CHAPTER 1

General introduction and outline of the thesis

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Infertility affects about 15% of all couples, caused by a male factor in about 30% of cases (Bhasin, 2007). Genetic factors are thought to account for 15-30% of male factor infertility (Ferlin *et al.*, 2007).

This chapter provides background information on karyotyping and chromosomal abnormalities, and the relation they have with male infertility. Next, an introduction to preimplantation genetic diagnosis is given, with details on chromosomal abnormalities in embryos. Lastly, a brief overview of the contents of this thesis is given.

Chromosomes

Around 1900, a series of experiments proved that chromosomes are the vectors of heredity. It took until the 1950s before the human diploid number was confirmed as 46 and the human karyotype as a XX/XY system (Gardner *et al.*, 2012).

Mitosis

Karyotyping is performed by culturing cells into mitosis. Mitosis is the process of somatic cell division during which the nucleus also divides. During mitosis each chromosome divides into two daughter chromosomes, one of which segregates into each daughter cell. Consequently, the number of chromosomes per nucleus remains unchanged. One of the phases of mitosis is metaphase, in which the chromosomes become aligned along the equatorial plane of the cell. At this point the chromosomes are maximally contracted and therefore most easily visible by microscope. Chromosomes consist of a centromere and a short (p) and long arm (q). By using different staining techniques on the cells in metaphase, different parts of chromosomes are shown in different colours (bands). A picture of all chromosomes, a karyogram, makes it possible to study the chromosomes, e.g. the number and structure. Increasing precision in banding techniques permitted smaller chromosomal aberrations to be observed (Gardner *et al.*, 2012).

Meiosis

Meiosis is the process of nuclear division which occurs during the final stage of gamete formation. During meiosis the diploid chromosome complement (46) is halved, so that each mature gamete receives a haploid complement of 23 chromosomes. Meiosis occurs in two cell divisions known as meiosis I and meiosis II, during which the gametes are formed. When the division of the chromosomes in meiosis I or II is unequal, chromosomally abnormal gametes are formed. Chromosomally abnormal gametes can also be formed in meiosis if the individual carries a chromosomal abnormality. After fertilization of a chromosomally abnormal gamete, an embryo with a chromosomal abnormality develops. A chromosomal abnormality that arises during conception will involve all the cells in the embryo and is called a constitutional abnormality. If an additional cell line arises after conception, due to errors in mitotic divisions, constitutional mosaicism results.

Chromosomal aberrations

Chromosomal aberrations can be balanced or unbalanced. In the balanced type, the normal amount of genetic material is present, although it is abnormally arranged. In translocations exchange of a part of the chromosome has occurred between two non-homologous chromosomes. A reciprocal translocation is formed when a break occurs in two different chromosomes, and the segments are exchanged to form two new derivative chromosomes (figure 1).

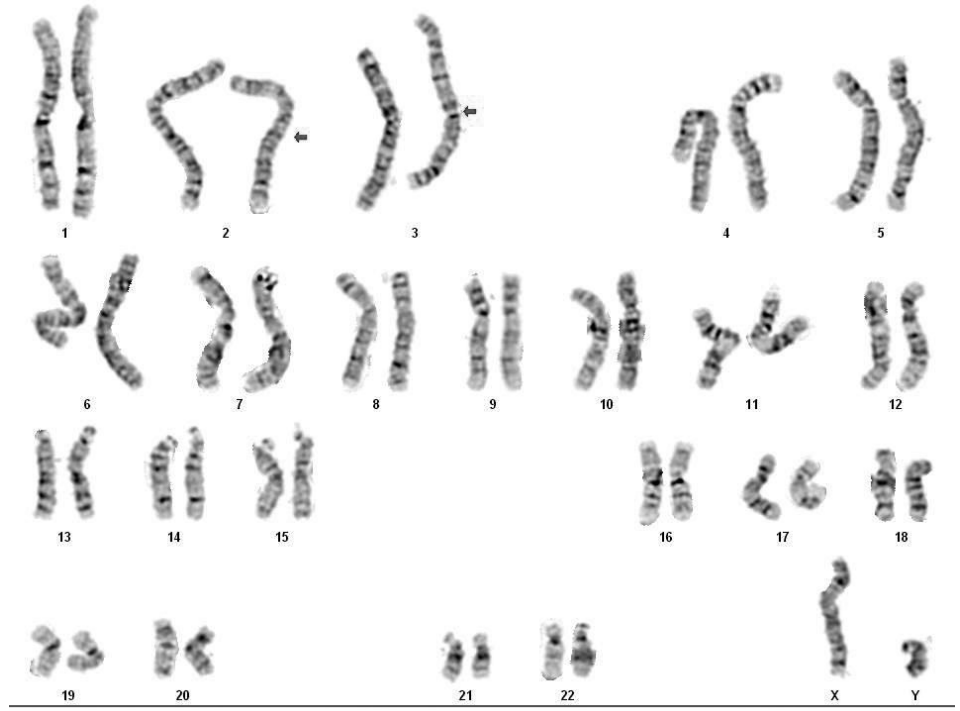

Figure 1: Reciprocal translocation. 46,XY, t(2;3)(p11.2;p21.3)

A Robertsonian translocation results from breakage of two acrocentric chromosomes at or close to their centromeres, with subsequent fusion of their long arms, resulting in one derivative chromosome. The total chromosome number is reduced to 45 (figure 2).

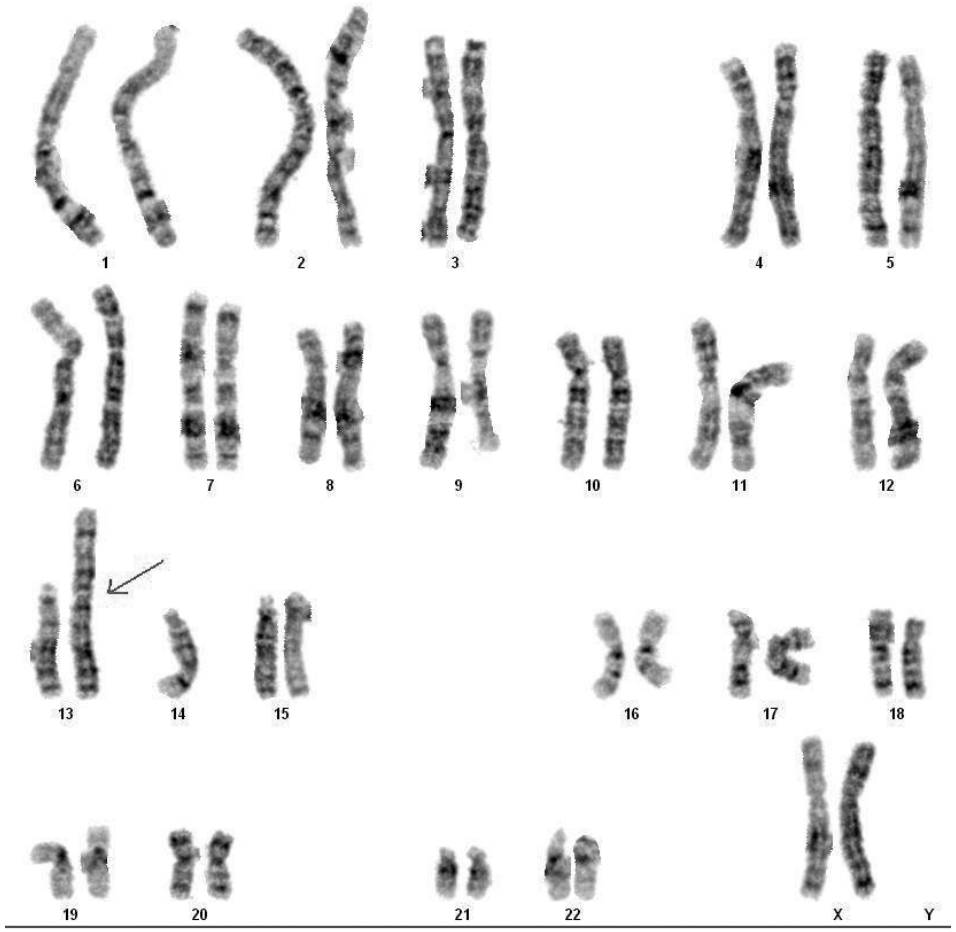

Figure 2: Robertsonian translocation. 45, XX, der(13;14)(q10;q10)

An inversion is a two-break rearrangement involving a single chromosome in which a segment is reversed in position, i.e. inverted. If the inversion segment involves the centromere, it is called a pericentric inversion. If it involves only one arm of the chromosome, it is known as a paracentric inversion.

In unbalanced chromosomal abnormalities, gain (duplication) or loss (deletion) of genetic material is present. This can involve small parts of chromosomes, or entire chromosomes (trisomies or monosomies), or even an entire set of chromosomes (triploidies). Unbalanced karyotypes usually affect a person's physical or mental health. In general, unbalanced sex chromosomes (gonosomal aberrations) influence the physical health less than autosomal rearrangements do, although fertility may be impaired.

The human genome is rich in variation. Clinically harmless variation can also be seen in the karyotype, especially of the chromosomal heterochromatic regions (*i.e.* the regions

predominantly containing non-coding DNA), and around the centromeres (*i.e.* small pericentric inversions). They are called chromosomal variants, or polymorphisms.

Prevalence of chromosomal abnormalities

The prevalence of chromosomal abnormalities in humans varies, depending on the population studied. Chromosome abnormalities are present in at least 10% of all spermatozoa and 25% of mature oocytes (Mueller and Young, 1998). Over 50% of embryos are chromosomally abnormal and do not survive beyond the first few days or weeks after fertilization. Between 15-20% of all recognized pregnancies end in spontaneous miscarriage (Fragouli *et al.*, 2011; Wells and Delhanty, 2000). Approximately 50% of all spontaneous miscarriages are due to aneuploidy and the incidence of chromosomal abnormalities in morphologically normal embryos is around 20% (Hook, 1992). From conception onwards the prevalence of chromosomal abnormalities falls rapidly. A study of 3000 amniocenteses revealed a prevalence of chromosomal aberrations of 0.94% (Artini *et al.*, 2011). At birth it has declined to 0.5-1% in liveborns (Nielsen and Wohlert, 1991), but in stillborn infants it is much higher (5%) (Hook, 1992).

Male infertility and chromosomal abnormalities

Since the 1950s chromosomal abnormalities were presumed to be the cause of infertility in patients with azoospermia or oligozoospermia. In 1959 it was discovered that men with Klinefelter's syndrome have 47 chromosomes and an XXY sex chromosomal constitution (Jacobs and Strong, 1959).

The association between male infertility and chromosomal abnormalities remained unclear until banding techniques had been introduced into daily clinical practice. Since then, several studies, preceded by Chandley *et al.* (1975), showed that the prevalence of chromosomal abnormalities was higher in infertile men (2.2%) compared with unselected male newborns (0.8%) (Nielsen and Wohlert, 1991).

The incidence of chromosomal abnormalities among infertile men (and women) is dependent on selection criteria and the definition of infertility. Some studies included oligozoospermic men or only azoospermic men, while other studies included both partners in an infertile couple. In some studies, infertility includes couples with recurrent miscarriage. For karyotyping different banding techniques have been used, a variable number of metaphases have been analysed and variant karyotypes have been listed as abnormal. Therefore there are no unbiased figures available for the frequency of chromosomal abnormalities in the adult population. Usually, Nielsen's study of newborns is used as a reference (Nielsen and Wohlert, 1991). In 2006, Ravel published a large study in 10.202 sperm donors of proven fertility. The prevalence of chromosomal abnormalities was 0.4% (Ravel *et al.*, 2006). Table 1 gives an overview of the prevalence studies in different groups of infertile men, in relation to sperm concentration. In infertile men, the prevalence of chromosomal abnormalities varied from 0.3% to 33.3%. In most studies, an inverse relation with sperm quality was reported, which is in agreement with earlier

studies in infertile men (Chandley, 1979). In general, the prevalence of chromosomal abnormalities was higher in populations of men with poor sperm quality, reaching a maximum of 21% in men with non-obstructive azoospermia (Ng *et al.*, 2009).

Table 1: Prevalence of chromosomal abnormalities in infertile men, an overview of the literature.

Population	Number of men studied	Prevalence of chromosomal abnormalities (%)	Reference
Infertile men, not specified	2242	14.3	(Hofherr *et al.*, 2011)
	668	8.2	(Yatsenko *et al.*, 2010)
	1210	3.7	(Pandiyan and Jequier, 1996)
	2749	3.6	(Hofherr *et al.*, 2011)
	694	2.02	(Van Assche *et al.*, 1996)
Men in IUI couples	582	0.3	(Riccaboni *et al.*, 2008)
	245	0	(Artini *et al.*, 2011)
Men in IVF couples	638	1.1	(Riccaboni *et al.*, 2008)
Men in ICSI couples			
ICSI, NOS	150	12	(Mau *et al.*, 1997)
	128	7	(Pauer *et al.*, 1997)
	134	4.5	(Krausz *et al.*, 1999a)
	1116	4.48	(Scholtes *et al.*, 1998)
	261	4.2	(Testart *et al.*, 1996)
	781	3.8	(Peschka *et al.*, 1999)
	305	3.3	(van der Ven *et al.*, 1998)
	305	3.2	(Haidl *et al.*, 2000)
	335	2.7	(Morel *et al.*, 2004)
	1426	2.2	(Riccaboni *et al.*, 2008)
	432	2.1	(Meschede *et al.*, 1998)
	1762	1.82	(Artini *et al.*, 2011)
ICSI, TFF	41	3.5	(Tuerlings *et al.*, 1998)
	34	0	(Kremer *et al.*, 1997)
ICSI, normospermia	10	10	(van der Ven *et al.*, 1997)
	27	7.4	(Bor *et al.*, 2002)
	430	3.02	(Gekas *et al.*, 2001)
	1559	0.96	(Clementini *et al.*, 2005)
Infertile men			
Normospermia (>20 M/ml)	30	10	(Ceylan *et al.*, 2009)
	359	2.2	(Yoshida *et al.*, 1997)
	295	1.7	(Matsuda *et al.*, 1989)
	90	1.1	(Cruger *et al.*, 2003)
	63	0	(Stegen *et al.*, 2012)
0-20 M/ml	2651	7.7	(Vincent *et al.*, 2002)
>0-20 M/ml	74	4.1	(Wang *et al.*, 2010)
	436	4	(Samli *et al.*, 2006)
	170	3.5	(Matsuda *et al.*, 1989)
	224	2.7	(Bor *et al.*, 2002)
	136	0.7	(Oliva *et al.*, 1998)
10-20 M/ml	112	2.68	(Yoshida *et al.*, 1997)
	34	0	(van der Ven *et al.*, 1997)
5-20 M/ml	259	3.39	(Clementini *et al.*, 2005)
	77	2.6	(Cruger *et al.*, 2003)
	464	2.37	(Gekas *et al.*, 2001)
	130	1.5	(Stegen *et al.*, 2012)
5-15 M/ml	4	0	(Martínez-Garza *et al.*, 2008)
5-10 M/ml	61	4.9	(Yoshida *et al.*, 1997)
	40	2.5	(van der Ven *et al.*, 1997)
1-10 M/ml	80	1.25	(Vutyavanich *et al.*, 2007)
0-5 M/ml	289	8	(Mohammed *et al.*, 2007)
	219	7.76	(Clementini *et al.*, 2005)
	750	5.6	(Foresta *et al.*, 2005)
2-5 M/ml	66	1.5	(Ng *et al.*, 2009)
1-5 M/ml	92	3.3	(Cruger *et al.*, 2003)
	39	2.56	(van der Ven *et al.*, 1997)
	227	2.2	(Stegen *et al.*, 2012)
>0-5 M/ml	30	13.3	(Ceylan *et al.*, 2009)
	231	6.9	(Yoshida *et al.*, 1997)
	73	6.85	(Akgul *et al.*, 2009)
	46	6.5	(Nagvenkar *et al.*, 2005)
	944	4.55	(Gekas *et al.*, 2001)
	64	3.7	(Han *et al.*, 2013)
	28	3.6	(Martínez-Garza *et al.*, 2008)
	865	3.5	(Tuerlings *et al.*, 1998)
	136	1.47	(Cavkaytar *et al.*, 2012)
	23	0	(Koşar *et al.*, 2010)
>0-2 M/ml	158	5.7	(Ng *et al.*, 2009)
0-1 M/ml	334	15.9	(Chiang *et al.*,. 2004)
>0-1 M/ml	24	8.3	(van der Ven *et al.*, 1997)
	89	2.2	(Vicdan *et al.*,. 2004)
	47	2.1	(Cruger *et al.*, 2003)
	111	1.8	(Kremer *et al.*, 1997)
	162	1.2	(Stegen *et al.*, 2012)
Azoospermia	30	33.3	(Ceylan *et al.*, 2009)
	358	18.71	(Gekas *et al.*, 2001)
	86	17.44	(Akgul *et al.*, 2009)
	42	14.3	(Nagvenkar *et al.*, 2005)
	244	13.1	(Yoshida *et al.*, 1997)
	383	12	(Samli *et al.*, 2006)
	77	11.7	(Cruger *et al.*, 2003)
	219	10.5	(Wang *et al.*, 2010)
	19	10.5	(Kremer *et al.*, 1997)
	50	10	(Vutyavanich *et al.*, 2007)
	50	10	(Oliva *et al.*, 1998)
	11	9.1	(van der Ven *et al.*, 1997)
	89	7.9	(Matsuda *et al.*, 1989)
	14	7.1	(Shamsi *et al.*, 2012)
	62	6.5	(Tuerlings *et al.*, 1998)
	49	6.1	(Bor *et al.*, 2002)

Table 1: Continued

	239	5.44	(Behulova *et al.*, 2011)
	92	5.4	(Koşar *et al.*, 2010)
NOA	71	21.1	(Ng *et al.*, 2009)
	50	16	(Martínez-Garza *et al.*, 2008)
	125	12	(Han *et al.*, 2013)
	196	11.22	(Cavkaytar *et al.*, 2012)
	119	4.2	(Vicdan *et al.*, 2004)

ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilization; M/ml, millions per milliliter; NOA, non-obstructive azoospermia; NOS, not otherwise specified; TFF, total fertilization failure.

In other studies, fertile men have been karyotyped as controls for the comparison of the prevalence of chromosomal abnormalities among infertile men. The prevalence in fertile men is 0.4%. Furthermore, female partners of infertile men have been karyotyped, and the prevalence of chromosomal abnormalities in the female partners varied from 0.8% to 13% (table 2).

Table 2: Prevalence of chromosomal abnormalities in women, fertile men and newborns, an overview of the literature.

Population	**Number of subjects studied**	**Prevalence of chromosomal abnormalities (%)**	**Reference**
Women in infertile couples	2710	1.3	(Riccaboni *et al.*, 2008)
Women in IUI couples	245	0.41	(Artini *et al.*, 2011)
Women in IVF and ICSI couples	2078	1.92	(Clementini *et al.*, 2005)
Women in ICSI couples	370	13	(Morel *et al.*, 2004)
	1164	9.79	(Scholtes *et al.*, 1998)
	150	6	(Mau *et al.*, 1997)
	436	5.5	(Meschede *et al.*, 1998)
	781	5.0	(Peschka *et al.*, 1999)
	1012	4.84	(Gekas *et al.*, 2001)
	305	3.3	(van der Ven *et al.*, 1998)
	305	3.2	(Haidl *et al.*, 2000)
	1762	1.53	(Artini *et al.*, 2011)
	261	1.2	(Testart *et al.*, 1996)
	122	0.8	(Pauer *et al.*, 1997)
Fertile sperm donors	10202	0.37	(Ravel *et al.*, 2006)
Fertile men			
Presenting for sperm analysis (normospermia)	303	0.3	(Foresta *et al.*, 2005)
Male partners of pregnant women	20	0	(Vicdan *et al.*, 2004)
Specifically chosen as control group	50	0	(Behulova *et al.*, 2011)
	76	0	(Shamsi *et al.*, 2012)
	96	0	(Han *et al.*,. 2013)
Newborns	34910	0.8	(Nielsen and Wohlert, 1991)

ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilization.

Studies on chromosomal abnormalities in spermatozoa of carriers of structural chromosomal rearrangements have been performed, with percentages of aneuploid spermatozoa close to 50% (Egozcue *et al.*, 2000). Most of these studies included only infertile men with chromosomal abnormalities and normozoospermic controls with a normal karyotype. In the latter the aneuploidy rates in spermatozoa ranged between 1-15% (Foresta *et al.*, 2002). However, a normal karyotype does not exclude having germ cell aneuploidy. Studies in infertile men with a normal karyotype showed that the sperm aneuploidy rate, especially for the sex chromosomes, was comparable to the rates in men carrying a chromosomal rearrangement (Giltay *et al.*, 2000; Maiburg *et al.*, 2012). This suggests that an altered intra-testicular environment not only damages spermatogenesis, but it may also disrupt the mechanisms controlling chromosomal segregation during meiosis (Calogero *et al.*, 2001). This is confirmed in a study in Klinefelter patients (Vialard *et al.*, 2012).

Male infertility and AZF deletions

Another genetic anomaly that can cause male infertility is a microdeletion in the azoospermia factor (AZF) region on the Y chromosome (Foresta *et al.*, 2002). A microdeletion is defined as a chromosomal deletion that may span several genes, but is not large enough to be detected using conventional cytogenetic methods (O'Flynn O'Brien *et al.*, 2010). Interstitial deletions of AZFa result in azoospermia. Interstitial deletions that include AZFb or AZFb plus AZFc usually result in azoospermia, although in some cases they cause severe oligospermia. Interstitial deletions that only include AZFc are the most common (6-12% in severely oligozoospermic and non-obstructive azoospermic men) and result in a variable infertility phenotype. Partial deletions of AZFc, including the most common (*gr/gr*), do not necessarily cause infertility, but are a risk factor for infertility. Genetic studies of ethnic groups produce diverse results because of the variations in their genomes that have evolved over generations to cope with environmental pressures specific to their region. For example, the *gr/gr* deletion was associated with spermatogenetic failure in studies conducted in the Netherlands and Australia, while there was no correlation found between the same deletion and spermatogenesis in Japanese, Chinese and German studies. The association with infertile phenotypes therefore depends on ethnicity and geographical region (O'Flynn O'Brien *et al.*, 2010).

Although heterogeneous results have been published (table 3), molecular testing reveals microdeletions of the Y chromosome in about 5-15% in otherwise healthy men with azoospermia or oligozoospermia and/or abnormal sperm morphology/motility for whom other causes of infertility have been excluded (Silber and Disteche, 1993). No symptoms other than infertility are known to be caused by AZF deletions. In men with retrievable spermatozoa, the presence or absence of deletions of the Y chromosome has no significant effect on pregnancy rates in their partners (van Golde *et al.*, 2001); the risk of birth defects is the same as for any infertile couple who achieves a pregnancy using ART. Y chromosome deletions are inherited in a Y-linked manner. The deletions

are usually *de novo* and therefore not present in the father of the proband. Despite their poor sperm quality, some men with an AZF deletion have spontaneously fathered sons who are infertile. Spontaneous conception will occur in about 4% of couples with severe oligozoospermia. In pregnancies achieved by ICSI, all male descendants inherit the deletion, with a high risk of infertility. Female fetuses from a father with a Y chromosome deletion have no increased risk of congenital abnormalities or infertility (Silber, 2011).

Table 3: Prevalence of AZF deletions in infertile men, an overview of the literature.

Population	Number of men tested	Prevalence of AZF-deletions (%)	Reference
Fertile men/controls	20	0	(Vicdan *et al.*, 2004)
	20	0	(Chellat *et al.*, 2013)
	50	0	(Dong *et al.*, 2012)
	50	0	(Behulova *et al.*, 2011)
	76	0	(Shamsi *et al.*, 2012)
	96	0	(Han *et al.*, 2013)
	100	0	(Cruger *et al.*, 2003)
	303	0	(Foresta *et al.*, 2005)
Infertile men			
NOS	20	15	(Babu *et al.*, 2002)
	143	14.69	(Song *et al.*, 2005)
	131	10.7	(Krausz *et al.*, 1999b)
	72	9.7	(Dada *et al.*, 2002)
	200	7	(Pryor *et al.*, 1997)
	112	6.25	(Shamsi *et al.*, 2012)
	202	4.95	(Clementini *et al.*, 2005)
	132	4	(Chen *et al.*, 2003)
	2749	4	(Hofherr *et al.*, 2011)
	98	3.1	(Quilter *et al.*, 2003)
	200	3.0	(Abid *et al.*, 2008)
	71	2.8	(Kunej *et al.*, 2003)
	81	2.47	(Selva *et al.*, 1997)
	1627	2.3	(Nap *et al.*, 1999)
	402	2.2	(Van Landuyt *et al.*, 2000)
Normospermia (>20 M/ml)	30	6.7	(Ceylan *et al.*, 2009)
	17	0	(Krausz *et al.*, 1999a)
	27	0	(Bor *et al.*, 2002)
	33	0	(van der Ven *et al.*, 1997)
	90	0	(Cruger *et al.*, 2003)
0- 20 M/ml	70	11.4	(Dada *et al.*, 2002)
	30	10	(Raicu *et al.*, 2003)
	74	9.5	(Wang *et al.*, 2010)
>0-20 M/ml	19	52.6	(Malekasgar and Mombaini, 2008)
	330	10.6	(Elfateh *et al.*, 2014)
	136	1.47	(Oliva *et al.*, 1998)
	31	0	(Chellat *et al.*, 2013)
10-20 M/ml	52	0	(van der Ven *et al.*, 1997)
5-20 M/ml	21	19.0	(Yao *et al.*, 2001)
	27	0	(Krausz *et al.*, 1999a)
	77	0	(Cruger *et al.*, 2003)
	81	0	(Bor *et al.*, 2002)
5-15 M/ml	4	0	(Martínez-Garza *et al.*, 2008)
5-10 M/ml	27	3.7	(van der Ven *et al.*, 1997)
1-10 M/ml	80	1.25	(Vutyavanich *et al.*, 2007)
0-5 M/ml	289	2.6	(Mohammed *et al.*, 2007)
2-5 M/ml	66	0	(Ng *et al.*, 2009)
>0-5 M/ml	28	14.3	(Martínez-Garza *et al.*, 2008)
	30	13.3	(Ceylan *et al.*, 2009)
	13	7.7	(Yao *et al.*, 2001)
	750	6.0	(Foresta *et al.*, 2005)
	136	2.2	(Cavkaytar *et al.*, 2012)
	70	1.42	(Rejeb *et al.*, 2008)
1-5 M/ml	37	8.1	(Pieri *et al.*, 2002)
	181	1.7	(Stahl *et al.*, 2010)
	26	0	(Krausz *et al.*, 1999a)
	47	0	(van der Ven *et al.*, 1997)
	92	0	(Cruger *et al.*, 2003)
	94	0	(Bor *et al.*, 2002)
>0-2 M/ml	158	8.2	(Ng *et al.*, 2009)
>0-1 M/ml	257	10.1	(Stahl *et al.*, 2010)
	111	6.3	(Kremer *et al.*, 1997)
	35	5.7	(Reijo *et al.*, 1996)
	113	4.4	(Dohle *et al.*, 2002)
	32	3.1	(van der Ven *et al.*, 1997)
	41	2.4	(Pieri *et al.*, 2002)
	89	2.25	(Vicdan *et al.*, 2004)
	149	1.3	(Bor *et al.*, 2002)
0-1 M/ml	334	9	(Chiang *et al.*, 2004)
	47	2.13	(Cruger *et al.*, 2003)
	42	0	(Krausz *et al.*, 1999a)
Azoospermia	31	51.6	(Malekasgar and Mombaini, 2008)
	30	33.3	(Ceylan *et al.*, 2009)
	50	16.0	(Oliva *et al.*, 1998)
	70	12.86	(Pieri *et al.*, 2002)
	74	12.1	(Yakin *et al.*, 2005)
	76	11.84	(Rejeb *et al.*, 2008)
	1193	10.4	(Stahl *et al.*, 2010)
	50	10	(Vutyavanich *et al.*, 2007)
	219	9.1	(Wang *et al.*, 2010)
	92	8.6	(Peterlin *et al.*, 2002)

Table 3: Continued

	37	8.1	(Dohle *et al.*, 2002)
	77	6.49	(Cruger *et al.*, 2003)
	22	4.5	(Krausz *et al.*, 1999a)
	226	3.35	(Behulova *et al.*, 2011)
	49	2	(Bor *et al.*, 2002)
	13	0	(van der Ven *et al.*, 1997)
	19	0	(Kremer *et al.*, 1997)
NOA	16	18.75	(Yao *et al.*, 2001)
	720	14.03	(Elfateh *et al.*, 2014)
	50	12	(Martínez-Garza *et al.*, 2008)
	196	9.69	(Cavkaytar *et al.*, 2012)
	1214	9.51	(Kumtepe *et al.*, 2009)
	71	8.5	(Ng *et al.*, 2009)
	119	4.2	(Vicdan *et al.*, 2004)
	49	2	(Chellat *et al.*, 2013)
	52	1	(Balkan *et al.*, 2008)

M/ml, millions per milliliter; NOA, non-obstructive azoospermia; NOS, not otherwise specified

Guidelines on genetic screening

Although men with balanced chromosomal abnormalities have a normal phenotype, their offspring is at increased risk of an unbalanced karyotype, which may result in a miscarriage or the birth of a child with congenital anomalies. ART enables infertile couples to have children, and IVF with ICSI is a treatment possibility for couples with severely compromised sperm parameters or in case of total fertilization failure. These developments have raised concerns of the consequences of ART. It has been assumed that, as ICSI bypasses the natural selection process, it could result in a greater chance of fertilization with a genetically abnormal sperm cell. This could mean that the number of miscarriages and children born with congenital anomalies, and transmission of infertility could increase.

Therefore, guidelines have been developed that address screening for chromosomal abnormalities in infertile patients. The clinical practice and guidelines that were current at the start of our research into the topic have been revised, but did not change much over the years.

The practice of chromosomal testing in connection with ART varies between countries. In Norway and Belgium (Soini *et al.*, 2006) chromosomal analysis before ICSI is offered to all couples; in Sweden, testing is offered only to men with non-obstructive oligozoospermia or azoospermia and in Finland it is offered to men with oligozoospermia or non-obstructive azoospermia and their female partners (Soini *et al.*, 2006). There are clinicians who suggest testing all men before ART because some aberrations can even be found in normospermic men (Foresta *et al.*, 2002), but most guidelines advise performing chromosome analysis only in selected cases. The selection is mostly based on the results of sperm analysis, as no other finding at physical examination or from a man's history is pathognomonic for chromosomal abnormalities. The American Society for Reproductive

Medicine (ASRM) recommends that karyotyping and Y chromosome analysis should be offered to men who have non-obstructive azoospermia or severe oligozoospermia (defined as < 5-10 million sperm/ml) prior to performing ICSI (AUA&ASRM, 2006). In the United Kingdom, the National Institute for Clinical Excellence (NICE) guideline states that men should be karyotyped if the indication for ICSI is a 'severe deficit of semen quality' or non-obstructive azoospermia. The definition of severe deficit of semen quality, however, is not given in the guideline. Testing for Y chromosome deletions should not be regarded as a routine investigation, although couples should be informed of the possibility (NICE, 2004). The European Association of Urology states that standard karyotype analysis should be offered to all men with damaged spermatogenesis who are seeking fertility treatment by IVF/ICSI. For men with severely damaged spermatogenesis, testing for Yq microdeletions before ICSI is desirable. However, they feel that it is reasonable to take into account the cost and limitations of current testing methods and to discuss this with the couple, as these men and their male children are unlikely to have any phenotypic abnormality other than impaired spermatogenesis (Dohle *et al.*, 2007). Karyotyping men with a total motile sperm count < 1 million was recommended by the Dutch Society of Obstetrics and Gynaecology in their guideline of 1999 and, irrespective of sperm quality, karyotyping was considered a prerequisite for ICSI treatment. Testing for AZF deletions could be considered (NVOG, 1999).

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) enables couples, both fertile and infertile, with a monogenetic or chromosomal defect (e.g. balanced translocation) to have an unaffected child and to reduce the risk of miscarriage. It is an alternative to prenatal diagnosis and pregnancy termination in case of an affected fetus. In PGD, the embryos resulting from an IVF(-ICSI) procedure are tested for the presence of the particular genetic abnormality. Unaffected embryos are transferred to the uterus or cryopreserved for later use, affected embryos are discarded.

In PGD aneuploidy screening, or preimplantation genetic screening (PGS), the same technique is applied. In this procedure, the couples do not carry a genetic defect, but are at high risk of aneuploid embryos, such as women of advanced maternal age. The embryos are tested for aneuploidies in multiple chromosomes.

Complete karyotyping of metaphase chromosomes in a single blastomere of early human embryos using traditional cytogenetic techniques is impossible due to time-constraints and technical limitations. Karyotyping requires dividing cells that are arrested in metaphase during culture. An alternative to karyotyping is fluorescent *in situ* hybridisation (FISH) using fluorochrome-labelled DNA probes that are complementary to DNA sequences specific to individual chromosomes. FISH enables enumeration of individual chromosomes and specific chromosomal regions even in interphase. FISH was first used on human blastomeres to discern the sex chromosomes in PGD for X-linked disorders (Wilton, 2002).

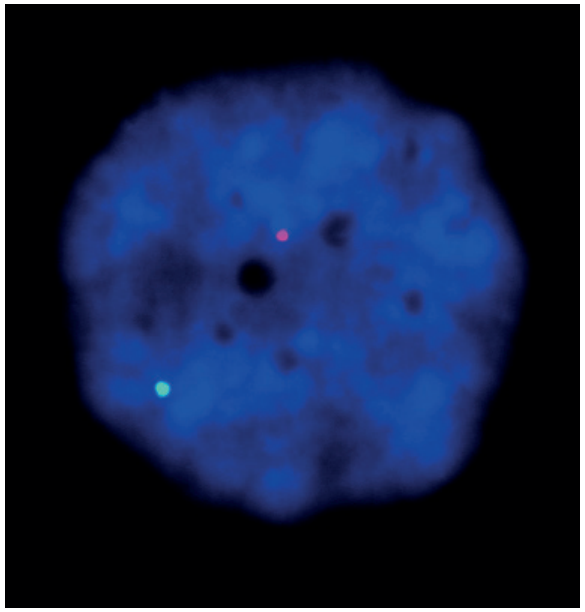

Figure 3: FISH on a XY blastomere. Green probe: X chromosome; Orange probe: Y chromosome.

Many FISH probes are nowadays available distributed over the chromosomes. However, only a restricted number of probes can be simultaneously applied to a single interphase nucleus because of the limited number of fluorochromes available (4) and the risk of misdiagnosis due to overlapping of signals.

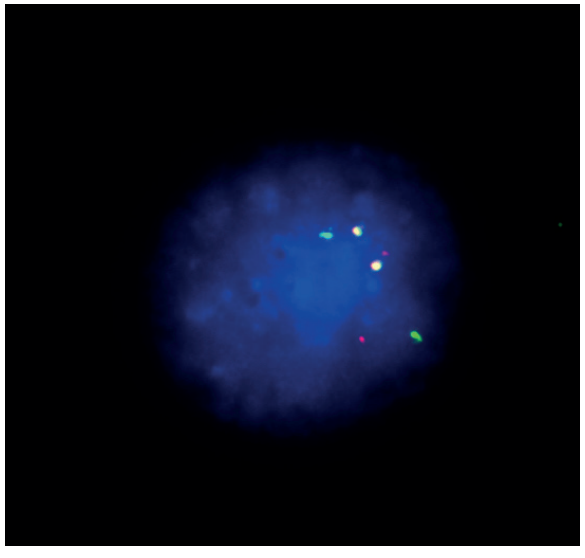

Figure 4: FISH on a blastomere with t(5;7). Green probe: telomere chromosome 5; Orange probe: telomere chromosome 7; Yellow probe: centromere chromosome 7.

Chromosomal abnormalities in cleavage stage embryos

Most of our current knowledge concerning the chromosomal constitution of human preimplantation embryos comes from the genetic analysis of cleavage stage embryos performed three days after fertilization, when embryos are usually composed of 6-10 blastomeres. Data obtained by such studies have indicated that only a minority of human embryos are chromosomally normal (Wilton, 2002). The chromosomal abnormalities may arise from an error during meiosis, resulting in an abnormal embryo and therefore a uniform abnormality in all cells, or from segregation errors during the first mitotic divisions (cleavage divisions). The latter event results in chromosomal mosaicism. Studies into aneuploidy in early human embryos have identified that 35-70% of IVF embryos are aneuploid in one or more blastomeres (Magli *et al.*, 2001). Especially embryos of poor morphology show high prevalences of aneuploidy, but even 17-43% of normally developing, good quality embryos are mosaic (Delhanty *et al.*, 1997; Magli *et al.*, 2007; Márquez *et al.*, 2000, Munné *et al.*, 1995; Munné and Cohen, 1998). Mosaicism has been reported to affect up to 91% of human preimplantation embryos when all cells are investigated (Vanneste *et al.*, 2009; Wells and Delhanty,2000). A large review on mosaicism in spare PGD and regular IVF embryos found an overall prevalence of mosaicism of 73% (van Echten-Arends *et al.*, 2011).Whether this high frequency of aneuploidy and mosaicism is also present in embryos that have developed *in vivo* is unknown, as these embryos cannot be studied in humans. However, several animal studies have shown lower rates of chromosomal abnormalities in embryos developed *in vivo* (8%), compared to culture *in vitro* (31%) (Sabhnani *et al.*, 2011).

In human IVF, the prevalence of mosaicism seems to be lower in embryos resulting from mild ovarian stimulation (37%), compared to conventional ovarian hyperstimulation (65%) (Baart *et al.*, 2007). This suggests that the artificial circumstances in IVF at least partially may induce mosaicism. Furthermore, embryo culture conditions may influence the susceptibility of the embryos to aneuploidy and mosaicism. A study in mice has shown a lower rate of mosaicism in embryos cultured in a 5% oxygen environment (52%), compared to embryos cultured at 20% oxygen (74%) (Bean *et al.*, 2002). The oxygen concentration to which embryos are exposed *in vivo* is about 5%, and in an ambient oxygen environment early embryos may be exposed to reactive oxygen species. These may have an effect on the segregation of the chromosomes in the first cleavage divisions, resulting in higher mosaicism rates in embryos grown in 20% oxygen. Especially in PGD, where an embryo is diagnosed as normal or abnormal based on the results of testing only one or two blastomeres, chromosomal mosaicism may lead to misdiagnosis. When embryos are incorrectly diagnosed as normal and transferred, or misdiagnosed as abnormal and discarded, this leads to lower success rates of PGD.

Pregnancy rates of PGD in translocation carriers

The PGD Consortium of ESHRE (European Society of Human Reproduction and Embryology) collects data from 57 international PGD centres. Data on PGD procedures and pregnancy rates are given per indication for PGD. From these data it can be gathered that the patients who have the lowest pregnancy rates per oocyte retrieval are those who have PGD for chromosomal imbalance (17%), especially for reciprocal translocations (14.5%) (Goossens *et al.*, 2012). This low pregnancy rate is mostly due to a high percentage of unbalanced embryos, and frequently no transferable embryos are available. Table 4 shows the percentage of started cycles with an embryo transfer per PGD indication (adapted from Harper *et al.*, 2010). In couples with reciprocal translocations only 57% of cycles had an embryo transfer, due to a chromosome imbalance in 70-80% of embryos (Harper *et al.*, 2010). Research into the reason for the high risk of unbalanced embryos has thus far not found the answer. Segregation studies have found differences in segregation patterns between male and female translocation carriers (Ko *et al.*, 2010; Lim *et al.*, 2008; Lledó *et al.*, 2010; Mackie Ogilvie and Scriven, 2002), but there is little difference in the number of PGD cycles with embryo transfers between male and female carriers (Harper *et al.*, 2012).

Translocation carriers have a high risk of recurrent miscarriages and conception of chromosomally abnormal pregnancies, and PGD is a method to decrease these risks. However, if the chance of conception with PGD is low, the couple may prefer to choose an alternative way of starting a family. Therefore, in counselling translocation carriers it would be helpful if, based on the cytogenetic characteristics of their translocation, a prediction of the outcome of PGD could be made.

*Table 4: Percentage of cycles with embryo transfer per PGD indication. Data adapted from ESHRE PGD consortium data collection XI (Goossens **et al.**, 2012).*

PGD indication	Started cycles with embryo transfer (% and 95% confidence interval)
Monogenetic disorders	79.2 77.1-81.4
X-linked diseases	78.1 69.8-86.4
Chromosomal abnormalities total	63.0 59.6-66.4
Robertsonian translocations	73.6 67.9-79.3
Reciprocal translocations	56.8 52.3-61.3

Outline of the thesis

The first part of the thesis focuses on screening for chromosomal abnormalities in infertile men.

Chapter 2 debates the policy of screening for chromosomal abnormalities solely based on sperm quality. Data of our retrospective cohort of infertile men were added to the data of other studies in the literature.

In *Chapter 3* sperm parameters, sex hormone levels, medical (andrologic) history, fertility history and family history were studied in a retrospective cohort, in order to identify possible (combinations of) risk factors for chromosomal abnormalities in infertile men.

Chapter 4 addresses the development of a screening policy. For the efficiency of a screening policy, both the prevalence of chromosomal abnormalities and the consequences of detecting (or not detecting) a chromosomal abnormality are important. The clinically most relevant consequences of a chromosomal abnormality are adverse pregnancy outcomes, i.e. conceiving a child with congenital anomalies, or the occurrence of miscarriage. This chapter describes the number of infertile men that need to be screened for chromosomal abnormalities to prevent one adverse pregnancy outcome.

The second part of the thesis, on chromosomal abnormalities in embryos, describes studies in cohorts of PGD embryos, in search for methods to improve the outcome of PGD.

Chapter 5 describes a pilot study in a cohort of human preimplantation embryos on the prevalence of chromosomal mosaicism. It evaluates whether the prevalence of mosaicism is lower in embryos cultured in a 5% oxygen environment, compared to embryos cultured at 20% oxygen, as found in a study in mice (Bean *et al.*, 2002).

Chapter 6 deals with a cohort of couples that underwent PGD for reciprocal translocations. The aim is to find cytogenetic factors that could be used as predictors for the ratio of balanced *versus* unbalanced embryos in couples that have PGD for reciprocal translocations. We hypothesized that there is an association between characteristics of the translocation, such as the ratio of the translocated segments or the place of the breakpoints, and the percentage of balanced embryos.

Chapter 7 provides a general discussion and future perspectives.

Chapter 8 is a summary of the thesis.

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