Small Supernumerary Marker Chromosomes in Human Infertility

Narjes Armanet^{a, b} Lucie Tosca^{a, b} Sophie Brisset^{a, b} Thomas Liehr^c
Gérard Tachdjian^{a, b}

^aService d'Histologie, Embryologie et Cytogénétique, Hôpital Antoine Bécère, Hôpitaux Universitaires Paris-Sud, Clamart, and ^bUniversité Paris-Sud, Le Kremlin-Bicêtre, France; ^cInstitute of Human Genetics, Jena University Hospital, Friedrich Schiller University, Jena, Germany

Key Words

FISH · Infertility · Microarray analysis · Small supernumerary marker chromosomes

Abstract

Small supernumerary marker chromosomes (sSMC) are structurally abnormal chromosomes that cannot be unambiguously identified by banding cytogenetics. The objective of this study was to provide an overview of sSMC frequency and characterization in a context of infertility and to review the literature describing sSMC in relation with male and female infertility. Therefore, a systematic literature review on sSMC associated with infertility was conducted by means of a PubMed literature and a sSMC database (<http://ssmc-tl.com/smc.html>) search. A total of 234 patients with infertility were identified as carriers of sSMC. All chromosomes, except chromosomes 10, 19 and the X, were involved in sSMC, and in 72% the sSMC originated from acrocentric chromosomes. Euchromatic imbalances were caused by the presence of sSMC in 30% of the cases. Putative genes have been identified in only 1.2% of sSMC associated with infertility.

The implication of sSMC in infertility could be due to a partial trisomy of some genes but also to mechanical effects perturbing meiosis. Further precise molecular and interphase-architecture studies on sSMC are needed in the future to characterize the relationship between this chromosomal anomaly and human infertility. © 2015 S. Karger AG, Basel

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [Zegers-Hochschild et al., 2014]. In about 22–28% of couples the cause of infertility remains unexplained [Kamath and Bhattacharya, 2012]. Patients with infertility are known to be a clinically heterogeneous group, as a variety of factors can influence fertility. Those can be hormonal, immunological, psychological, anatomical (internal or external genital malformation), related to age, exercise, obesity, infectious disease, resulting from surgery, or be associated with defined abnormalities in the gametes, for example aberrant semen param-

eters [Shah et al., 2003]. It is difficult to accurately assess the genetic contribution to reduced fertility as most, if not all, of the above factors are likely to have a genetic component. Nevertheless, specific genotypes and karyotypes have been associated with infertility phenotypes, and studies of specific genes in humans and model systems defined the nature of the polygenic and multifactorial basis of infertility. Infertility phenotypes have been associated with specific genetic conditions, such as mutations in the cystic fibrosis (*CFTR*) gene, mutations or microdeletions in specific Y chromosome genes, or the presence of constitutional numerical or structural chromosomal aberrations [Shah et al., 2003]. The latter, such as sex chromosome aberrations, and the presence of constitutional inversions, translocations, or small supernumerary marker chromosomes (sSMC) [Liehr et al., 2004] can lead both to infertility and repeated abortions [Shah et al., 2003].

sSMC are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by banding cytogenetics alone. They are equal in size to or smaller than a chromosome 20 of the same metaphase spread [Liehr, 2011]. sSMC are present in 0.075% of unselected prenatal individuals and in 0.044% of consecutively studied postnatal ones [Liehr and Weise, 2007]. The apparently reduced rate might be in part connected to the fact that 30–50% of pregnancies with a sSMC detected are terminated [Warburton, 1991; Kumar et al., 1997; Cavani et al., 2003]. In developmentally retarded patients, the sSMC rate is elevated to 0.288%. The frequency of sSMC in individuals presenting infertility is 0.125% [Liehr, 2011]. Distinguishing male from female, there seems to be a 7.5:1 difference in the sSMC frequency for this special group [Liehr and Weise, 2007]. It is known that sSMC may lead to reduced fertility in males without additional clinical sSMC-related symptoms [Chandley et al., 1975; Manvelyan et al., 2008a]. It is also currently thought that spermatogenesis impairment due to the presence of chromosomal abnormalities including sSMC is the main cause of oligoasthenozoospermia (OAT) [Mau et al., 1997; Koç et al., 2009]. However, little is really known about the mechanism causing this kind of infertility.

By a systematic literature search on sSMC associated with infertility, we reviewed the cases of patients presenting sSMC linked with male or female reproduction difficulties and discussed the relationship between sSMC and spermatogenesis impairment, implantation difficulties, repeated abortions, and other reproductive disorders.

Materials and Methods

This review focuses on patients presenting sSMC associated with male or female reproduction difficulties. Therefore, a systematic literature search on sSMC associated with infertility was conducted by means of a PubMed literature search (using relevant terms and their combinations, e.g. sSMC, infertility, array-CGH, FISH, reproduction, abortion, miscarriage, OAT) and the sSMC database [Liehr, 2015]. The period of our literature search spanned from March 2013 to December 2014 and included peer reviewed publications and congress abstracts. Studies analyzing sSMC and discussing the relationship of sSMC and spermatogenesis impairment, implantation difficulties, repeated abortions, and other reproductive disorders were considered. All cases were characterized for their chromosomal origin and genetic content by FISH and for 21 cases by microarray analysis.

Results

The results obtained in the 234 sSMC cases with infertility are summarized in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000438718). All cases were characterized for their chromosomal origin and genetic content by FISH and for 21 cases by array-CGH. All human chromosomes, except chromosomes 10, 19, and X, were involved in sSMC (fig. 1). The majority (72%) of sSMC derived from an acrocentric chromosome (170 cases), and 53% of sSMC originated from chromosome 14 or 15 (125 cases). The reasons for which cytogenetic studies were performed in the 234 patients are listed by case in online supplementary table 1. They are also summarized in figure 2 where the cases are divided into 4 groups and by gender as follows: (a) unexplained infertility, comprising patients for which no etiology was found or for which details on the symptoms are unavailable; (b) OAT/others, comprising males with OAT or similar reasons of infertility associated with numerically and/or morphologically altered sperm; (c) RAB, comprising patients with repeated abortions, and (d) amenorrhea, including females suffering from primary or secondary amenorrhea or similar clinical conditions like premature ovarian failure (POF).

Overall, the most common indication was unexplained infertility, followed by RAB and amenorrhea in females and OAT/others in males. All kind of infertilities are more likely to appear when the sSMC originates from an acrocentric chromosome.

The parental origin incidence of sSMC in cases with infertility is summarized in figure 3. The relevant information was available for 41 of the 234 cases of online

Fig. 1. Chromosomal distribution of sSMC in 234 infertile patients depicted by gender, based on the data of on-line supplementary table 1.

Fig. 2. Distribution of causes of infertility in the 234 patients with sSMC divided by gender, based on the data of online supplementary table 1. OAT = Oligoasthenoteratozoospermia; RAB = repeated abortions.

Table 1. Overview of the 8 cases that were precisely characterized by microarray analysis

Study	Karyotype	sSMC	Array analysis	Chromosomal regions and DNA positions	Number of genes	Fertility problem
Sheth et al. [2011] (case P-1)	47,XX,+mar(100%)	sSMC(3)	no imbalance detected, 44K oligonucleotide	3p11.2q11.1	0	repeated abortions
Guediche et al. [2012a] (case 3)	47,XX,+mar(100%)	sSMC(15)	no imbalance detected, 180K oligonucleotide	15q11.1	0	primary infertility
Bertini et al. [2012]	47,XX,+mar(100%)	sSMC(15)	no imbalance detected, 44K oligonucleotide	15q11.1	0	POF
Guediche et al. [2012a] (cases 1+2)	47,XY,+mar(100%) and 37% in sperm	sSMC(15)	gain of 3.6 Mb, 105K oligonucleotide	15q11.2 20,102,341–23,699,901	18	OAT
Baldwin et al. [2008] (case 18)	47,XX,+mar(80%)	sSMC(18)	no imbalance detected, 44K oligonucleotide	18p11.21q11.1	0	not specified
Guediche et al. [2012a] (case 4)	47,XX,+mar(100%)	sSMC(21)	gain of 0.266 Mb, 180K oligonucleotide	21p11.1 10,866,741–11,133,106	6	POF
Guediche et al. [2012b]	48,XX,+mar1,+mar2 [24]/47,XX,+mar1 or 2[5]/46,XX[3]	sSMC(6) and sSMC(20)	gains of 9 Mb on sSMC(6) and 3.3 Mb on sSMC(20), 244K oligonucleotide	6p11.2q12 57,354,689–66,400,962 20p11.21q11.1 22,833,806–26,156,226	42	repeated abortions
Dutta et al. [2014]	47,XY,+mar(100%)	sSMC(22)	no imbalance detected, 300K SNP array	22q11.1	0	repeated abortions

OAT = Oligoasthenoteratozoospermia; POF = premature ovarian failure.

Fig. 3. Parental origin of sSMC in the cases listed in online supplementary table 1, provided that information was available.

supplementary table 1. Unexpectedly, 48.8% of the sSMC detected that are linked with fertility problems were inherited. Maternally derived sSMC occurred 2.4 times more frequently than paternally derived sSMC (fig. 3). A total of 88.2% of inherited sSMC were derived from an acrocentric chromosome (online suppl. table 1). In so-called familial cases the sSMC was proven to have been carried through more than one generation, but neither information on paternal nor maternal passage was provided.

Among the 234 cases of sSMC related to infertility, 138 were published in PubMed, and among them, 8 were precisely characterized by microarray analysis with the description of the chromosomal breakpoints and the genes included (table 1). After microarray analysis exploration in 5 cases, neither genomic imbalance nor genes were found in the studied sSMC [Baldwin et al., 2008; Sheth et al., 2011; Bertini et al., 2012; Guediche et al., 2012a; Dutta et al., 2014]. For the 3 others cases microarray analysis showed genomic imbalances and highlighted genes mapped on the sSMC [Guediche et al., 2012a, b] (table 1).

So far, sSMC meiosis segregation in the sperm of carriers has been studied in 10 males [Martin et al., 1986; Mennicke et al., 1997; Cotter et al., 2000; Wiland et al., 2005; Paetzold et al., 2006; Oracova et al., 2009; Guediche et al., 2012a; Perrin et al., 2012]. These previous studies concerned mainly sSMC(15) and showed a frequency of sperm nuclei containing sSMC varying from 6 to 50%.

Discussion

In this review, we summarized studies concerning sSMC associated with infertility, including spermatogenesis impairment, amenorrhea, POF, implantation difficulties, and repeated abortions.

To our knowledge, more than 5,500 cases of sSMC have been previously described and analyzed by banding cytogenetic analysis and FISH [Liehr, 2015], and 234 concern sSMC associated with infertility in otherwise clinically healthy persons. Among these studies, only 8 have studied the size, chromosomal regions, and genes involved by microarray analysis [Liehr, 2015].

Frequency and Chromosomal Origin of sSMC in Infertile Patients

Studies showed that the frequency of sSMC detected in infertile patients is higher than in the general population (0.125 vs. 0.043%). This frequency is also different between male (0.165%) and female infertility (0.022%) [Liehr and Weise, 2007]. In this present review, 210 new cases have been added since the publication by Liehr and Weise [2007]. Among the 234 patients in our review, 85 are females (36%) and 149 are males (64%) (online suppl. table 1). Based on the 234 cases reported, an involvement of 21 of the 24 human chromosomes has been described in connection with infertility and sSMC formation. We found that 72% (170 cases) of sSMC detected in association with infertility derived from an acrocentric chromosome. This rate is also similar for the overall population (~70% according to Liehr [2015]). sSMC(15) has often been reported at an increased incidence in infertile males with oligo- or azoospermia, suggesting a causal effect [Cotter et al., 2000]. Consequently, it was not unexpected that sSMC(15) was the most frequently sSMC observed (fig. 1).

Parental Origin of the sSMC

In the 234 cases, 51.2% (21 cases, the relevant information was available for 41 patients) of sSMC were described as de novo (fig. 3). Among the inherited sSMC (17 cases),

the maternal transmission is more frequent than the paternal one [70% (12 cases) vs. 30% (5 cases)]. The preferentially maternal transmitting mode suggests either a reduced fertility in male carriers or that the sSMC is excluded during spermatogenesis. Moreover, unpaired chromatin potentially causes a meiotic arrest during spermatogenesis that is less marked in females [Reinholdt et al., 2009].

A tendency towards gender-specific differences in the offspring has also been previously described: a maternally inherited sSMC is more likely to lead to fertility problems in sons, while a paternally inherited sSMC is more likely to lead to infertility in daughters [Manvelyan et al., 2008a]. This was confirmed by the present review as the majority of sSMC detected in females were paternally transmitted and the majority of sSMC detected in males were maternally transmitted.

sSMC and Uniparental Disomy in Infertility

A combination of sSMC and uniparental disomy (UPD) is rarely encountered, especially in a context of infertility. According to the Liehr database, only 46 sSMC cases with UPD are reported [Liehr, 2015], which are not associated with infertility. Nevertheless, UPD has to be considered especially in prenatal cases with sSMC. Every sSMC, irrespective of its chromosomal origin, may be principally connected with UPD. UPD of chromosomes 6, 7, 14, 15, 16, and 20 is most often reported. Maternal UPD is approximately 9 times more frequent than paternal UPD, and UPD in connection with a parentally inherited sSMC is, if existent, a rare event. The gender type and shape of sSMC have no effect on UPD formation [Liehr et al., 2011].

Meiosis Studies

During meiosis, a 1:1 segregation ratio for the sSMC would be predicted. Studying meiosis segregation in the sperm of sSMC carriers allows evaluating this theoretical expectation. So far, sSMC segregation in meiosis has been studied by FISH in 10 males [Martin et al., 1986; Mennicke et al., 1997; Cotter et al., 2000; Wiland et al., 2005; Paetzold et al., 2006; Oracova et al., 2009; Guediche et al., 2012a; Perrin et al., 2012]. All these studies showed a significantly increased frequency of sperm aneuploidy due to sSMC compared to control donors.

Martin et al. [1986] found in 2 fertile males that the frequency of spermatozoa with sSMC was not significantly different from 50%. Similarly, Mennicke et al. [1997] found the sSMC in approximately 50% of spermatozoa in a male carrying a sSMC(15). Four other studies

on meiotic segregation of sSMC(15) showed a lower proportion (from 6 to 37%) of spermatozoa with sSMC(15) [Cotter et al., 2000; Paetzold et al. 2006; Oracova et al., 2009; Guediche et al., 2012a]. Wiland et al. [2005] detected 8.25% of spermatozoa with sSMC in a male having a mosaicism for this sSMC in 4% of his lymphocytes. These data indicate an important variability in the segregation of sSMC during meiosis. The inter-individual variations observed probably depend on the origin and size of the sSMC, the variable proportion of normal/abnormal cells in lymphocytes, and the varying amounts of euchromatin in the sSMC. The addition of all these factors to some form of selection process against the sSMC during spermatogenesis makes adequate comparisons of patients very difficult. The reason why heterochromatic sSMC cause infertility is still debated. The chromosomal alteration does not cause the phenotypic effects through the overexpression or the silencing of a gene/s which is disrupted in the rearrangement, but the cytogenetic alteration per se causes the phenotype by a mechanical effect during meiosis. Moreover, the association between sSMC and a sexual vesicle could lead to meiosis arrest and produce severe spermatogenetic impairment, explaining oligo- and asthenozoospermia [Perrin et al., 2012].

Several early reports suggested the possibility of an interchromosomal effect resulting in an increase in chromosomal non-disjunction during meiosis associated with sSMC [Cotter et al., 2000]. Similarly, Martin et al. [1986] reported an increase in aneuploidy in sperm (sex chromosome aneuploidy and monosomies of chromosomes 18, 21, and 22) from a sSMC carrier. However, a previous review of the literature concluded that there was no increase in trisomic conceptions or miscarriages for sSMC carriers [Steinbach and Djalali, 1983]. Cotter et al. [2000] also examined the frequency of disomy 18 in the sperm of a sSMC(15) carrier. This frequency was not significantly higher from the control group or other published reports. Synaptonemal complex analysis of oligozoospermic sSMC carriers showed that the sSMC preferentially associated with the XY bivalent at meiosis [Jaafar et al., 1994]. Similar associations of the trivalent or quadrivalent (in Robertsonian or reciprocal translocation carriers) with the XY bivalent were observed in patients with infertility and spermatogenesis impairment. In fertile translocation carriers, such associations were less likely to occur [Cotter et al., 2000]. Even if oligozoospermia is also associated with sperm autosomal aneuploidy, whatever is the karyotype, the association of autosomal chromosomal material with the XY bivalent seems to correlate with impairment

of meiosis/spermatogenesis [Solari, 1999]. These suggestions are supported by a recent study that hypothesized that the presence of sSMC is associated with infertility by inducing an interchromosomal effect [Guediche et al., 2012a]. Indeed, some male carriers presented infertility whereas others showed normal fertility with 1:1 segregation of their sSMC. Three-dimensional interphase FISH studies in sperm of sSMC carriers might help elucidating the influence of nuclear architecture on fertility [Manvelyan et al., 2008b; Klein et al., 2012].

Mantzouratou et al. [2009] studied the meiotic and mitotic behavior of a ring/deleted chromosome 22 in human embryos determined by preimplantation genetic diagnosis (PGD) for a maternal carrier. Twelve embryos were analyzed, and the data showed that no embryos were completely normal or balanced for chromosome 22 by day 5. In this case, the ring chromosome is very stable in the mother as she is phenotypically normal and has the r(22) in all metaphases and interphases studied in her lymphocytes. The couple concerned in this case presents with a poor prognosis in terms of receiving a karyotypically normal child.

Genetic Content of sSMC in Infertile Patients

The varying degrees of phenotypic abnormalities observed are most probably due to the different DNA sequences of the sSMC. Numerical and structural chromosomal abnormalities are a common cause of human diseases including reproductive failure. They are traditionally identified by karyotyping, which has low resolution and limited ability to detect gains and losses of chromosomal material. The use of array-CGH allows the detection of chromosome abnormalities at a higher resolution [Lapierre and Tachdjian, 2005].

Only 3 cases of sSMC studied by microarray analysis and associated with male or female infertility showed genomic imbalances and highlighted duplicated genes (table 1). The first case presented a 3.6 Mb sSMC(15) associated with a severe OAT [Guediche et al., 2012a]. In this sSMC, 18 genes were mapped including the *POTEB* gene (*POTE* ankyrin domain family member B) that has been identified to be expressed in human testis, particularly in a specific cell type in spermatogenesis, the primary spermatocytes [Ise et al., 2008]. The high and specific expression of *POTEB* in primary spermatocytes, some of which are undergoing apoptosis, suggests a role in inducing programmed cell death, and the duplication of this gene could explain the semen alteration of this patient. He also has a brother that presented the same sSMC(15) with a duplication of *POTEB* without semen alteration. The po-

tential impact of sSMC on the predisposition to infertility depends also on others parameters, like the genetic background of the patients or if the sSMC is de novo or not.

The second case presented a POF at the age of 33 associated with a sSMC(21) [Guediche et al., 2012a]. Array-CGH data showed 6 duplicated genes including 5 genes belonging to the *BAGE* (B melanoma antigen) family which are expressed in some ovarian tumors [Zhang et al., 2010]. In the current study, mRNA expression showed that *BAGE* was not related to menopause [Zhang et al., 2010]. This patient did not present ovarian cancer, and the duplicated genes have not been described in association with POF.

The third case presented 2 sSMC associated with repeated abortions [Guediche et al., 2012b]. In the 3.3-Mb DNA fragment of sSMC(20), 35 genes were mapped. Among them, the *THBD* gene codes for thrombomodulin, an endothelial-associated anticoagulant protein involved in the control of hemostasis and inflammation at the vascular beds [Anastasiou et al., 2012]. This protein is also a cofactor of the protein C anticoagulant pathway which is expressed mainly on the endothelial surface of blood vessels and in placental syncytiotrophoblast cells [Stortoni et al., 2012] and therefore could be involved in the pathogenesis of pregnancy loss in this case.

Further studies by microarray analysis of the genes present in sSMC and their involvement in human infertility are needed to gain more information about their possible role in the observed symptoms.

Preimplantation Genetic Diagnosis for sSMC Associated with Infertility

The diagnosis of a sSMC, like that of any other chromosomal abnormality, has a profound impact on the whole family involved. In the case of infertility, the diagnosis of a sSMC must be evoked as one of the possible causes of the problems of the couple.

Preimplantation genetic diagnosis (PGD) is often used for selection of normal and balanced embryos for transfer during in vitro fertilization [Munne, 2002; Simopoulou et al., 2003; Munne, 2005; Otani et al., 2006]. Indeed, using appropriate specific probes, PGD can detect sSMC in preimplantation embryos from sSMC carriers as well as aneuploidy in the embryos.

Two studies reported the use of PGD for sSMC carriers. The first paper concerns a sSMC that was identified as a familial heterochromatic dicentric derivative of chromosome 15 in a phenotypically normal male presenting primary infertility [Oracova et al., 2009]. Investigations

of somatic cells combined with sperm analysis suggested genetic counseling of the couple. FISH analysis of sperm and blastomeres (PGD) was used to determine the segregation ratio of the sSMC and aneuploidy of chromosomes 13, 15, 16, 18, 21, 22, X, and Y. sSMC(15) was observed in 41% of preimplantation embryos, and the frequency of chromosomally normal embryos in PGD analysis was 40%. According to Oracova et al. [2009], it seems that the presence of sSMC(15) does not adversely affect the early development of the embryos' morphology.

The second study concerns a supernumerary ring chromosome 22 that derived from an interstitial deletion with one of the breaks occurring at the centromere [Mantzouratou et al., 2009]. Ring/del cases can be considered to form a special subgroup among sSMC. In the case of the ring/del situation, the additional material is compensated for by the deletion, and the phenotype is normal. The couple requested PGD following the birth of a son with a mosaic karyotype. In his lymphocytes, one cell line had a copy of the ring 22 chromosome in addition to the normal 46,XY complement while in other cells the ring has been lost. Twelve embryos were analyzed, and the data showed that no embryos were completely normal or balanced for chromosome 22 by day 5.

In cases of previous repeated abortions in a couple with a sSMC carrier, PGD can be considered. It can avoid the transfer of embryos carrying the sSMC, as its presence could probably be the reason of implantation failures or early embryo development perturbations. In other cases, when a member of a couple presents a sSMC associated with OAT or amenorrhea or when the infertility remains unexplained, the indication of a prenatal diagnosis in case of a pregnancy can be discussed. Considering the fact that the parent who carries the sSMC has a normal phenotype (except for infertility), the probability that the fetus presents abnormalities is low. Nevertheless, the counselor has to provide information about all known facts on sSMC and to consider the possible occurrence of additional chromosomal anomalies. Furthermore, sensitivity problems of microarray analyses in conjunction with low mosaicism rates and complex sSMC can be difficult for diagnosis. Also, resolution problems of some array platforms in the pericentromeric and boundary regions of heterochromatin can complicate analysis.

sSMC are frequently complex, and whole array-CGH characterization of the sSMC provides informed genetic counseling [Reddy et al., 2013]. For some cases, it can be speculated that the chromosomal aberration led to an even more imbalanced, and subsequently unviable, situation in the potential offspring than that present in the

sSMC carriers themselves. The parents need to be aware that their future child could also present the same infertility problems as they are. Finally, if appropriate, the problem of uniparental disomy should be discussed [Liehr et al., 2011].

In conclusion, the implication of sSMC in infertility could be due to partial trisomy of some genes but also to mechanical effects perturbing meiosis. The genes included in these regions have to be precisely identified as their presence on the sSMC can disrupt their expression. A single microarray analysis has the capacity to generate information allowing the rapid identification of the specific gene content of sSMC at a high resolution, therefore de-

fining the genotype-phenotype correlations associated with sSMC and infertility. Microarray analysis is also a precious tool for a precise and prudent genetic counseling as it informs on the sSMC content.

Further precise molecular and interphase-architecture studies on sSMC are needed in the future to characterize the relationship between this chromosomal anomaly and human infertility.

Acknowledgments

This work was supported in parts by the Else Kröner-Fresenius-Stiftung (2011_A42).

References

- Anastasiou G, Gialeraki A, Merkouri E, Politou M, Travlou A: Thrombomodulin as a regulator of the anticoagulant pathway: implication in the development of thrombosis. *Blood Coagul Fibrinolysis* 23:1–10 (2012).
- Baldwin EL, May LF, Justice AN, Martin CL, Ledbetter DH: Mechanisms and consequences of small supernumerary marker chromosomes: from Barbara McClintock to modern genetic-counseling issues. *Am J Hum Genet* 82:398–410 (2008).
- Bertini V, Viola D, Vitti P, Simi P, Valetto A: An idiopathic (15) associated with POF (premature ovarian failure): molecular cytogenetic definition of a case and review of the literature. *Gene* 503:123–125 (2012).
- Cavani S, Malcarne M, Arlanian A, Stagni L, Piombo G, et al: Prenatal and postnatal identification of 93 supernumerary small chromosomes. *Ann Genet* 46:231 (2003).
- Chandley AC, Edmond P, Christie S, Gowans L, Fletcher J, et al: Cytogenetics and infertility in man. I. Karyotype and seminal analysis: results of a five-year survey of men attending a subfertility clinic. *Ann Hum Genet* 39:231–254 (1975).
- Cotter PD, Ko E, Larabell SK, Rademaker AW, Martin RH: Segregation of a supernumerary del(15) marker chromosome in sperm. *Clin Genet* 58:488–492 (2000).
- Dutta UR, Vempally S, Ranganath P, Dalal A: A novel combined 15q11.2 duplication and a bisatellited supernumerary marker derived from chromosome 22: molecular characterization of the marker. *Gene* 539:162–167 (2014).
- Guediche N, Tosca L, Kara Terki A, Bas C, Lecerf L, et al: Array comparative genomic hybridization analysis of small supernumerary marker chromosomes in human infertility. *Reprod Biomed Online* 24:72–82 (2012a).
- Guediche N, Tosca L, Nouchy M, Lecerf L, Cornet D, et al: Small supernumerary marker chromosomes derived from chromosomes 6 and 20 in a woman with recurrent spontaneous abortions. *Eur J Med Genet* 55:737–742 (2012b).
- Ise T, Das S, Nagata S, Maeda H, Lee Y, et al: Expression of POTE protein in human testis detected by novel monoclonal antibodies. *Biochem Biophys Res Commun* 4:603–608 (2008).
- Jaafar H, Gabriel-Robez O, Vignon F, Flori E, Rimpler Y: Supernumerary chromosomes and spermatogenesis in human male carrier. *Hum Genet* 94:74–76 (1994).
- Kamath MS, Bhattacharya S: Demographics of infertility and management of unexplained infertility. *Best Pract Res Clin Obstet Gynaecol* 26:729–738 (2012).
- Klein E, Manvelyan M, Simonyan I, Hamid AB, Guilherme RS, et al: Centromeric association of small supernumerary marker chromosomes with their sister-chromosomes detected by three-dimensional molecular cytogenetics. *Mol Cytogenet* 5:15 (2012).
- Koç A, Onur O, Ergün MA, Perçin F: Supernumerary marker chromosome 15 in a male with azoospermia and open bite deformity. *Asian J Androl* 11:617–622 (2009).
- Kumar C, Kleymann SM, Samonte RV, Verma RS: Marker chromosomes in fetal loss. *Hum Reprod* 12:1321–1324 (1997).
- Lapierre JM, Tachdjian G: Detection of chromosomal abnormalities by comparative genomic hybridization. *Curr Opin Obstet Gynecol* 17:171–177 (2005).
- Liehr T: Small Supernumerary Marker Chromosomes (sSMC), A Guide for Human Geneticists and Clinicians (Springer, Berlin 2011).
- Liehr T: Small Supernumerary Marker Chromosomes. <http://ssmc-tl.com/sSMC.html> (accessed May 6, 2015).
- Liehr T, Weise A: Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med* 19:719–731 (2007).
- Liehr T, Claussen U, Starke H: Small supernumerary marker chromosomes (sSMC) in humans. *Cytogenet Genome Res* 107:55–67 (2004).
- Liehr T, Ewers E, Hamid AB, Kosyakova N, Voigt M, et al: Small supernumerary marker chromosomes and uniparental disomy have a story to tell. *J Histochem Cytochem* 59:842–848 (2011).
- Mantzouratou A, Mania A, Apergi M, Laver S, Serhal P, Delhanty J: Meiotic and mitotic behaviour of a ring/deleted chromosome 22 in human embryos determined by preimplantation genetic diagnosis for a maternal carrier. *Mol Cytogenet* 2:3 (2009).
- Manvelyan M, Riegel M, Santos M, Fuster C, Pellestor F, et al: Thirty-two new cases with small supernumerary marker chromosomes detected in connection with fertility problems: detailed molecular cytogenetic characterization and review of the literature. *Int J Mol Med* 21:705–714 (2008a).
- Manvelyan M, Hunstig F, Bhatt S, Mrasek K, Pellestor F, et al: Chromosome distribution in human sperm – a 3D multicolor banding-study. *Mol Cytogenet* 1:25 (2008b).
- Martin RH, Hildebrand KA, Yamamoto J, Peterson D, Rademaker AW, et al: The meiotic segregation of human sperm chromosomes in two men with accessory marker chromosomes. *Am J Med Genet* 25:381–388 (1986).
- Mau UA, Bäckert IT, Kaiser P, Kiesel L: Chromosomal findings in 150 couples referred for genetic counselling prior to intracytoplasmic sperm injection. *Hum Reprod* 12:930–937 (1997).

- Mennicke K, Diercks P, Schlieker H, Bals-Pratsch M, al Hasani S, et al: Molecular cytogenetic diagnostics in sperm. *Int J Androl* 20:11–19 (1997).
- Munne S: Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. *Reprod Biomed Online* 4:183–196 (2002).
- Munne S: Analysis of chromosome segregation during preimplantation genetic diagnosis in both male and female translocation heterozygotes. *Cytogenet Genome Res* 111:305–309 (2005).
- Oracova E, Musilova P, Kopečna O, Rybar R, Vozdova M, et al: Sperm and embryo analysis in a carrier of supernumerary inv dup(15) marker chromosome. *J Androl* 30:233–239 (2009).
- Otani T, Roche M, Mizuiki M, Colls P, Escudero T, Munne S: Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online* 13:869–874 (2006).
- Paetzold U, Schwanitz G, Schubert R, Van der Ven K, Montag M: Sperm analysis, genetic counseling and therapy in an infertile carrier of a supernumerary marker chromosome 15. *Adv Med Sci* 51:31–35 (2006).
- Perrin A, Nguyen MH, Delobel B, Gueganic N, Basinko A, et al: Characterization and meiotic segregation of a supernumerary marker chromosome in sperm of infertile males: case report and literature review. *Eur J Med Genet* 55:743–754 (2012).
- Reddy KS, Aradhya S, Meck J, Tiller G, Abboy S, Bass H: A systematic analysis of small supernumerary marker chromosomes using array CGH exposes unexpected complexity. *Genet Med* 15:3–13 (2013).
- Reinholdt LG, Czechanski A, Kamdar S, King BL, Sun F, Handel MA: Meiotic behavior of aneuploid chromatin in mouse models of Down syndrome. *Chromosoma* 118:723–736 (2009).
- Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK: The genetic basis of infertility. *Reproduction* 126:13–25 (2003).
- Sheth F, Andrieux J, Ewers E, Kosyakova N, Weise A, et al: Characterization of sSMC by FISH and molecular techniques. *Eur J Med Genet* 54:247–255 (2011).
- Simopoulou M, Harper JC, Fragouli E, Mantzouratou A, Speyer BE, et al: Preimplantation genetic diagnosis of chromosome abnormalities: implications from the outcome for couples with chromosomal rearrangements. *Prenat Diagn* 23:652–662 (2003).
- Solari AJ: Synaptonemal complex analysis in human male infertility. *Eur J Histochem* 43:265–276 (1999).
- Steinbach P, Djalali M: Ineffectivity of accessory bisatellited marker chromosomes in inducing meiotic nondisjunction. *Hum Genet* 64:402–403 (1983).
- Stortoni P, Cecati M, Giannubilo SR, Sartini D, Turi A, et al: Placental thrombomodulin expression in recurrent miscarriage. *Reprod Biol Endocrinol* 8:1 (2012).
- Warburton D: De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet* 49:995–1013 (1991).
- Wiland E, Jarmuz M, Kurpisz M: Segregation of the marker chromosome der(20) in the sperm of a male with karyotype 46,XX[96]/47,XY+mar[4]. *Med Sci Monit* 11:CS9–CS15 (2005).
- Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, et al: International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. *Fertil Steril* 101:366–378 (2014).
- Zhang S, Zhou X, Yu H, Yu Y: Expression of tumor-specific antigen MAGE, GAGE and BAGE in ovarian cancer tissues and cell lines. *BMC Cancer* 10:163 (2010).