Italian Journal of
Gynæcology & Obstetrics
March 2017 - Vol. 29 - N. 1 - Quarterly - ISSN 2385 - 0868

Routine karyotiping in infertile couples: is it really mandatory? Proposal from experience on 7,196 infertile Italian couples

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ABSTRACT

The aim of the study was to establish whether karyotyping of both partners should be carried out on every infertile couple regardless of the cause of their infertility. In fact in Italian infertility centers karyotyping is often performed in all couples besides the universally accepted indications : hypergonadotropic amenorrhea, premature menopause, recurrent miscarriage, severe male infertility . This approach is labour and cost-expensive.

We evaluated the incidence of chromosomal abnormalities in a retrospective cohort of 7,196 infertile couples, referred for assisted reproduction, to define evidence-based criteria to recommend karyotyping during infertility assessment.

A total of 185 pathologic karyotypes were identified (1,28%), 120 in men (1.66 %) and 65 in women (0.90 %). The most frequent abnormalities were reciprocal translocations both in men (29/120, 24.16%) and in women (24/65, 36.92%). The incidence of chromosome abnormalities in infertile males was: 16.95% in azoospermic males, 4.70% in severely oligozoospermic, 1.83 % in mildly oligozoospermic, and 0.78% in normal semen group.

Our data definitely indicates that, outside the universally accepted indications, the incidence of abnormal karyotype is not different from the general population. Our results do not support the indication to prescribe a karyotype analysis in every couple undergoing Assisted Reproductive Technology.

Keywords: karyotype; chromosome abnormalities; infertility; Assisted Reproductive Technology; ART

SOMMARIO

Lo scopo dello studio è stato quello di stabilire se l'esame del cariotipo di entrambi i partner debba essere eseguito su ogni coppia infertile indipendentemente dalla causa d'infertilità.

Nei Centri Italiani, il cariotipo è spesso effettuato in tutte le coppie al di fuori delle indicazioni universalmente riconosciute: amenorrea ipergonadotropa, menopausa precoce, aborto abituale, grave fattore maschile. Questo approccio porta ad un aumento dei costi e tempi del percorso.

Abbiamo quindi eseguito un'analisi retrospettiva, valutando l'incidenza di anomalie cromosomiche in una coorte di 7.196 coppie infertili, inviate per riproduzione assistita, per definire criteri basati sull'evidenza volti a indicare l'esecuzione del cariotipo durante la valutazione diagnostica.

Sono stati indentificati 185 cariotipi patologici (1,28%), dei quali 120 nei maschi (1,66%) e 65 nelle femmine (0,90%).

Le incidenze delle anomalie cromosomiche nelle quattro categorie maschili sono state: 16,95% nei maschi con azoospermia, 4,70% in caso di severa oligozoospermia, 1,83% nell'oligozoospermia moderata e 0,78% nel gruppo con esame seminale normale.

In conclusione i nostri dati indicano in maniera chiara che, al di fuori delle indicazioni universalmente accettate, l'incidenza delle anomalie del cariotipo in pazienti infertili non differisce da quella della popolazione generale. I dati quindi non supportano l'indicazione a prescrivere l'analisi del cariotipo in ogni coppia che si sottoponga a tecniche di riproduzione assistita.

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DOI: 10.14660/2385-0868-58

INTRODUCTION

According to literature, it can be speculated that about 15% of male and 10% of female infertile patients have some genetic alterations, part of which is represented by chromosome abnormalities[1]. Indications to perform a karyotype analysis are well established in definite conditions[2]. For example, many studies have demonstrated a higher incidence of chromosome abnormalities in males with severe sperm impairment. Therefore, testing for karyotype is mandatory in males with infertility due to a severe sperm factor[3,4]. Among women, genetic tests are also part of the diagnostic work-up in case of hypergonadotropic amenorrhea, since amenorrhea could have a possible genetic aetiology[5,6]. Karyotype is also indicated in both partners in cases of recurrent pregnancy loss[7].In 2002, Foresta et al.[1] set up a proposal of guidelines for the genetic testing of infertile couples. They suggested that a karyotype analysis should be performed in both members of a couple undergoing Assisted Reproduction Technique (ART). However the General Guideline and Quality Assurance for Cytogenetic from the E.C.A. of 2012[8] recommends that karyotyping should be used only in couple with infertility of unknown aetiology. According to the guideline from N.I.C.E., published in 2013[9], testing should be done in specific cases with a severe male infertility. Therefore there is no consensus between geneticists whether routine karyotyping of both partners should be carried out on every infertile couple regardless of the cause of their infertility. In the absence of robust data, in Italy there is not a general agreement and the evaluation protocols offered to infertile couples and the prescribing behaviour between professionals vary among different Italian regions.

The aim of the study was to establish incidence of chromosomal abnormalities in a retrospective cohort of 7,196 infertile couples to define evidence-based criteria to recommend karyotyping during assessment of peoples with fertility problems.

MATERIALS AND METHODS

Since 1995 all couples referred to the two public IVF Units in Genoa (Italy) for primary infertility, lasting two years or longer, have been tested for karyotype in the Regional Genetic Laboratory, regardless of the aetiology of their infertility. The retrospective study was approved by local institutional review board.

The results of the tests performed between 1995 and 2015 were retrospectively collected and subsequently matched with the databases of the two IVF centers. Only the results of couples where both partners were analysed were selected for this study.

In addition a specific retrospective subgroup analysis was conducted on data of males referred for andrological consultation for pure male infertility factor. This subgroup included patients with azoospermia or oligozoospermia confirmed by at least two consecutive semen analyses. Males with normal semen or with sporadic semen alterations were used as control group. Semen analyses were performed during the study according to the indications present in the WHO manuals in use at the time of sample collection (WHO 1987, 1999, 2010)[10].

Azoospermia, severe oligozoospermia, and mild oligozoospermia were respectively defined as: the total absence of sperm cells in seminal fluid, a sperm cell count of <5 millions/mL, and a sperm cell count between 5 and 20 million/mL. Centrifugation of specimens was always performed in every case of azoospermia, according to international guidelines.

Karyotype analysis was performed on peripheral blood lymphocytes according to standard procedures.

Metaphases were stained with quinacrine mustard (Sigma-Aldrich, Steinheim, Germany) and analysed at a resolution level of 400 bands for all patients as indicated in the Italian Guidelines for the Cytogenetic Diagnosis[11] in use at the time of sampling (1995, 2007, 2013).

Breakpoints of reciprocal translocations and inversions and characterization of supernumerary chromosome markers were confirmed by fluorescent in situ hybridization (FISH). FISH, was performed according to manufacturer's instructions by using commercial probes, or it was performed as described by Lichter and Cremer when using in-house made single copy DNA probes[12].

RESULTS

We collected data from 7,196 couples (14,392 subjects). A total of 185 pathologic karyotypes were diagnosed (1.28%), 120 in men (1.66%) and 65 in women (0.90%). In one couple both partners showed an abnormal karyotype (0.013%).

Tables 1 and 2 show all the abnormal karyotypes diagnosed in male and female patients respectively.

Table 1.
Abnormal karyotypes diagnosed in males (n° 120)

Abnormality	n.	Abnormality	n.
Aneuploidies	**28**	**Robertsonian translocations**	**22**
45,X[5]/46,XY[95]	1	45,XY,der(13;14)(q10;q10)	15
45,X[10]/46,XY[90]		45,XY,der(13;14)(q10;q10),inv(19)(p11q12)	1
47,XXY	13	45,XY,der(14;15)(q10;q10)	2
47,XXY/46,XY	1	45,XY,der(14;21)(q10;q10)	1
47,XYY	11	45,XY,der(14;22)(q10;q10)	2
47,XYY[37]/46,XY[63]	1	45,XY,der(21;22)(q10;q10)	1
Reciprocal translocations	**29**	**Inversions**	**23**
46,XY,t(1;10)(p32;p14)	1	46,XY,inv(1)(p34.3q12)	2
46,XY,t(1;11)(p33;p14)	1	46,XY,inv(5)(p12q13.1)	1
46,XY,t(1;11)(p22;q22)	1	46,XY,inv(5)(q15.1q21)	1
46,XY,t(1;13)(p13.3;q12)	1	46,XY,inv(7)(q21.2q32)	1
46,XY,t(1;13)(p21;q26.3)	1	46,XY,inv(10)(p11.2q22)	1
46,XY,t(1;5)(q41;q21)	1	46,XY,inv(10)(p12.1q21.2)	1
46,XY,t(2;20)(p16;p12.3)	1	46,XY,inv(11)(p14.3q22)	1
46,XY,t(2;20)(p23;p11.2)	1	46,XY,inv(12)(p11.2q13.3)	1
46,XY,t(2;21)(q12;p11.2)	1	46,XY,inv(19)(p13.3q13.1)	1
46,XY,t(3;9)(p14;q21)	1	46,X,inv(Y)(p11.2q11.2)	5
46,XY,t(3;6;13)	1	46,X,inv(Y)(p11.2q11.22)	1
46,XY,t(3;13)(q11.1;q33)	1	46,X,inv(Y)(p11.2q11.222)	1
46,XY,t(5;11)(q31;q21)	1	46,X,inv(Y)(p11.2q11.23)	4
46,XY,t(6;11)(q23;p14)	1	46,X,inv(Y)(p11.3q11.1)	1
46,XY,t(6;12)(q21;q24.1)	1	46,X,inv(Y)(p11.31q12)	1
46,XY,t(7;21)(p21.3;q21.3)	1	**Deletions**	**5**
46,XY,t(9;11)(q21.1;q25)	1	46,X,del(Y)(q11.21)	1
46,XY,t(9;13)(q33;q12)	1	46,X,del(Y)(q12)	4
46,XY,t(9;15)(q22;q22)	1	**Various**	**7**
46,XY,t(9;20)(q12;p11.1)	1	46,XY,dup(13)(q12)	1
46,XY,t(11;22)(q23.3;q11.2)	1	46,XY,ins(8;1)	1
46,XY,t(13;18)(q12;p11.2)	1	46,XY,16ps	1
46,XY,t(14;18)(q24;p11.32)	1	46,X,Yqs	1
46,XY,t(15;19)(q25;q13.33)	1	46,X,idic(Y)(q11.21;q11.21)	2
46,XY,t(16;20)(q21;p11.2)	1	45,X/46,X,idic(Y)(q11.21;q11.2)	1
46,XY,t(20;22)(q12;q12)	2	**Markers**	**6**
46,X,t(Y;5)(p11.2;q12)	1	47,XY,+mar/46XY	1
46 XY,t(Y;9)(q12;q12)[90]/46,XY[10]	1	47,XY,+dic(14 o 22)(q11.1;q11.1)	2
		47,XY,+dic(14 or 22)(q11.1;q11.1)[40]/46,XY[60]	1
		47,XY,+idic(15;15)(q11.1;q11.1)	2

Table 2.
Abnormal karyotypes diagnosed in females (n°65)

Abnormality	n°	Abnormality	n°
Aneuploidies	**17**	**Reciprocal translocations**	**24**
45,X/46,XX	1	46,XX,t(1;2)(q43;p24)	1
45,X[5/46,XX[95]	1	46,XX,(1;5)(q23;q13)	1
45,X[8]/46,XX[92]	1	46,XX,t(1;7)(p36.3;q32)	1
45,X[16]/46,XX[84]	1	46,XX,t(1;10)(q25;q24)	1
45,X/47,XXX/46,XX	1	46,XX,t(1;11)(p36.1;p15)	1
45,X[4]/47,XXX[3]/46,XX[93]	1	46,XX,t(2;10)(q11.2;q25.2)	1
45,X[66]/47,XXX[32]/46,XX[2]	1	46,XX,t(2;12)(q32;q23)	1
47,XXX	7	46,XX,t(3;6)(q27;q24)	1
47,XXX[7]/46,XX[93]	1	46,XX,t(3;7)(p22.1;p21.1)	1
47,XXX[91]/46,XX[9]	1	46,XX,t(3;14)(q25;q24)	1
47,XXX,t(12;19)(q21.3;p13.3)	1	46,XX,t(3;16)(p21;p12)	1
Markers	**4**	46,XX,t(3;16)(q25;q21)	1
47,XX,+mar(1)[50]/46,XX[50]	1	46,XX,t(4;8)(q31.1;p23.1)	1
47,XX,+dic(14;15)(q11;q11)	1	46,XX,t(4;14)(q28;q21)	1
47,XX,+idic(15;15)(q11.2;q11.2)	1	46,XX,t(4;19)(q12;q13.1)	1
47,XX,+ mar.ish der(16)(q12.1)[36]/46,XX[64]	1	46,XX,t(5;16)(q31.3;p11.2)	1
Robertsonian translocations	**12**	46,XX,t(5;9)(q12;p21)	1
45,XX,der(13;14)(q10;q10)	8	46,XX,t(6;18)(q21;q11.2)	1
45,XX,der(14;21)(q10;q10)	1	46,XX,t(9;17)(p13;q22)	1
45,XX,der(14;22)(q10;q10)	2	46,XX,t(10;13)(q24;q32)	1
45,XX,der(21;22)(q10;q10)	1	46,XX,t(10;19)(p12;q13.1)	1
Inversions	**4**	46,XX,t(11;22)(q23.3;q11.2)	1
46,XX,inv(2)(p11.2;q13)	1	46,XX,t(14;19)(q24.3;p13.1)	1
46,XX,inv(9)(p12;q21.11)	1	46,X,t(X;9)(q22;q12)	1
46,XX,inv(12)(p11.2q13.3)	1	**Various**	**3**
46,XX,inv(19)(p13.1q12)mat	1	46,XX,r(21)(p13q22.3)[68]/46,XX[32]	1
Deletions	**1**	46,XX,der(1)(p32)	1
46,X,del(X)(p21.2)	1	46,X,der(X)(Xqter-Xq21:3::Xp11.23-Xqter)	1

The frequency of the various abnormalities is detailed in **Table 3**. The most frequently recovered abnormalities were reciprocal translocations both in men (29/120, 24.16%) and in women (24/65, 36.92%).

Table 3.
Incidence of various chromosome abnormalities

	Men (n°7196)	Women (n°7196)
Aneuploidies and mosaicisms	28	17
Markers	6	4
Reciprocal Traslocations	29	24
Robertsonian Translocations	22	12
Inversions	23	4
Deletions	5	1
various	7	3
Tot	120/7196 (1.66%)	65/7196 (0.90%)

The incidence of chromosome abnormalities in the four male groups, divided according to their sperm count, was: 16.95% in azoospermic males, 4.70% in severely oligozoospermic, 1.83% in mildly oligozoospermic, and 0.78% in the group with normal semen analysis **(Tab. 4)**. Results changed slightly but not significantly after exclusion of 47, XYY cases **(Tab. 4)**.

Table 4.
Chromosome abnormalities according to severity of male factor infertility including and excluding 47,XYY

DIAGNOSIS	N°	Pathologic Karyotype	%	N° 47,XYY	without 47,XYY
Azoospermia	171	29	16.95%	0	16.95%
Severe oligozoospermia	637	30	4.70%	1	4.55%
Moderate/mild oligozoospermia	1035	19	1.83 %	2	1.64%
Other males	5353	42	0.78 %	9	0.61%
total	7196	120	1.66%	12	1.50%

DISCUSSION

The frequency of chromosome abnormalities in live births of the general population is reported to be 0.92%[13]). Since a proportion of newborns with abnormal karyotype will not gain the adult age, due to the severity of their pathology, it can be inferred that the frequency of abnormal karyotype among healthy adults should not be higher than 0,9%.Moreover in a large-scale survey of over 10,000 sperm donors 38 karyotype aberrations (0.37%) were diagnosed, including 21 balanced chromosomal rearrangements (0.2%). Semen parameters were always normal, suggesting that not all chromosomal aberrations have consequences on spermatogenesis[14] and that a minimum incidence of abnormal karyotype

around 2/1000 (0.02%) should also be expected in the population of normal fertility.

Male factor infertility has been associated with karyotype anomalies. Routine karyotyping of infertile men with unexplained azoospermia and oligozoospermia (<10 million/ml) is today recommended[15]. In a review of 11 studies involving 9,766 azoospermic and oligozoospermic men, sex and autosomal anomalies were found respectively in 4.2 and 1.5%[16].

Recently two large studies from the USA reported an incidence of karyotype abnormalities in infertile males varying from 8.2% (668 analysed cases)[17] to 14.3% (2,242 analysed cases)[18]. According to the largest study in cytogenetics of male infertility[19] the more severe the oligozoospermia is, the higher the frequency of chromosomal abnormalities is. Patients with less than 10 million spermatozoa/ml show a 10 times higher incidence (4%) of abnormalities with respect to the general population. In patients with <5 million spermatozoa/ml the percentage of abnormal karyotypes is 7–8%, and in patients with non-obstructive azoospermia the percentage rises to 15–16%. The most frequent abnormalities in azoospermia and severe male factor infertility patients are sex chromosome aneuploidies. Klinefelter syndrome (47,XXY) represents the most common abnormality, followed by Y chromosome terminal deletions (Yq-) and structural autosomal abnormalities. About 20% of 47,XXY patients presents different grade of mosaicisms (47, XXY/46, XY, 47,XXY/45,X/46,XY). On the other hand, in oligozoospermic patients the abnormalities are mainly structural autosomal rearrangements (Robertsonian translocations, reciprocal translocations, paracentric inversions and marker chromosomes).

In the present study all subjects, men and women, referred to the two IVF Units after at least 2 years of unsuccessful attempts underwent an evaluation of their karyotype, regardless of the aetiology of their infertility. This allowed us to have a really unbiased group of infertile couples.

There are four Italian studies similar to this one. The first study reported data on 2,078 couples referred for ART, finding abnormal results in 2.02% of males and in 1.92 % of females[20].

The second study analysed the incidence of chromosome abnormalities according to the type of ART planned for the couple[21]. The authors found a higher incidence of pathological karyotypes in male partners of couples scheduled for ICSI (2.2%) than in those who were about to be treated with IVF (1.1%) or IUI (0.3 %). In females the incidence of pathological results was overall 1.3% and it did not vary according to the ART procedure.

A third Italian group studied 1,146 couples scheduled for ART. The authors reported a frequency of abnormalities of 1.52% (1.83% in men and 1.2% in women). Interestingly they also evaluated the outcome of pregnancies obtained in couples with karyotype abnormalities and found that 41% of pregnancies ended in a miscarriage[22].

A more recent Italian study, which reported on 1,762 infertile couples scheduled for ART, found an incidence of chromosome abnormalities of 1.82% in males and 1.53 % in females[23]. The data of the four studies are summarized in **Tab. 5**.

Table 5.
Comparison among results reported by this and other four Italian studies

	N° Women analysed N°	Abnormal karyotype %	N° Men Analysed	Abnormal kariotype %
Clementini et al 2005	2078	1.92%	2078	2.02%
Riccaboni et al 2008	2710	1.3%	2710	1.5%
Tiboni et al 2010	1146	1.2%	1146	1.83%
Artini et al 2011	1762	1.53%	1762	1.82%
This study 2016	7196	0.90 %	7196	1.66%

To the best of our knowledge this study represents the largest cohort of infertile couples tested for chromosomal abnormalities (7,196 couples). Overall, in contrast to the results reported by other groups, we did not found an increase in abnormal karyotypes in women (0.90%), with respect to the general population. A possible explanation could be that all the referred couples were tested, regardless of the diagnosis, so avoiding any selection bias.

Our data confirmed a higher incidence of chromosomal abnormalities (1.66%) in males with semen abnormalities. In agreement with previous reports, karyotype abnormalities were higher in azoospermic (16.95%) and severely oligozoospermic patients (4.70%), than in mild oligozoospermic cases (1.83%). This data were confirmed also after exclusion of 47, XYY males (Tab. 4), as the proportion of these subjects is increased in infertile population[24].

Recent studies show that the 47, XYY patients may display altered meiotic segregation, increased sperm apoptosis and necrosis, which could lead to semen abnormalities and subsequent infertility[25,26].

On the other hand, the male subgroup with normal or sporadically abnormal semen analysis showed an incidence of abnormal karyotypes similar to the general population.

Previous recommendations have suggested to perform a karyotype analysis on every couple undergoing an IVF treatment[1]. This was justified by the hypothesis that infertile couples undergoing ART could have a higher incidence of chromosome abnormalities and by the potential advantages in cases of pathological results, such as the opportunity to perform PGT (preimplantation genetic testing) on the embryos, to avoid the risk of miscarriage and unexpected adverse neonatal outcome. Those arguments could be questionable as the incidence of abnormal results is higher only in the subgroup of patients with semen abnormalities. Moreover karyotype analysis is labour and cost-expensive thus increasing the Italian Public Health burdening.

Our study, on a very large cohort of infertile couples, did not demonstrate a higher incidence of chromosomal abnormalities in males without semen abnormalities and in women.

These findings indicate that karyotyping should be performed only in selected infertile couples on the basis of an accurate clinical assessment. A multidisciplinary team (e.g. reproductive gynaecologist and geneticist) could be the more advisable approach to evaluate infertile patients thus to prescribe karyotyping and other genetic tests only when appropriate, allowing cost saving and the best allocation of available resources.

ETHICAL APPROVAL

All procedures performed in this retrospective study were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

REFERENCES

1) Foresta C, Ferlin A, Gianaroli L, Dallapiccola B (2002) **Guidelines for the appropriate use of genetic tests in infertile couples**. Eur J Hum Genet 10:303–312

2) Krausz C (2011) **Male infertility: Pathogenesis and clinical diagnosis**. Best Practice & Research Clinical Endocrinology & Metabolism 25:271–285

3) Van Assche E, Bonduelle M, Tournaye H, Joris H, Verhejen G, Devroey P, Van Steirtheghem A, Liebaers I (1996) **Cytogenetic of infertile men**. Hum Reprod 1:1–24

4) Robert I, McLachlan and Moira K, O'Bryan (2010) **State of the Art for Genetic Testing of Infertile Men**. J Clin Endocrinol Metab, 95: 1013-1024

5) Hens L, Devroey P, Van Waesberghe L, Bonduelle M, Van Steirtheghem AC, Liebaers I (1989) **Chromosomes studies and fertility treatment in women with ovarian failure**. Hum Reprod 36:81–91

6) Speroff, L. (1999) **Women's healthcare in the 21st century**. Maturitas 32, 1–9

7) Crosignani PG, Rubin BL (2000) **Optimal use of infertility diagnostic tests and treatments**. The ESHRE Capri workshop group. Hum Reprod 15:723–732

8) Hastings R., Howell R., Bricarelli F. D., Kristoffersson U., Cavani S. **General Guidelines and Quality Assurance for Cytogenetics**. In: E.C.A. - European Cytogeneticists Association [online]. 2. vyd. 2012

9) National Collaborating Centre for Women's and Children's Health (UK) Fertility. **Assessment and Treatment for People with Fertility Problems London.** Royal College of Obstetricians & Gynaecologists; (2013) Feb. National Institute for Health and Clinical Excellence: Guidance .

10) World Health Organization (2010) **Laboratory Manual for the Examination and Processing of Human Semen**. 5th edn. WHO, http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/index.html

11) SIGU, **Linee Guida per la Diagnosi Citogenetica 2013**, http://www.sigu.net/show/attivita/5/1/citogenetica

12) Lichter P, Cremer T (1992) **Chromosome analysis by non-isotopic in situ hybridization**. In: Rooney DE, Czepulkowski BH, eds. Human Cytogenetics A Practical Approach. New York: IRL Press 157-192

13) Jacobs PA, Browne C, Gregson N, Joyce C, and White H (1992) **Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding**. J Med Genet. Feb 2; 29: 103–108

14) Ravel C, Berthaut I, Bresson JL, Siffroi JP. and and the Genetics Commission of the French Federation of CECOS (2006) **Prevalence of chromosomal abnormalities in phenotypically normal and fertile adult males: large-scale survey of over 10,000 sperm donor karyotypes**. Hum. Reprod. 21: 1484-1489

15) ASRM (2012) **Diagnostic evaluation of the infertile male: a committee opinion The Practice Committee of the American Society for Reproductive Medicine**. Fertil Steril Aug; 98: 294-301

16) Johnson MD (1998) **Genetic risks of intracytoplasmic sperm injectionin the treatment of male infertility: recommendations for genetic counseling and screening**. Fertil Steril 70:397–411

17) Yatsenko AN, Yatsenko SA, Weedin JW, Lawrence AE, Patel A, Peacock S, Matzuk MM, Lamb DJ, Cheung SW, Lipshultz LI (2010) **Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men**. J Urol. Apr;183(4):1636-42

18) Hofherr S, Wiktor A, Kipp B, Dawson D, Van Dyle D (2011) **Clinical diagnostic testing fort the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience**. J Assist Reprod Genet, Nov; 28(11): 1091-8

19) Vincent MC, Daudin M, De MP, Massat G, Mieusset R, Pontonnier F, Calvas P, Bujan L, Bourrouillout G. (2002) **Cytogenetic investigations of infertile men with low sperm counts: a 25-year experience**. J Androl. Jan-Feb;23(1):18-22

20) Clementini E, Palka C, Iezzi I, Stuppia L, Guanciali-Franchi P, Tiboni GM (2005) **Prevalence of chromosomal abnormalities in 2,078 infertile couples referred for assisted reproductive techniques**. Hum Reprod 2005; 20:437–42

21) Riccaboni A, Lalatta F, Caliari I, Sonetti S, Somigliana E, Ragni G (2008) **Genetic screening in 2710 infertile candidate couples for assisted reproductive techniques: result of application of Italian Giudelines for the appropriate use of genetic test**. Fertil Steril, Apr; 89: 800-808

22) Tiboni GM, Verna I, Giampietro F, Leonzio E, Impicciatore GG (2011) **Cytogenetic findings and reproductive outcome of infertile couples referred to an assisted reproduction program**. Gynecol Endocrinol. Sep;27:669-674

23) Artini PG, Papini F, Ruggiero M, Bartalini G, De Leo V, Scaravelli G, Piomboni P, Cela V (2011) **Genetic screening in Italian infertile couples undergoing intrauterine insemination and in vitro fertilization techniques: a multicentric study**. Gynecol Endocrinol. Jul;27(7):453-7. doi: 10.3109/09513590.2011.579207. Epub 2011 May 24.

24) Martin RH (2008) **Cytogenetic determinants of male fertility**. Human Reproduction Update 14:379-390

25) Moretti E, Anichini C, Sartini B, Collodel G (2007) **Sperm ultrastructure and meiotic segregation in an infertile 47, XYY man**. Andrologia. Dec;39(6):229-34

26) Wong EC, Ferguson KA, Chow V, Ma S (2008) **Sperm aneuploidy and meiotic sex chromosome configurations in an infertile XYY male**. Hum Reprod. 23:374