Chromosomal alterations and male infertility

A. Antonelli*, L. Gandini**, P. Petrinelli*, L. Marcucci*, R. Elli*, F. Lombardo**, F. Dondero**, and A. Lenzi**

*Laboratory of Human Cytogenetics, Section of Molecular Genetics, Department of Cellular Biotechnology and Hematology; **Laboratory of Seminology and Reproductive Immunology, Section of the Training Center in Andrology of the European Academy of Andrology, Department of Pathophysiology, "La Sapienza" University, Rome, Italy

ABSTRACT. Reduced male fertility can be caused by genetic factors affecting gamete formation or function; in particular, chromosome abnormalities are a possible cause of male subfertility as shown by their higher frequency in infertile men than in the general male population. Meiotic studies in a number of these males have shown spermatogenesis breakdown, often related to alterations in the process of chromosome synapsis. Indeed, any condition that can interfere with X-Y bivalent formation and X-chromosome inactivation is critical to the meiotic process; furthermore, asynapsed regions may themselves represent a signal for the meiotic checkpoint that eliminates spermatocytes with synaptic errors. We performed cytogenetic, hormonal and seminal studies in 333 infertile patients selected because azoospermic, severely oligozoospermic or normozoospermic with failure to fertilize the partner's oocytes in an *in vitro* fertilization (IVF) program. Our findings: 1) confirm the high incidence of chromosomal anomalies among infertile males; 2) highlight the relevance in male infertility of quantitative/positional modifications of the constitutive heterochromatin; and 3) underline the relevance of cooperation between andrologists and cytogenetists prior to every kind of assisted reproduction, above all prior to intracytoplasmic sperm injection, in which selective hurdles eliminating abnormal germ cells are bypassed.

(J. Endocrinol. Invest. 23: 677-683, 2000)

STATE OF ART

The relationship between male infertility and anomalies of the constitutional karyotype has become even more relevant since the development of the intracytoplasmic sperm injection (ICSI) as the most advanced therapy in male infertility. In fact, one of the debates on the widespread use of ICSI is that this technology could lead to the production of abnormal fetuses through the use of chromosomally unbalanced spermatozoa that have avoided all the steps of the natural selection pathway (1). Reduced fertility affects approximately 15% of couples worldwide and in about 40% of these a male factor can be identified as the cause of childlessness (2). Genetic factors affecting gamete formation or function are responsible for a proportion of these cases (3). In particular, chromosome abnormalities have been known for some time to be a possible cause of male subfertility (4, 5). Indeed, the overall incidence of chromosome anomalies in infertile men is higher than in the general male population, ranging between 2.2% and 8.6%. This value increases to about 16% in azoospermic males due to the presence of patients with Klinefelter syndrome (47,XXY) in this group. Besides sex chromosome abnormalities, a variety of structural chromosome anomalies, such as Robertsonian translocations, reciprocal translocations, accessory marker chromosomes and inversions, are found in the karyotype of infertile males. Meiotic studies in a number of these males have shown spermatogenesis breakdown, often related to alterations in the process of chromosome synapsis. Indeed, as suggested by Lifschytz and Lindsley (6), any condition that can interfere with X-Y bivalent formation and X-chromosome inactivation is critical to the meiotic process. Presumably, the transcriptionally inactive status of the X-chromosome during male meiotic prophase may constitute a control mechanism for normal spermatogenesis. Furthermore, asynapsed regions,

Key-words: Male infertility, semen analysis, chromosome, heterochromatine.

Correspondence: Prof. Andrea Lenzi, Dipartimento di Fisiopatologia Medica, Servizio di Seminologia e Immunologia della Riproduzione, Policlinico Umberto I, 00161 Roma, Italy.

E-mail: lenzia@uniroma1.it

even if they do not interfere with X-Y bivalent, may in themselves represent a signal for the meiotic checkpoint that eliminates spermatocytes with synaptic errors via p53-independent apoptosis, as shown in mice (7).

Regarding the numerical abnormalities of sex chromosomes, the most common chromosome constitution is 47,XXY, mostly associated with azoospermia. Indeed, the possibility of XXY cells producing gametes seems so unlikely that the rare non-azoospermic Klinefelter patients seen are possibly undetected XXY/XY mosaics (5). It is not yet known why XXY cells cannot carry out meiosis (8). The considerable overlap between the testicular pathologies in trisomies caused either by an additional X chromosome or by an extra 21 suggests that this may be consequent to pairing problems in pachytene of a supernumerary chromosome rather than specific for X-chromosome disomy (9). Thus, the excess of disomic XY spermatozoa observed in XXY men (10) and in XXY mice (11) may be the result of mis-segregation in XY spermatocytes or of selection against XXY spermatocytes bearing the XX bivalent.

On the other hand, patients with a supernumerary Y chromosome, although more frequently observed among infertile men than in newborn males (4), have a spermatogenic profile ranging from severe impairment to apparent normality as in the general population (5). Different explanations for "normal variability" were put forward. As suggested by early meiotic studies (12, 13), XYY spermatogonia tend to lose the supernumerary Y chromosome before or during meiosis thus ensuring the correct continuation of the meiotic process. Alternatively, XYY cells enter meiosis preferentially forming a YY bivalent plus an X univalent or forming an XY bivalent plus a Y univalent (10); both conditions, by not interfering with sex vescicle formation and X inactivation, allow meiosis to proceed normally.

With regard to the different anomalies of chromosome structure correlated to spermatogenic impairment, the data available show that they share the ability to cause disturbance in the spermatogenic process. This possibly occurs through contacts between asynapsed active autosomal material and sex chromosomal chromatin; as a result, interference in (meiotic? X chromosome?) function should determine spermatogenesis breakdown (6). Rearranged chromosomes that can form a meiotic asymmetrical multivalent have a high probability of being asynapsed; Robertsonian translocations are rearrangements at special risk – 8.5 fold more frequent in infertile males than in newborns (4) – and reciprocal translocations involving one acrocentric chromosome. In the latter, the nucleolus organizing region (NOR)-carrying heterochromatic short arm has a high probability of being asynapsed, remains transcriptionally active during meiosis and shows contacts with the X-Y bivalent in a high proportion of the translocation carriers spermatocytes (13-15). Also the accessory marker chromosomes formed by acrocentric short arms – 5-fold more frequent in infertile males than in newborns (4) – presumably affect spermatogenesis during pachytene by interfering with X-chromosome inactivation through contacts between the sex vescicle and NOR-carrying short arms (16). Finally, autosomal inversions – 8-fold more frequent in infertile males than in newborns (4) – can be correlated to spermatogenic impairment taking into account the meiotic behavior of the inversion bivalent. In fact, small inverted segments may be asynapsed because of the difficulty in loop-forming, whereas large inverted segments may form loops that are not properly synapsed. In addition, the heterochromatic blocks possibly present in the inverted segment are usually delayed in their pairing, and this may have a particular disruptive effect on the alignment and synapsis of adjacent euchromatic segments (17). In this respect, pericentric inversion involving only the heterochromatin of chromosomes 1 and 9 can represent a particular risk for male infertility. This is true for chromosome 1 inversions, regardless of the breakpoint positions (8, 18), but not for chromosome 9 inversions. 9ph is common in the general population and is considered a "normal variant", although contradictory data are reported about its effect on reproductive fitness in males (19-21). One explanation could be that inverted chromosome 9 is likely to have different breakpoints and/or heterochromatic blocks of different size (22). In this respect, chromosomal regions made of constitutive heterochromatin may play a relevant role. As recently pointed out by investigations in other species, such as *Drosophila melanogaster*, heterochromatin has a wide range of actions influencing chromosome pairing and segregation, together with gene expression and nuclear organization (23). Therefore, variations in amount/position of heterochromatin play a role in disturbing synapsis of the adjacent regions (17).

OUR EXPERIENCE

In order to investigate the role of chromosomal abnormalities in male infertility, we performed a study in a selected group of 333 infertile patients, male partners of infertile couples living in Rome and surrounding areas, attending the Outpatients Department of our Seminology Unit. Patients underwent clinical andrological examination, seminal analysis and laboratory screening for hormones (FSH, LH, PRL and

Table 1. Karyotypes, mean±SD of age and sperm parameters of the 269 infertile non-azoospermic patients.

Karyotypes	Patients (no.)	Age (years)	Conc./ml (no.x10^6)	Forward motility (%)	Atypical forms (%)
Normal	231	33.3±6.1	17.7±18.6	16.0±14.0	75.0±16.8
Abnormal	26	30.3±4.3	10.5±12.6	13.3±15.0	81.7±16.7
Variant	12	32.4±7.2	26.9±27.1	22.5±20.5	67.3±23.6

T). The selection criteria for cytogenetic studies based upon seminal analyses were the presence of at least one of the following conditions: azoospermia; severe oligozoospermia (sperm concentration <5x10^6/ml); oligozoospermia (sperm concentration 5-20x10^6/ml) and normozoospermia (sperm concentration >20x-10^6/ml) with failure to fertilize the partner's oocytes in an IVF program (at least 4 oocytes, all classified as mature, unsuccessfully inseminated).

Semen analysis

Semen analyses were carried out according to the World Health Organization standards (24). All the semen samples were analyzed by the same biologists without any knowledge of the karyotype. The following variables were used for this study: sperm concentration (no.x10^6/ml), total and forward sperm motility (%), sperm morphology (% atypical forms). Several sub-classes were identified on the basis of the sperm parameters; 5 sub-classes for sperm concentration: a) azoospermia; b) <5x10^6/ml; c) 5-10x10^6/ml; d) 11-19x10^6/ml; e) ≥20x10^6/ml; 4 subclasses for sperm forward motility: a) <10%; b) 10-24%; c) 25-50%; d) >50%; and 4 subclasses for atypical forms: a) 100-91%; b) 90-81%; c) 80-70%; d) <70%.

Cytogenetic studies

Peripheral lymphocytes obtained from infertile males were cultured in RPMI 1640 supplemented with 10% fetal calf serum, penicillin, streptomycin and 0.1 ml of phytohemagglutinin (PHA) M (Murex Biotech). Metaphase chromosomes were prepared from PHA-stimulated lymphocyte cultures. Chromosome preparations were carried out according to standard protocols. Three cultures were set up for each patient. Chromosome identification was performed by R banding. For this purpose bromodeoxyuridine (Budr) (final concentration 10^{-3} M) was added to cultures 6 hours before harvesting and chromosome preparations were stained with Giemsa (RBG) or Acridine Orange (RBA).

Differential stainings for active NORs was performed by Ag-I. CBG banding was used when variations of amount/position of constitutive heterochromatin were suspected. The heteromorphism extent of the constitutive heterochromatin of chromosomes 1, 9, 16 and Y were evaluated according to Hsu *et al*. (25).

Statistical analysis of data was performed by Student *t* test or by χ^2 test (RxC Table).

Results and comments

In our group of 333 patients 42 had an abnormal karyotype and 17 had a "variant" karyotype, *i.e.* karyotype showing variations of chromosome structure usually referred to as "normal" in the literature,

Table 2. Hormonal data of the infertile males studied.

	Normal karyotype				Abnormal karyotype				Variant karyotype			
Sperm conc./ml no.x10^6	FSH mU/ml	LH mU/ml	T mg/ml	PRL ng/ml	FSH mU/ml	LH mU/ml	T mg/ml	PRL ng/ml	FSH mU/ml	LH mU/ml	T ng/ml	PRL ng/ml
0	18.2±10.8	13.2±8.8	5.6±1.9	9.3±9.5	25.8±11.5	15.6±9.0	3.0±3.0	9.7±3.8	12.6±11.5	7.1±4.9	5.0±2.5	11.7±3.8
<5	11.3±6.1	7.5±4.8	6.7±3.7	10.2±4.3	10.2±4.2	8.9±4.6	5.4±1.3	8.9±4.9	8.6±8.1	7.3±8.1	4.9±1.7	6.3±1.1
5-10	9.0±4.5	6.7±4.1	6.6±2.5	6.9±6.2	6.7±0.8	6.7±1.1	7.1±2.4	5.7±2.7	-	-	-	-
11-19	6.0±2.8	4.7±1.9	6.4±3.2	6.5±2.9	10.6±8.0	6.6±1.9	6.9±329	8.5±3.0	17.0*	14.0*	4.2*	7.9*
≥20	5.5±2.7	4.6±2.2	6.5±15.7	9.3±22.1	5.3±1.7	5.5±1.6	5.3±2.4	7.1±2.4	5.9±1.6	6.1±1.9	4.8±1.5	9.4±2.6

Values are mean±SD; *class represented by a single subject.

Table 3 - Seminal profiles and karyotypes of interest.

Patient	Karyotipe	Sperm conc. (x10^6/ml)	Total motility (%)	Forward motility (%)	Abnormal morphology (%)
X-chromosome abnormalities					
15 patients	47,XYY	0	-	-	-
Y-chromosome abnormalities					
C.L.	47,XYY	0.5	0	0	100
D.V.	47,XYY	1.5	20	10	70
S.G.	47,XYY	15	50	20	73
A.R.	46,X,del(Y)(q12)	3	0	0	88
Robertsonian translocation					
D.P.*	45,der(13;14)(q10;q10)	0.4	0	0	100
D.F.	45,der(13;14)(q10;q10)	1	5	0	100
T.A.	45,der(13;15)(q10;q10)	1.5	10	5	85
V.G.	45,der(13;14)(q10;q10)	6	25	10	70
R.M.	45,der(13;14)(q10;q10)	12	30	20	78
R.R.	45,der(13;14)(q10;q10)	13	0	0	95
M.S.**	45,der(13;14)(q10;q10)	15	20	10	95
G.L.	45,der(14;21)(q10;q10)	18	35	20	73
M.A.**	45,der(13;14)(q10;q10)	25	50	40	60
D.A.	45,der(13;14)(q10;q10)	25	35	20	78
Reciprocal translocations					
P.R.	46,t(12;22)(q13;p11)	0	-	-	-
P.G.	46,t(1;7)(q44;q12.2)	0.1	0	0	100
M.P.	46,t(14;20)(p10;q10)	0.1	0	0	100
N.M.**	46,t(2;3)(p12;q26)	0.7	0	0	100
T.Y.	46,t(9;17)(p12;q24)	8	30	10	82
A.A	46,t(1;2)(p22;q23)	55	55	55	40
Supernumerary bisatellited marker chr.					
L.F.	47,+mar	0.5	0	0	100
P.L.*	47,+mar	9	25	15	68
T.C.	47,+mar	9	30	30	75
S.M.	47,+mar	22	45	40	56
Pericentric inversion					
G.A.°	46,inv(1)(p34q12)	0.5	0	0	100
Heterochromatin inversions					
D.Ma*	46,1ph	12	35	25	60
D.Mi*	46,1ph	15	25	15	78
C.T.	46.9ph	0	-	-	-
U.F.	46.9ph	0	-	-	-
M.D.	46.9ph	0.1	0	0	100
R.G.	46.9ph	1	30	20	7
C.Mau.*	46.9ph	2	20	10	87
F.S.	46.9ph	4	25	15	90
M.M.	46.9ph	12	10	5	79
C.Mas.*	46.9ph	27	55	55	48
P.S.	46.9ph	80	50	50	55

*Maternally inherited; **paternally inherited; °*de novo.*

such as Yqh+, 9ph, double satellites on the short arm of an acrocentric chromosome. The frequency of each type of variant chromosome in our infertile group was not significantly different from that observed in unselected groups (26), with the exception of 9ph which is three times more frequent (19, 27). The frequency of azoospermia in our sample was 19.2% (15.7%, 38% and 29.4% respectively among men with normal, abnormal and variant karyotype). As expected, most (94%) of the azoospermic patients with abnormal karyotype are Klinefelter patients.

Table 1 shows the seminal parameters of the non-azoospermic patients grouped according to their karyotype: normal, abnormal and variant. The statistical analysis of the data by the Student *t* test showed that a significant difference in sperm concentration exists between patients with normal and abnormal karyotype. For a more detailed analysis, we evaluated the distribution of patients belonging to the 3 groups in different sub-classes of seminal parameters (see *Semen analysis*). The results showed that chromosomal variants have only a minimal influence on seminal parameters (and this only on the worst class of sperm concentration), whilst chromosomal abnormalities play a negative role in sperm concentration and morphology, but have no impact on sperm motility: 1) 42% of males with abnormal karyotype (*vs* 26% of those with normal karyotype) fall into the worst class sperm concentration (>0 and <5x10^6/ml); 2) 38% of males with abnormal karyotype (*vs* 19% of males with normal karyotype) fall into the worst class for atypical forms (100-91%); 3) only 17% of males with abnormal karyotype (*vs* 33% of men with normal karyotype) show both normal sperm concentration (>20x 10^6/ml) and normal rate of atypical forms (<70%); 4) 71% of infertile men with normal karyotype and 80% of infertile men with abnormal karyotype (not significantly different rates) show sperm motility lower than 25% .

Hormonal studies, as expected, showed an inverse correlation between gonadotropin concentration and sperm concentration (Table 2); the highest values were found in the azoospermic patients, especially in those affected by Klinefelter syndrome. These data show a correlation between spermatogenesis impairment and gonadotropins concentration, in particular in patients with chromosomal alterations, in agreement with Foresta *et al.* (28). Prolactin and testosterone values remained in the normal range.

To better correlate seminal parameters to the different chromosome constitutions, we grouped the infertile males showing similar chromosome abnormalities (Table 3). We also included heterochromatin inversions of chromosome 9, because of their still debated role in male infertility. The main considerations we can draw are:

1) Robertsonian translocations are the most frequent autosomal rearrangement in our group (3%), whereas it is rare in the general population (0.07-0.22%) (29). Independently of the chromosomes involved, the majority of the patients showed abnormal seminal parameters with individual differences that may depend on different breakpoints and/or different genetic background (30);
2) the 6 patients carrying reciprocal translocations had different seminal characteristics, ranging from normality to azoospermia. This correlates well with the expected meiotic behavior of the chromosomes involved in each translocation based on the breakpoint localizations. Three examples are shown in Figure 1. Indeed the t (1;2) carrier is normozoospermic whereas the (1;7) carrier showed the worst seminal profile of the translocation carriers according to the shortness of one arm in the quadrivalent configuration expected in patient meiosis;

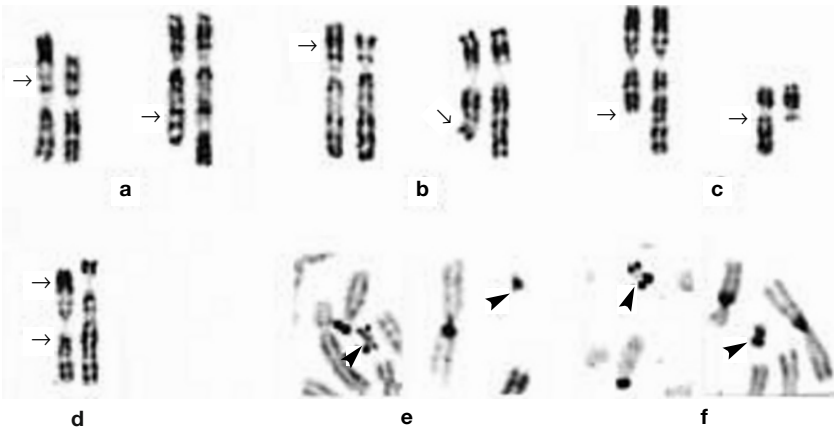

Fig. 1 - *Examples of chromosomal rearrangements associated with different degrees of seminal impairment. a, b, c: the reciprocal translocations observed respectively in the patients A.A., N.M. and P.G. The arrows indicate the breakpoints; d: the pericentric inversion of chromosome 1 observed in the patient G.A. The arrows indicate the breakpoints; e, f: the supernumerary marker chromosomes observed respectively in the patients S.M. and P.L. Both markers are bisatellited (see the specific staining of NORs, on the left of each figure), but show different amount of pericentromeric heterochromatin (see C-positive regions, on the right of each figure). The arrows indicate the marker chromosomes.*

3) of the 4 supernumerary bisatellited marker chromosomes studied, one (patient S.M.) showed a single centromeric C-band and the other three had a bipartite centromeric C-band. According to the classification proposed by Steinbach *et al.* (31), they are presumed to be without phenotipic effects, but all were carried by infertile men. In particular, the presence of the marker characterized by a single centromeric C-band is associated with the less severe spermatogenic impairment (Fig. 1);
4) 7 of the 9 patiens carrying the inversions that involve the pericentromeric heterochromatin of chromosome 9 show azoospermia or severe oligoasthenoteratozoospermia, although 9ph is referred to as variant in the literature.

In conclusion, the incidence of chromosomal anomalies among the infertile men in this series (7.8%) strongly suggests that cytogenetic screening should be a part of preparatory evaluation prior to every kind of assisted reproduction. Therefore, constitutional chromosome analysis is to be recommended, above all prior to ICSI, in which selective hurdles eliminating abnormal germ cells are bypassed. This kind of investigation is a pre-requisite for evaluating sperm aneuploidy frequency in order to avoid unexplained failures and to minimize the risk of transmitting chromosomal abnormalities potentially responsible for increased male infertility and even for multiple congenital anomalies of the conceptus. Therefore, close cooperation between andrologists and cytogenetists could ensure good selection of the infertile male at risk undoubtedly resulting in a positive cost/benefit ratio (32, 33).

ACKNOWLEDGMENTS

This work was partially supported by a grant of "La Sapienza" University, Rome. We are grateful to M. Proietti and L. Verni for their skilful technical participation in this work.

REFERENCES

1. Johnson M.D.
Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: recommendations for genetic counseling and screening.
Fertil. Steril.1998, *70:* 397-411.
2. Bahsin S., de Krestser D.M., Baker H.W.
Clinical review 64: pathophysiology and natural hystory of male infertility.
J. Clin. Endocrinol. Metab. 1994, *79:* 1525-1529.
3. Chandley A.C.
Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility.
Hum. Reprod. 1998, *13:* 45-50.
4. Van Assche E., Bonduelle M., Tournaye H., Joris H., Verheyen G., Devroey P., Van Stirteghem A., Liebaers I.
Cytogenetics of infertile men.
Hum. Reprod. 1996, *11* (Suppl. 4): 1-26.
5. Egozcue S., Blanco J., Vendrell J.M., Garcia F., Veiga A., Aran B., Barri P.N., Vidal F., Egozcue J.
Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion.
Hum. Reprod. Update 2000, *6:* 93-105.
6. Lifschytz E., Lindsley D.L.
The role of X-chromosome inactivation during spermatogenesis.
Proc. Natl. Acad. Sci. USA 1972, *69:* 182-186.
7. Odorisio T., Rodriguez T.A., Evans E.P., Clarke A.R., Burgoyne P.S.
The meiotic checkpoint monitoring synapsis eliminates spermatocytes via p53-independent apoptosis.
Nat. Genet. 1998, *18:* 257-2661.
8. Vogt P.H.
Molecular basis of male (in)fertility.
Int. J. Androl. 1997, *20* (Suppl. 3): 2-10.
9. Mittwoch U.
Sex determination and sex reversal: genotype, phenotype, dogma and semantics.
Hum. Genet. 1992, *89:* 467-479.
10. Blanco J., Rubio C., Simon C., Egozcue J., Vidal F.
Increased incidence of disomic sperm nuclei in a 47,XYY male assessed by fluorescent in situ hybridization (FISH).
Hum. Genet. 1997, *99:* 413-416.
11. Mroz K., Hassold T.J., Hunt P.A.
Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors.
Hum. Reprod. 1999, *14:* 1151-1156.
12. Burgoyne P.S.
Evidence for an association between univalent Y chromosome and spermatocyte loss in XYY mice and men. Cytogenet. Cell Genet. 1979, *23:* 84-89.
13. Gabriel-Robez O., Rumpler Y.
The meiotic pairing behaviour in human spermatocytes carrier of chromosome anomalies and their repercussions on reproductive fitness. II. Robertsonian and reciprocal translocations. A European collaborative study.
Ann. Genet. 1996, *39:* 17-25.
14. Rosenmann A., Wahrman J., Richler C., Voss R., Persitz A., Goldman B.
Meiotic association between the XY chromosomes and unpaired autosomal elements as a cause of human male sterility.
Cytogenet. Cell Genet. 1985, *39:* 19-29.
15. Yu W.R., Gabriel-Robez O., Croquette M.F., Rigot J.M., Rumpler Y.

X-Y quadrivalent association and sterility in a man carrier of a reciprocal autosomal translocation involving the whole arm of an acrocentric chromosome t(2;15)(q21,3;cen).
Andrologia 1995, *27:* 171-174.

16. Jaafar H., Gabriel-Robez O., Vignon F., Flori E., Rumpler Y.
Supernumerary chromosomes and spermatogenesis in a human male carrier.
Hum. Genet. 1994, *94:* 74-76.
17. Batanian J., Hulten M.A.
Electron microscopic investigations of synaptonemal complexes in an infertile human male carrier of a pericentric inversion inv(1) (p32q42). Regular loop formation but defective synapsis including a possible interchromosomal effect.
Hum. Genet. 1987, *76:* 81-89.
18. Chandley A.C., McBeath S., Speed R.M., Yorston L., Hargreave T.D.
Pericentric inversion in human chromosome 1 and the risk for male sterility.
J. Med. Genet. 1987, *24:* 325-334.
19. Yamada K.
Population studies of INV(9) chromosomes in 4,300 Japanese: incidence, sex difference and clinical significance.
Jpn. J. Hum. Genet. 1992, *37:* 293-301.
20. Teo S.H., Tan M., Knight L., Yeo S.H., Ng I.
Pericentric inversion 9: incidence and clinical significance.
Ann. Acad. Med. Singapore 1995, *24:* 302-304.
21. Kleiman S.E., Yogev L., Gamzu R., Hauser R., Botchan A., Lessing J.B., Paz G., Yavetz H.
Genetic evaluation of infertile men.
Hum. Reprod. 1999, 14: 33-38.
22. Samonte R.V., Conte R.A., Ramesh K.H., Verma R.S.
Molecular cytogenetic characterization of breakpoints involving pericentric inversions of human chromosome 9.
Hum. Genet. 1996, 98: 576-580.
23. Wakimoto B.
The spreading influence of heterochromatin.
Trends Genet. 1997, 13: 349.
24. World Health Organization.
Laboratory manual for the examination of human semen and semen-cervical mucus interaction.
Cambridge University Press, Cambridge, UK, 2000.
25. Hsu L.Y., Benn P.A., Tannenbaum H.L., Perlis T.E., Carlson A.D.
Chromosomal polymorphisms of 1, 9, 16, and Y in 4 major ethnic groups: a large prenatal study.
Am. J. Med. Genet. 1987, *26:* 95-101.
26. Walzer S., Gerald P.S.
A chromosome survey of 13751 consecutive male newborns.
In: Hook E.B., Porter I.H. (Eds.), Population cytogenetics: studies in human.
Academic Press, New York, 1977, pp. 45-61.
27. Colls P., Blanco J., Martinez-Pasarell O., Vidal F., Egozcue J., Marquez C., Guitart M., Templado C.
Chromosome segregation in a man heterozygous for a pericentric inversion, inv(9)(p11q13), analyzed by using sperm karyotyping and two-color fluorescence in situ hybridization on sperm nuclei.
Hum. Genet. 1997, *99:* 761-765.
28. Foresta C., Galeazzi C., Bettella A., Stella M., Scandellari C.
High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome.
J. Clin. Endocrinol. Metab. 1998, 83: 203-205.
29. Tawn E.J., Earl R.
The frequencies of constitutional chromosome abnormalities in an apparently normal adult population.
Mutat. Res. 1992, *283:* 69-73.
30. Page S.L., Shin J-C., Han J-Y., Choo K.H.A., Shaffer L.G.
Breakpoint diversity illustrates distinct mechanisms for Robertsonian translocation formation.
Hum. Mol. Genet. 1996, *5:* 1279-1288.
31. Steinbach P., Djalali M., Hansmann I., Kattner E., Meisel-Stosiek M., Probeck H.D., Schmidt A., Wolf M.
The genetic significance of accessory bisatellited marker chromosomes.
Hum. Genet. 1983, *65:* 155-164.
32. Pandiyan N., Jequier A.M.
Mitotic chromosomal anomalies among 1210 infertile men.
Hum.Reprod. 1996, *11:* 2604-2608.
33. Bourrouillou J., Calvas P., Bujan L., Mieusset R., Mansat A., Pontonnier F.
Mitotic chromosomal anomalies among infertile men.
Hum. Reprod. 1997, *12:* 2337-2338.