.5</td>
<td>11.8±5.9</td>
<td>1.6±2.1</td>
<td></td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td>45.9-49.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 continued

<table>
<thead>
<tr>
<th>Group 4: Carriers of Chromosomal Abnormalities (CA) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td># of cells</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA2</td>
</tr>
<tr>
<td>CA3</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
<tr>
<td>(95% CI)</td>
</tr>
</tbody>
</table>

<sup>a</sup> p<0.05, reduced when compared with controls Mann-Whitney test  
<sup>b</sup> p<0.05, increased when compared with controls Mann-Whitney test  
<sup>c</sup> p<0.05, Significantly increased when compared with pooled controls, Fisher exact test  
<sup>d</sup> p<0.05, Significantly decreased when compared with pooled controls, Fisher exact test  
<sup>e</sup> individual value beyond the 95% confidence interval of the control group  

(95% CI)
4.3.3 Analysis of synaptic errors

Two types of synaptic errors were commonly observed in the analysis of pachytene cells. These included gaps in the SC, in which both SYCP1 (transverse elements) and SYCP3 (lateral elements) staining was absent (Figure 4.2 C), as well as asynapsis of the SC, in which only the SCYP3 was observed (Figure 4.2 C). The mean frequency, of cells with gaps in the SC, was 8.9% in control men, with a range from 1%-21% (Table 4.2). The mean frequency of gaps was not significantly different from controls in the NOA and OA groups, however, a significant increase in the mean frequency of gaps was observed in the CA group (p<0.05, Mann-Whitney test) and each individual man in the CA group showed an increase in gaps when compared with pooled controls (p<0.05, Fisher test). Two additional individuals in the NOA and OA groups showed an increased frequency of gaps when compared with pooled controls (NOA7, OA3) (p<0.05, Fisher test). The mean frequency, of cells with unsynapsed regions of the SC, was 1.4% in control men with a range from 0%-3% (Table 4.2). There was no difference in the mean frequency of cells with unsynapsed regions between groups. However, four individual showed a significantly higher frequency when compared with controls (NOA1, OA4, CA1, CA3) (p<0.05, Fisher test).

4.3.4 Chromosome-specific recombination and sperm aneuploidy

Following the immunoflorescent analysis of pachytene cells, we hybridized slides with FISH probes for chromosomes 13, 18 and 21, in order to determine chromosome specific rates of recombination on those chromosomes (Figure 4.2 E, F). We examined 223 pachytene nuclei from 5 control men, and 547 pachytene nuclei from 9 men in the infertile groups (Table 4.3). Two individuals showed significantly altered chromosome-specific rates of recombination. In the control group, chromosome 13 displayed single recombination foci in 9.4% of cells and two or more recombination foci in 90.6% of cells. The proportion of nuclei with single recombination foci was increased in OA4 to 29.4% with a corresponding decrease in nuclei, with two or more foci, to 70.6%. In CA2, chromosome 13 displayed single recombination foci in 71.4% of nuclei and two or more recombination foci in 21.4% of nuclei. In the control group, chromosome 18 displayed single recombination foci in 13% of pachytene nuclei and two or more foci in 87% of
foci. In contrast, OA4 and CA2 displayed increases in the proportion of cells with single foci with 27% and 21.4% respectively. With respect to chromosome 21, control men showed an absence of recombination foci in 0.4% of nuclei and the presence of single foci in the remaining 99.6% of nuclei. In CA2, 35.7% of nuclei showed an absence of recombination on chromosome 21, with a corresponding 64.3% showing single recombination foci.
# Table 4.3 Analysis of crossovers on chromosomes 13, 18 and 21

<table>
<thead>
<tr>
<th># of cells</th>
<th>Chromosome 13 [% (n)]</th>
<th>Chromosome 18 [% (n)]</th>
<th>Chromosome 21 [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 foci</td>
<td>1 foci</td>
<td>≥ 2 foci</td>
</tr>
<tr>
<td><strong>Group 1: Control Men (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>38</td>
<td>0% (0)</td>
<td>10.5% (4)</td>
</tr>
<tr>
<td>C2</td>
<td>50</td>
<td>0% (0)</td>
<td>20% (10)</td>
</tr>
<tr>
<td>C3</td>
<td>14</td>
<td>0% (0)</td>
<td>14.3% (2)</td>
</tr>
<tr>
<td>C4</td>
<td>61</td>
<td>0% (0)</td>
<td>6.6% (4)</td>
</tr>
<tr>
<td>C5</td>
<td>60</td>
<td>0% (0)</td>
<td>1.7% (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>223</td>
<td>0%</td>
<td>9.4% (21)</td>
</tr>
<tr>
<td><strong>Infertile Men (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOA1</td>
<td>61</td>
<td>0% (0)</td>
<td>9.8% (6)</td>
</tr>
<tr>
<td>NOA2</td>
<td>86</td>
<td>0% (0)</td>
<td>7% (6)</td>
</tr>
<tr>
<td>NOA5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NOA7</td>
<td>76</td>
<td>0% (0)</td>
<td>3.9% (3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>223</td>
<td>0%</td>
<td>6.7% (15)</td>
</tr>
<tr>
<td><strong>Group 2: Non Obstructive Azoospermia (NOA) (n=4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OA3</td>
<td>90</td>
<td>0% (0)</td>
<td>12.2% (11)</td>
</tr>
<tr>
<td>OA4</td>
<td>85</td>
<td>0% (0)</td>
<td>29.4% (25)</td>
</tr>
<tr>
<td>OA6</td>
<td>86</td>
<td>0% (0)</td>
<td>12.8% (11)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>261</td>
<td>0%</td>
<td>18% (47)</td>
</tr>
<tr>
<td><strong>Group 3: Obstructive Azoospermia (OA) (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>16</td>
<td>0% (0)</td>
<td>12.5% (2)</td>
</tr>
<tr>
<td>CA2</td>
<td>14</td>
<td>7.1% (1)</td>
<td>71.4% (10)</td>
</tr>
<tr>
<td>CA3</td>
<td>33</td>
<td>0% (0)</td>
<td>12.1% (4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>63</td>
<td>1.6% (1)</td>
<td>25.4% (16)</td>
</tr>
</tbody>
</table>

*p<0.05, Significantly different when compared with pooled controls, Fisher exact test*
Recombination is thought to play a key role in the proper segregation of chromosomes at meiosis. As aberrant recombination may cause non-disjunction, and an increase in sperm disomy, we analyzed the frequency of disomy in the sperm for the chromosomes 13, 18, 21 and the sex chromosomes (Table 4.4). The mean frequency of disomy, in the sperm, from the control, OA and CA groups respectively, were: 0.44%, 0.29% and 0.39% for XX and YY; 0.43%, 0.48% and 0.32% for XY; 0.19%, 0.45% and 0.04% for chromosome 18; 0.6%, 0.6% and 0.71% for chromosome 13; and 0.5%, 0.58% and 1.2% for chromosome 21 (Table 4.4). Although we observed an increase in chromosome 13 and 21 disomy in the CA group and chromosome 18 disomy in the OA group, no difference in the mean frequency of disomy, for each of the chromosomes examined, between each of the infertile groups and the control group was statistically significant (p>0.05, Kruskall-Wallace test). Nevertheless, significant increases in disomy were observed in several individuals: OA3 (chromosomes 18, 21), OA4 (chromosome 18), CA1 (chromosomes 13, 21), CA2 (XX and YY), and CA3 (XX and YY, chromosome 21) when compared with controls.

Table 4.4 Disomy rates in sperm of control men and infertile men with normal and abnormal karyotypes.

<table>
<thead>
<tr>
<th>Group 1: Control men (n=4)</th>
<th>Frequency of disomy [% (n)]</th>
<th>Group 2: Non Obstructive Azoospermia (NOA) (n=4)</th>
<th>Frequency of disomy [% (n)]</th>
<th>Group 3: Obstructive Azoospermia (OA) (n=3)</th>
<th>Frequency of disomy [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of cells</td>
<td>XX or YY</td>
<td>XY</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.37 (4)</td>
<td>0.37 (4)</td>
<td>0.28 (3)</td>
<td>0.89 (7)</td>
</tr>
<tr>
<td></td>
<td>1025</td>
<td>0.58 (6)</td>
<td>0.3 (3)</td>
<td>0.19 (2)</td>
<td>1174</td>
</tr>
<tr>
<td></td>
<td>1015</td>
<td>0.49 (5)</td>
<td>0.79 (8)</td>
<td>0.2 (2)</td>
<td>1010</td>
</tr>
<tr>
<td></td>
<td>1034</td>
<td>0.48 (5)</td>
<td>0.39 (4)</td>
<td>0.3 (3)</td>
<td>1199</td>
</tr>
<tr>
<td></td>
<td>1003</td>
<td>0.3 (3)</td>
<td>0.3 (3)</td>
<td>0</td>
<td>1007</td>
</tr>
<tr>
<td>Mean</td>
<td>0.44</td>
<td>0.43</td>
<td>0.19</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.35-0.54)</td>
<td>(0.26-0.6)</td>
<td>(0.09-0.29)</td>
<td>(0.4-0.82)</td>
<td>(0.36-0.66)</td>
</tr>
<tr>
<td></td>
<td>No sperm</td>
<td>Insufficient sperm</td>
<td>No sperm</td>
<td>Insufficient sperm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NOA1</td>
<td>NOA2</td>
<td>NOA5</td>
<td>NOA7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No sperm</td>
<td>Insufficient sperm</td>
<td>No sperm</td>
<td>Insufficient sperm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OA3</td>
<td>0.19 (2)</td>
<td>0.39 (4)</td>
<td>0.39 (4)</td>
<td>1023</td>
</tr>
<tr>
<td></td>
<td>OA4</td>
<td>0.29 (3)</td>
<td>0.57 (6)</td>
<td>0.57 (6)</td>
<td>791</td>
</tr>
<tr>
<td></td>
<td>OA5</td>
<td>0.39 (4)</td>
<td>0.49 (3)</td>
<td>0.4 (4)</td>
<td>1027</td>
</tr>
<tr>
<td>Mean</td>
<td>0.29</td>
<td>0.48</td>
<td>0.45</td>
<td>0.6</td>
<td>0.58</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.18-0.4)</td>
<td>(0.37-0.59)</td>
<td>(0.34-0.56)</td>
<td>(0.49-0.71)</td>
<td>(0.29-0.87)</td>
</tr>
</tbody>
</table>
Table 4.4 continued

<table>
<thead>
<tr>
<th>Group 4: Carriers of Chromosomal Abnormalities (CA) (n=3)</th>
<th># of cells</th>
<th>Frequency of disomy</th>
<th># of cells</th>
<th>Frequency of disomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>2518</td>
<td>0.56 (14)³</td>
<td>0.4 (10)</td>
<td>0.08 (2)</td>
</tr>
<tr>
<td>CA2</td>
<td>10172</td>
<td>0.03 (3)</td>
<td>0.11 (11)</td>
<td>0.03 (3)</td>
</tr>
<tr>
<td>CA3</td>
<td>224</td>
<td>0.59 (2)²</td>
<td>0.45 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.39</td>
<td>0.32</td>
<td>0.04</td>
</tr>
</tbody>
</table>

(95% CI) (0.03-0.75) (0.11-0.53) (0.00-0.085) (0.07-1.35) (0.56-2.2)

³p<0.001 when compared to the pooled control value for the same chromosome, Chi-square test
²p<0.05 when compared to the pooled control value for the same chromosome, Chi-square test

In order to observe the relationship between the absence of recombination on specific chromosomes and sperm aneuploidy, we correlated recombination and sperm aneuploidy, in each chromosome studied, among all men (control and infertile groups). Previous studies have observed a correlation between absence of recombination in the sex body and XY disomy (Ferguson et al., 2007; Sun et al., 2008). However, this study found no such correlation, with respect to the sex chromosomes ($r=0.386$, $p>0.05$, Pearson test) or with respect to chromosomes 13, 18, and 21.

4.3.5 Meiotic behaviour of chromosomal rearrangements

During meiosis, chromosome rearrangements are believed to adopt unique pairing structures in order to syanapse the homologous regions of the derivative and normal chromosomes. While genome wide recombination in carriers of CA was not reduced compared with controls, we were interested in the frequency of recombination in the chromosomes involved in the CA. In CA2 (rob[13;21]) we noted significantly decreased recombination on both chromosome 13 and 21, with a significant increase in the proportion of cells with no recombination in the chromosome 21 bivalent (Table 4.3). With respect to CA3 (inv[1]), we used FISH following immunostaining to determine the frequency of recombination on chromosome 1 in CA3 and a control (Table 4.5). CA3 showed a significantly decreased proportion of cells with four recombination foci and a
significantly increased proportion of cells with two or less recombination foci (Fishers exact test).

| Table 4.5 Analysis of crossovers on chromosome 1 in the inv(1) carrier |
|-----------------------------|------------------|------------------|------------------|------------------|
|                            | # of cells       | ≤2 foci          | 3 foci           | 4 foci           | ≥5 foci          |
| CA3 (Inv[1])               | 34               | 38.2% (13)       | 32.3% (11)       | 36% (9)         | 4% (1)          |
| Control                    | 100              | 0                | 22% (22)         | 66% (66)        | 12% (12)        |

*a p<0.05 significantly different when compared with control, Fishers exact test

In order to determine the meiotic relationship of the chromosome abnormalities with the sex body, we hybridized pachytene stage spermatocytes with FISH probes for the chromosome(s) involved in the abnormality and for the sex body (Figure 4.3). With respect to CA2 (t[13;21]), of 32 cells examined, we observed a trivalent in 84% of cells and of these cells 33.3% showed an association of the trivalent with the sex body (Figure 4.3 A, B). Among 32 cells examined in CA1 (+mar), we found 62.5% had an observable marker at the pachytene stage. Of these cells, 50% showed an association with the sex body (Figure 4.3 C, D). With respect to CA3 (inv[1]), of 25 cells examined, 92% showed a large centric region of chromosome 1 lacking SYCP1 staining, indicative of a large region of asynapsis. Of these cells, 56% contained small intermittent regions of SYCP1 staining within the large gap, possibly indicative of the presence of an inversion loop. A previous study examining the synaptonmeal complex in an inversion carrier found no presence of an inversion loop (Gabriel-Robez et al., 1988). Of the cells examined, 50% showed an association of the centric region of chromosome 1 with the sex body (Figure 4.3 E, F). Of the 8% of cells, which showed complete syanpse along the chromosome 1 bivalent, none showed an association with the sex body.
Figure 4.3 Immunostaining and FISH analysis of chromosome abnormalities.

Immunostaining and FISH on the rob(13;21) (A, B), the +mar (C, D) and the inv(1) (E, F) allowed the determination of chromosome specific recombination and any association between the CA and the sex chromosomes. (B) The rob(13;21) was analyzed with LSI 13 (green), LSI (21) and X/Y (blue). (D) The mar carrier was analyzed with 14/22 centromeric probe (red) and X/Y (blue) probes. (F) The inv(1) carrier was analyzed with 1p-tel (red), 1q-tel (green) and X/Y (blue) probes.
Table 4.6 Association of chromosome abnormalities with the sex body

<table>
<thead>
<tr>
<th></th>
<th># of cells</th>
<th>Proportion of cells displaying association with the sex body % (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1 (+mar)</td>
<td>20</td>
<td>50% (10)</td>
</tr>
<tr>
<td>(chromosome 21)</td>
<td>98</td>
<td>3.1% (3)</td>
</tr>
<tr>
<td>CA2 (t[13;21])</td>
<td>27^b</td>
<td>33% (9)</td>
</tr>
<tr>
<td>(chromosome 13)</td>
<td>85</td>
<td>4.7% (4)</td>
</tr>
<tr>
<td>CA3 (Inv[1])</td>
<td>34</td>
<td>50% (17)</td>
</tr>
<tr>
<td>(chromosome 1)</td>
<td>100</td>
<td>10% (10)</td>
</tr>
</tbody>
</table>

a mar observed in 20 of 32 cells analyzed
b trivalent observed in 27 of 32 cells analyzed
c p<0.05, significantly different from controls, Fisher's exact test

4.3.6 Comparison of spermatocytes derived from testicular and ejaculate samples

Spermatocytes analyzed in CA1 and CA2 were derived from ejaculate samples. In order to determine if spermatocytes from ejaculate (ES) and testicular samples (TS) were equivalent, we compared spermatocytes from an ejaculate sample from an individual whose testicular sample had been previously examined (Ma et al., 2006b). In the ES sample, we studied 50 pachytene nuclei, in which the mean recombination in the ES was 40.9 compared with 42.0 in the TS (Table 4.7). No significant difference was observed with respect to the mean frequency of recombination (p>0.05, Mann Whitney test), the frequency of recombination in the XY bivalent, the proportion of cells lacking an autosomal MLH1 focus, or the proportion of cells with gaps or unpaired regions (p>0.05, Fisher exact test) (Table 4.7). In the ES, we examined 281 prophase spermatocytes and staged the cells as leptotene, zygotene, zygotene/pachytene or pachytene. When the proportions of cells in each stage were compared with 610 cells examined in the TS, no stage was significantly different (Fisher exact test) (Table 4.8). As well, FISH for chromosomes 13, 18 and 21 was performed on ES spermatocytes to determine chromosome specific frequencies of recombination (Table 4.9). No difference in the frequency of recombination on chromosomes 13, 18 and 21 was observed when the ES sample was compared with the TS sample (Fisher exact test).
### Table 4.7 Comparison of MLH1 foci and synaptic errors between testicular and ejaculate derived spermatocytes

<table>
<thead>
<tr>
<th></th>
<th>#of cells analyzed</th>
<th>Mean recombination foci per cell</th>
<th>Range of recombination foci</th>
<th>Recombination in XY bivalent (%)</th>
<th>Proportion of cells with an autosomal bivalent lacking an MLH1 focus (%)</th>
<th>Proportion of cells with gaps (%)</th>
<th>Proportion of cells with unpaired regions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOA10 from testicular sample</td>
<td>51</td>
<td>42.0±4.7</td>
<td>30-56</td>
<td>0</td>
<td>11.8</td>
<td>21.6</td>
<td>11.8</td>
</tr>
<tr>
<td>NOA10 from ejaculate sample</td>
<td>50</td>
<td>40.9±4.1</td>
<td>31-52</td>
<td>0</td>
<td>8.0</td>
<td>20.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Recombination not significant, using Mann Whitney test (P=0.1917)
Proportions of synaptic errors not significant, using Fisher exact test (All P values >1.0)

### Table 4.8 Comparison of progression through prophase between testicular and ejaculate derived spermatocytes

<table>
<thead>
<tr>
<th></th>
<th>Test. Path.</th>
<th># of cells</th>
<th>Leptotene (%)</th>
<th>Zygotene (%)</th>
<th>Zygotene/Pachytene (%)</th>
<th>Pachytene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOA10 from testicular sample</td>
<td>Maturation arrest</td>
<td>610</td>
<td>21.3</td>
<td>6.6</td>
<td>9.8</td>
<td>62.3</td>
</tr>
<tr>
<td>NOA10 from ejaculate sample</td>
<td>Maturation arrest</td>
<td>281</td>
<td>16.2</td>
<td>8.1</td>
<td>11.0</td>
<td>63.7</td>
</tr>
</tbody>
</table>

No stage was significantly different, using Fisher exact test (All P values >0.5)
Table 4.9 Comparison of crossovers on chromosomes 13, 18 and 21 between testicular and ejaculate derived spermatocytes

<table>
<thead>
<tr>
<th># of cells</th>
<th>Chromosome 13</th>
<th>Chromosome 18</th>
<th>Chromosome 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 foci</td>
<td>1 foci</td>
<td>2 foci</td>
</tr>
<tr>
<td>NOA10 from testicular sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>0%</td>
<td>31%</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(14)</td>
<td>(31)</td>
</tr>
<tr>
<td>NOA10 from ejaculate sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0%</td>
<td>37%</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(10)</td>
<td>(17)</td>
</tr>
</tbody>
</table>

None were significantly different, using Fisher exact test (All P values >0.5)

4.4 Discussion

Recent studies have begun to describe the meiotic defects present in infertile men (Gonsalves et al., 2004; Ma et al., 2006b; Ferguson et al., 2007; Ferguson et al., 2009). In this study, we hoped to further classify the meiotic defects effecting infertile men as well as study any relationship between chromosome specific recombination and sperm aneuploidy. A limited number of immunofluorescent studies, on reciprocal translocation carriers, have shown disrupted meiotic behaviour in CA men, though this behaviour may be distinct from that observed in karyotypically normal infertile men (Oliver Bonet et al., 2005; Sun et al., 2007; Ferguson et al., 2008). We examine recombination and synapsis in three previously unstudied varieties of CA, and comment on the recombination on the involved chromosomes, as well as any association of these CA with the SB. A previous immunofluorescent study has shown that spermatocytes derived from the ejaculate can be used to study the SC in pachytene spermatocytes (Wong et al., 2008). We report the first cases of analysis, of recombination, in ejaculate-derived spermatocytes. We hoped to determine if spermatocytes derived from the ejaculate were equivalent, with respect to the frequency of recombination and synaptic errors, to those derived from testicular samples.
4.4.1 Defects in progression through prophase, recombination and synapsis

In infertile men, previous studies have observed either a complete arrest of cells at zygotene (Gonsalves et al., 2004; Topping et al., 2006) or an impairment of the progression through meiosis, with an increase in early stage cells and a reduction in cells at pachytene stage (Ma et al., 2006b; Ferguson et al., 2007; Sun et al., 2007). We observed no cases with the former phenotype, however, the later phenotype was represented in individual members of the OA, NOA and CA groups, who showed significantly reduced proportions of cells at pachytene and an increase in the proportion of cells at either leptotene, zygotene or zygotene/pachytene suggestive of an impairment of the progression through prophase.

Decreased frequencies of recombination, in men with NOA, have been observed previous studies (Gonsalves et al., 2004, Sun et al., 2004, 2007). In the three NOA cases studied here, we observed no decrease in recombination in the NOA group, similar to other recent findings (Topping et al., 2006; Sun et al., 2008). The discrepancy, between the findings reported here and those previously observed in our lab (Ferguson et al., 2008), could be due to the limited number of individuals in our NOA study group. Furthermore, unlike the previous study from our lab, the NOA group described here includes an individual with a Y-deletion. It is possible that men with Y-deletions and men with idiopathic NOA are associated with different meiotic errors, and as such, we may not expect to see reduced recombination in men with Y-deletions. Nevertheless, when we combined our results, for NOA men, with the previous NOA men studied in our lab, we observed a significantly lower mean level of recombination compared with the control group. We did observe a decrease in recombination in men from the OA group, however, as these men are presumed to have normal spermatogenesis, it is curious that this group displayed reduced recombination while the NOA group, for whom reduced recombination has been suggested as a cause of their impaired spermatogenesis, displayed no such reduction. An altered testicular environment, due to the presence of an obstruction, could explain some of the differences observed in the OA when compared with controls.
Previous studies have noted reduced recombination in the sex body in infertile men (Gonsalves et al., 2004; Topping et al., 2006; Sun et al., 2007; Ferguson et al., 2007; Sun et al., 2008). We observed a significantly increased proportion of cells lacking recombination foci in the sex body, in both the NOA and CA groups. It has been suggested that the absence of recombination in the sex body could disrupt the segregation of the sex chromosomes and lead to activation of meiotic checkpoints and meiotic arrest. If this hypothesis were true it could explain the disrupted spermatogenesis observed in the NOA group. Previous studies have noted a significantly increased proportion of cells, in NOA men, lacking recombination in an autosomal bivalent (Sun et al., 2008). However, we observed no difference in the proportion of cells lacking recombination in an autosomal bivalent.

The presence of synaptic errors during meiosis has been implicated in infertility (Chandly et al., 1986). While NOA and OA groups showed no difference in the mean proportion of cells with synaptic errors, individuals in both infertile groups showed a statistically increased proportion of cells with synaptic errors (NOA1, NOA7, OA4, OA3). However, similarly elevated proportions of synaptic errors were observed in the control group (C2, C4, C5) suggesting a high degree of natural variability in the presence of synaptic errors. NOA1, a carrier of an AZFc Y chromosome microdeletion, showed an increase in the proportion of cells with asynapsed regions. A previous study examined synaptic errors in three carriers of AZFc deletions, found significantly higher frequencies of cells with gaps and asynapsed regions (Gioffroy-Siraudin et al., 2007). Authors have suggested that the synaptic errors are related to a secondary effect, resulting from a change in the testicular environment due to the presence of the AZFc microdeletion (Guichaoua et al., 2005). The study also noted a significant increase in the proportion of early stage prophase cells relative to the proportion of late stage prophase cell, a finding not observed in our study. This inconsistency could be related to marked variability observed, in the effect of AZFc deletions on spermatogenesis.

We observed a slight reduction in global recombination in the CA group. However, these men were also characterized by large increases in the proportion of cells with synaptic errors. As proper synapsis is a prerequisite for successful recombination, it is not
clear if reduced recombination levels are attributable to the increases in gaps and asynapsis.

4.4.2 Chromosome specific recombination and sperm aneuploidy

Recombination is believed to be crucial for the proper segregation of chromosomes (Koehler et al., 1996). It is possible, that errors in recombination, in infertile men, are responsible for the observed increase in sperm disomy seen in this population. Several studies have combined immunofluorescent and FISH analysis of pachytene nuclei to characterize the recombination patterns on specific chromosomes in normal males (Tease and Hulten, 2004; Codina-Pascual et al., 2006; Sun et al., 2006). However, fewer studies have combined these techniques with an analysis of sperm aneuploidy in the same men (Ferguson et al., 2008, Sun et al., 2008). These studies have indicated a correlation between the absence of recombination and the presence of sperm disomy, however, this relationship has been limited to the sex chromosomes. Individuals, in our study, displayed reduced recombination for the sex chromosomes (NOA1, NOA2, NOA7, CA1, CA2, CA3), chromosome 13 (OA4, CA2), chromosome 18 (OA4, CA2) and chromosome 21 (CA2). Increased sperm disomy was observed for the sex chromosomes (CA1, CA3), chromosome 13 (CA2), chromosome 18 (OA3, OA4) and chromosome 21 (OA3, CA1, CA2, CA3). However, we observed no correlation between the proportion of cells with reduced recombination and the frequency of sperm disomy.

Among carriers of CA, increases in sperm disomy, of chromosomes not involved in the abnormality, have been termed an interchromosomal effect (ICE) as they are thought to arise as a result of the presence of the abnormality. It is unknown, however, if the ICE takes effect through disruption of recombination on other chromosomes thereby altering their segregation. CA1 (mar[22]) displayed significantly increased frequencies of disomy for XX and YY disomy as well as chromosome 21. While CA1 did show reduced recombination in the sex body, no such reduction was observed in chromosome 21. With respect to the chromosomes not involved in the rearrangement, CA2 (rob[13;21]) showed a decrease in recombination in the 18 bivalent. However, no significant increase in sperm disomy was observed for chromosome 18. In CA3 (inv[1]), sperm disomy for XX and YY as well as chromosome 21 was significantly increased. We observed slight decreases
in recombination in the sex body and the chromosome 21 bivalent, though the later was not significant. In all three CA carriers, we observed drastically higher proportions of cells with synaptic errors, which could account, in part, for the increases in sperm disomy in some of these men.

4.4.3 Meiotic behaviour in carriers of CA

The examination of recombination in carriers of CA has been limited, including only a few studies in carriers of reciprocal translocations (Ferguson et al., 2008; Oliver-Bonet et al., 2005; Pigozzi et al., 2005; Sun et al., 2005). Of these four studies, only one has found a decrease in global recombination when compared with controls (Sun et al., 2005). In three carriers of CA, we found a significant reduced frequency of recombination in two of the three CA carriers when compared with controls. Additionally, we were interested in the frequency of recombination, in the chromosomes involved in the CA, in CA2 and CA3. In both carriers, we found a decrease in recombination in the involved chromosomes, similar to previous studies (Oliver-Bonet et al., 2005; Pigozzi et al., 2005; Ferguson et al., 2008). Authors have suggested that the high degree of asynapsis noted in these carriers is the most likely explanation for the reduction in recombination (Sun et al., 2005). Indeed, we observed a significant increase in the frequency of asynapsis and gaps in the SC in CA2 and CA3.

The infertility observed in carriers of CA abnormalities has been attributed to both the presence of asynapsed regions, primarily around the breakpoints of rearrangements, as well as the association of the CA with the sex body. Fidelity of synapse is thought to be crucial for the progression of spermatogenesis, as unpaired regions are thought to be picked up by the pachytene checkpoint, leading to apoptosis and meiotic arrest (Odorisio et al., 1998). Association of the CA with the sex body is thought to provide a “safe environment” for the asynapsed regions of the CA, by spreading of MSCI, preventing their detection by meiotic checkpoints. However, the association may also disrupt spermatogenesis through decreased expression in the chromosomes of the CA, and disruption of MSCI increasing expression on the X chromosome (Homolka et al., 2007). The mechanism whereby CA are attracted to the SB is unknown. However, authors have noted a higher tendency for SB interaction in rearrangements containing acrocentrics,
possibly due to the acrocentrics natural tendency to associate with the sex body (Luciani et al., 1987).

If the interaction of the CA with the SB disrupts the normal behaviour of the sex chromosomes, we would expect to observe, in carriers of CA in which there is a large degree of association with the sex body, reduced recombination in the sex body and an increase in sex chromosome disomy in the sperm. In all three CA carriers, we observed a significantly lower proportion of cells with sex body recombination. In Chapter III, we noted the presence of interchromosomal effects in chromosomes 21 and the sex chromosomes in CA1 and CA3. If the association of the CA with the sex body disturbs the normal segregation of the sex chromosomes, we might expect to see an increase in XY disomy in the sperm from these carriers. It is curious that we also noted increased XX and YY disomy, as these sperm chromosome complements result from errors in meioses II disjunction. Previous authors have suggested that disturbance during MI may carry over and cause non-disjunction in MII (Sun et al., 2008). Acrocentric chromosomes have been shown to preferentially associate with the sex body, possibly accounting for the observed ICE in chromosome 21. With respect to CA2, we observed no ICE in any of the chromosomes studied.

If the association of the CA with the SB disrupts spermatogenesis we might expect to observe lower sperm parameters in carriers that show a greater frequency of association between the CA and the SB. Indeed, several studies have noted a greater degree of SB association in carriers with more impaired fertility (Johannison et al., 1993; Gabriel Robez and Rumpler, 1996). Our results are generally supportive of this relationship, as CA1 and CA2 showed reduced sperm concentration (oligospermia) and an association with the sex body ranging from 33-50%. However, CA3 displayed a more severely reduced sperm concentration and yet had a comparable frequency of association between the CA and SB. It is possible that other meiotic errors, such as asynapsis of the inverted region, account for the greater degree of spermatogenic disruption in CA3.
4.4.4 Comparison of spermatocytes derived from ejaculate and testicular samples

We report the first examination of recombination and synapsis in spermatocytes derived from the ejaculate. Studies of spermatocytes from testicular samples have helped determine the frequency of recombination and synaptic defects in both fertile and infertile men. However, these studies have been severely limited by the necessity of retrieving samples during testicular biopsies or vasectomy reversals. In general, only men with complete absence of sperm in the ejaculate (azzospermia) are offered testicular biopsy for the purpose of retrieving sperm. As such, studies of men with milder forms of infertility have generally not been done. Furthermore, it is unclear if men undergoing vasectomy reversals represent an appropriate control population. It is possible that a vasectomy may affect meiotic behaviour through an alteration in the testicular environment.

We compared spermatocytes from an ejaculate sample from an infertile man, with a testicular sample from the same individual previously reported in Ma et al. (2006b). We found no significant difference in the: proportion of cells at various stages of prophase, the global frequency of recombination and synaptic errors, or the frequency of chromosome specific recombination on chromosomes 13, 18, 21.

These results suggest that spermatocytes found in the ejaculate are representative of those found in testicular biopsies. The ability to perform meiotic analysis on pachytene cells derived from the ejaculate has the potential to greatly increase the scope of meiotic studies and allow the examination of as yet unstudied population groups. However, there may be limitations to the technique. It remains unknown what proportion of the population have spermatocytes in the ejaculate. It is conceivable that spermatocytes are only released into the lumen of the seminiferous tubule as a result of meiotic distress, and as such, spermatocytes may not be present, in sufficient numbers for study, in the ejaculate of men with normal spermatogenesis.
4.5 References


CHAPTER V: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Summary and Conclusions

Chromosomal abnormalities are associated with infertility, with the frequency of CA ten times higher in the infertile population (de Braekeeler and Dao, 1991). In addition to having quantitatively fewer sperm, carriers of CA are at risk for the production of chromosomally abnormal sperm due to the aberrant segregation of the chromosomes involved in the abnormality. Studies have determined the patterns of segregation in the most common varieties of CA. Nevertheless, many rearrangements have yet to be studied and, indeed, the segregation patterns of whole classes of CA, such as CCR and sSMC, have either never been studied or only very limitedly. Furthermore, while it has been suggested that CA may disrupt the segregation of other chromosomes through an interchromosomal effect, the existence of such a process remains contentious. Lastly, while CA are believed to impair spermatogenesis through the disruption of meiosis, it is unknown if they show the same altered meiotic behaviour observed in karyotypically normal infertile men. The cumulative results presented in this thesis suggest that carriers of CA display different meiotic behaviour from both fertile men as well as karyotypically normal infertile men, with respect to both the production of chromosomally abnormal and the presence of errors in synapsis and recombination.

In Chapter II we used FISH to examine, in carriers of CA, the segregation of the chromosomes involved in the CA, by determining the frequency of each segregation mode in the sperm. We observed significantly increased levels of sperm aneuploidy in all carriers, with the exception of the carriers of mosaic aneuploidy. The segregation patterns of the carriers of reciprocal, X:Autosomal, and Robertsonian translocations were comparable to reports of similar rearrangements in the literature, as well as to theoretical models of segregation. The inversion carrier displayed a recombinant frequency similar to that observed in previous reports. With respect to the CCR carrier, we observed a lower than expected frequency of unbalanced chromosome complements. The two sSMC studied, were present in 11.5% and 13.5% of sperm studied, values far less than the 50% theoretically predicted. This result suggests that the marker is either lost prior to meiosis or that sperm carrying the marker are selected against during spermatogenesis. The
meiotic analysis, of one sSMC carrier in Chapter IV, suggests both mechanisms may be active. In the sSMC carrier, of 32 meiotic cells examined, 62.5% showed the presence of a marker chromosome, while in the sperm the marker was found in only 11.5% of sperm. In the two carriers of mosaic aneuploidy, we observed no increase in sperm disomy for the additional chromosome. The accumulated evidence, including reports that have noted similarly low levels of sperm disomy in mosaic carriers, seem to suggest that, in mosaic carriers of aneuploidy, the only spermatocytes capable of undergoing spermatogenesis are those that are not carrying the additional chromosome. It is also possible that the low level of the additional chromosome is due to the tissue specificity of the mosaicism and that the mosaic cell line does not exist in the testis.

Previous studies examining the presence of an interchromosomal effect related to CA have been contradictory. Our results, outlined in Chapter III, are similarly inconclusive, with six of twelve carriers showing evidence of an interchromosomal effect. However, of the carriers studied, those CA that show an ICE appear to be restricted to the larger rearrangements such as the reciprocal translocations and CCR. A possible explanation of this relationship is that larger rearrangements are more likely to physically interact with other bivalents, thereby disrupting their segregation, while smaller rearrangements are less likely to have such interactions. The exception is the ICE observed in the 47,XY,+mar carrier which does not seem to fit this pattern. In Chapter IV, we observed globally reduced recombination, as well as increases in synaptic errors in this carrier, which may explain the increased disomy observed in other chromosomes. However, in Chapter IV, we also observed asynapsis and reduced sex chromosome recombination in the t(13;21) carrier, for whom no ICE were observed in Chapter II. Among all CA carriers, we noted that ICE were observed most frequently in acrocentric chromosomes 13 and 21. Previous studies have noted a preference for both CA (Chandley et al. 1986; Luciani et al., 1984; Johannisson et al., 1983) and acrocentric bivalents (Solari and Tres, 1970; Stahl et al., 1984) to associate with the sex body, which may account for the ability for CA to preferentially disrupt the segregation of acrocentric chromosomes. In addition to comparing sperm disomy in carriers of CA with fertile controls, we compared CA carriers with infertile men with OAT. Generally, we observed higher levels of sperm aneuploidy among OAT men than carriers of CA. We suggest that
this difference is indicative of the different causes of infertility between the two groups. Previous studies (Ma et al., 2006; Ferguson et al., 2007; Ferguson et al., 2009), have found, in some infertile men, altered global recombination, and an increase in the frequency of autosomal bivalents lacking any recombination foci and have suggested that this is a contributor to their infertility. As recombination is strongly linked with non-disjunction, a general decrease in recombination may contribute to both reduced sperm parameters as well as increased sperm aneuploidy. In contrast, in carriers of CA, this study, as well as others, seems to suggest that asynapsis, as well as association of the CA with the sex body, is the cause of disruption of spermatogenesis. While we observed global synaptic errors, such as gaps and asynapsis, in the CA carriers, a relationship between synaptic errors and non-disjunction has not been established. As such, increased sperm disomy, in CA carriers, may be limited to other chromosomes that are interacting with the sex body.

Recently developed immunocytogenetic techniques have allowed us to examine the detailed behaviour of spermatocytes undergoing meiosis both globally, and, with the aid of FISH, on specific chromosomes. In Chapter IV, we examined the progression through prophase, as well as recombination and synapsis, in five control men, three men with OA, three men with impaired spermatogenesis and three carriers of CA. We observed no significant difference in the proportion of cells, at various stages of prophase, between groups. Decreased genome-wide recombination was observed in all three carriers of OA and in two of the three carriers of CA. An increased proportion of cells with gaps in the SC or with asynapsed regions of the SC were noted in two of the OA group, two of the NOA group and all of the CA group, however, the proportion of cells with synaptic errors was markedly higher in carriers of CA. An increased proportion, of cells lacking recombination between the sex chromosomes, was observed in NOA and CA groups. When recombination was examined on chromosomes 13, 18 and 21, only one OA individual showed significantly altered recombination. We observed no correlation between the frequencies of absence of recombination on a specific chromosome with sperm disomy for that chromosome. We were also interested in examining the meiotic behaviour of the involved chromosomes in two of the carriers of CA. Using FISH, we examined the frequency of recombination on the involved chromosomes and found
reduced recombination in both carriers. In all three carriers of CA, we noted a significantly increased frequency of cells in which there was association between the CA and the sex body. Lastly, we describe the first examination of spermatocytes derived from the ejaculate as opposed to testicular samples. Samples of both varieties, from one individual, were compared, and no significant difference was observed with respect to recombination, synapsis or progression through prophase.

In summary, carriers of CA have distinctly different patterns of sperm chromosome complements, and display altered meiotic behaviour, when compared with control men and karyotypically normal infertile men. The chromosomal rearrangements studied generally displayed unbalanced segregation modes at frequencies comparable to similar rearrangements. This supports the notion that the segregation of the rearranged chromosomes at meiosis is dependant on the relative sizes of the translocated and centric segments. In carriers of numerical abnormalities we observed the abnormality in far fewer sperm than would be expected, supporting the notion that numerical abnormalities are readily lost pre and post meiotically. We observed elevated sperm disomy in some carriers of CA, possibly suggestive of an ICE. However, karyotypically normal infertile men displayed even higher rates of sperm aneuploidy, a difference possibly accounted for by the different causes of infertility between the two groups. We observed meiotic defects in both infertile men and men with CA that are likely involved in these individuals infertility. Reduced global recombination was observed more commonly in OA men while men, while all NOA men displayed reduced recombination between the sex chromosomes. Carriers of CA displayed slightly reduced recombination, though more striking was the increase in synaptic errors observed in this group. Furthermore, carriers of CA displayed reduced recombination on the chromosomes involved in the CA and an association between the CA and the sex body. Clearly, further studies will be needed to confirm our findings in other carriers of CA, as well as to address new questions that have arisen in this work.


5.2 Future Directions

The work presented in this thesis has only begun to address the relationship between chromosomal abnormalities, meiotic defects, male infertility and the production of chromosomally abnormal sperm. Studies on the chromosome segregation have been useful in determining the risks presented by various rearrangements. However, numerous sperm studies in reciprocal and Robertsonian translocations show surprising heterogeneity among similar or even identical translocations. Conceivably, both the genetic background of the patient, as well as environmental factors, play a role in this variability. Studies of identical translocations in genetically related individuals have shown less variation in patterns of segregation (Estop et al., 1992; Rousseaux et al., 1995; Cora et al., 2002) suggesting that the genetic background can affect segregation patterns. Further studies, examining chromosome segregation in conjunction with meiotic studies, will be useful in determining if any specific meiotic behaviours can account for the variation in chromosome segregation modes. The presence of an interchromosomal effect, in relation to CA, remains contentious. Further sperm studies of ICE in conjunction with meiotic analysis will provide insight into the meiotic mechanisms that may play a role in ICE. However, the magnitude of all ICE observed has been limited, especially when compared to the chromosome imbalances resulting from the segregation of the involved chromosomes, suggesting that the clinical risk associated with ICE is minimal.

Evidence has accumulated, pointing to an interaction between the CA and the SB and a resulting disruption of meiosis. Furthermore, recent studies have suggested that unsynapsed regions, of rearrangements, are silenced through MSUC, a process similar, or which possibly includes MSCI. However, relatively little is known regarding the mechanisms and consequences of such a process. It remains unclear if MSUC and MSCI are indeed part of the same process or just similar processes. Further studies should be done examining the interaction between unsynapsed regions of rearrangements and the sex body. Additionally, studies describing the proteins that localize to the sex body and to unsynapsed chromosomes during meiosis will help to determine if there are differences between MSCI and MSUC. As of yet, MSUC has only been observed in large regions of
asynapsis in reciprocal translocations. However, numerous studies have shown that all varieties of CA show some interaction with the sex body. Meiotic studies of other varieties of CA will help to elucidate the relationship between sex body interaction, silencing of unsynapsed chromatin and disruption of spermatogenesis. Furthermore, determining the minimum size of asynapsis necessary for silencing. If MSUC also acts on smaller regions of asynapsis, it may have consequences for men with infertility and especially men with CA, as the results in Chapter IV show that these men may have increased rates of small asynapsed regions.

Despite evidence that meiotic errors are implicated in infertility in infertile men and translocation carriers, the cause of these meiotic errors remains largely unknown. Early studies in screening meiotic genes for mutations have been met with some success (Miyamoto et al., 2003; Zhang et al., 2006), while others have been less promising (Westerveld et al., 2005; Christensen et al., 2005; Zhang et al., 2008). The cause of meiotic defects may not be entirely genetic, as it is also possible that environmental factors may play a role. Very little is known regarding the impact of chemical exposures on meiotic events such as synapsis or recombination. A recent study has found that exposure to BPA, a component of polycarbonate plastics, has dramatic effects on synapsis and recombination in mice oocytes (Susiarjo et al., 2007). Nevertheless, almost nothing is known regarding the effects of BPA or other chemicals on meiosis in males.

The analysis of meiotic defects in spermocytes derived from the ejaculate represents an exciting new direction in the study of meiosis in men. Studies utilizing spermocytes from ejaculate samples may allow us to analyze previously unstudied groups of infertile men, especially those with milder forms of infertility, including carriers of CA. While men with CA often have reduced sperm parameters, complete azoospermia is less common. As such, it is difficult to retrieve testicular samples from these carriers, as they are unlikely to undergo testicular biopsies for the purpose of retrieving sperm. For that reason, prior studies of meiosis in men with CA have been limited to carriers of reciprocal translocations with azoospermia. The use of ejaculate samples will allow us to study a wide variety of CA, including Robertsonian translocations, inversions, CCR as well as numerical abnormalities. If spermotyctes can
be retrieved from men with proven fertility, comparisons can be made with testicular samples retrieved from men undergoing vasectomy reversals, to determine if, in fact, this population is an appropriate control. Nevertheless, we expect that the use of this new technique will dramatically alter the study of meiosis in the human male, providing vast new opportunities for the examination of the meiotic process in men with both disrupted and normal spermatogenesis.
5.2 References


APPENDIX I: ETHICS APPROVAL CERTIFICATES

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board –
Room 210, 828 West 10th Avenue,
Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD
APPROVAL

<table>
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<th>PRINCIPAL INVESTIGATOR:</th>
<th>INSTITUTION / DEPARTMENT:</th>
<th>UBC CREB NUMBER:</th>
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<td>Sai Ma</td>
<td>UBC/Medicine, Faculty of Obstetrics &amp; Gynaecology</td>
<td>H06-03490</td>
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INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

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CO-INVESTIGATOR(S):

Victor Chow
Mark K. Nigro
SPONSORING AGENCIES:

Canadian Institutes of Health Research

PROJECT TITLE:

Investigation of meiotic defects in infertile men undergoing assisted reproductive technologies

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: January 9, 2008

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

REB FULL BOARD
MEETING REVIEW DATE:

January 9, 2007

DOCUMENTS INCLUDED IN THIS APPROVAL:

<table>
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CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

Approval of the Clinical Research Ethics Board by:

UBC C&W Research

Children’s & Women’s Health Centre of British Columbia

A2-136, 950 West 28th Avenue
Vancouver, BC V5Z 4H4
Tel: (604) 875-3103 Fax: (604) 875-2496
Email: cwrebc@cw.bc.ca
Website: http://www.cfr.ca/research_support
> Research Ethics
ETHICS CERTIFICATE OF MINIMAL RISK APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR: Sai Ma

DEPARTMENT: UBC/Medicine, Faculty of Obstetrics & Gynaecology

UBC C&W NUMBER: H06-03490

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

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<td>Children's and Women's Health Centre of BC (incl. Sunny Hill)</td>
<td>Child &amp; Family Research Institute</td>
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Other locations where the research will be conducted: N/A

CO-INVESTIGATOR(S):

Victor Chow
Mark K. Nigro

SPONSORING AGENCIES:

- Canadian Institutes of Health Research (CIHR) - "Investigation of Meiotic Defects as an Underlying Cause of Male Factor Infertility"
- Canadian Institutes of Health Research (CIHR) - "Investigation of meiotic defects in infertile men undergoing assisted reproductive technologies"

PROJECT TITLE:

Investigation of meiotic defects in infertile men undergoing assisted reproductive
REMINDER: The current UBC Children's and Women's approval for this study expires:
February 4, 2010

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CERTIFICATION:

In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
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3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the UBC Children's and Women's Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approved by one of:

Dr. Marc Levine, Chair
Dr. Mason Bond, Associate Chair
**ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT**

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Other locations where the research will be conducted:

N/A

**CO-INVESTIGATOR(S):**

Victor Chow  
Mark K. Nigro

**SPONSORING AGENCIES:**

- Canadian Institutes of Health Research (CIHR) - "Detection of Chromosomal Defects in Sperm from Infertile Men"
- Various Sources - "Detection of chromosomal defects in sperm from infertile men- PG reference Orsil 06-1931"

**PROJECT TITLE:**

Detection of Chromosomal Defects in Sperm from Infertile Men

**REMINDER:** The current UBC CREB approval for this study expires: July 14, 2009

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the specified clinical trial site. This approval and the views of this Research Ethics Board have
been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the
University of British Columbia Clinical Research Ethics Board and the accompanying
documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the Clinical Research Ethics Board by: