# Correlation between Chromosomal Variants and Male Infertility in a Population of Brazilian Infertile Men

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## Abstract

Heterochromatin polymorphism is considered a variant of a normal karyotype but is more frequent in infertile men. The aim of this study was to evaluate the correlation between heterochromatic variants and male infertility and to discuss the possible mechanisms of how heterochromatic polymorphism might affect spermatogenesis.

**Methods:** Cytogenetic analysis was undertaken in a group of 392 infertile men from the Andrology Outpatient Clinic of the Human Reproduction Service of the ABC School of Medicine. Additionally, C-banding was performed in men with heterochromatin polymorphism, and NOR-banding in men with satellites variations.

**Results:** 47 patients of the sample showed chromosomal variants (12% of the sample). Considering these men, 8 presented idiopathic infertility, where 19 presented severe oligozoospermia, 18 had non-obstructive azoospermia, 2 presented recurring pregnancy loss. The most frequent chromosome involved was chromosome 9, observed in 37.5% of the cases. Increased heterochromatin of chromosome 9 isolated was present in 8 men and pericentromeric inversion of chromosome 9 isolated was present in 7 men. Both aberrations were found in one man. Increased heterochromatin of chromosome 16 was found isolated in 6 cases and associated to other variation in 3 cases. For chromosome Y, variation in heterochromatin was found in 6 cases and associated to other variation in two cases and for chromosome 1, increased heterochromatin was found only associated to other variations. Satellites' variation of chromosome 14 was found isolated in one case and associated in other case, of chromosome 21 was found isolated in 3 cases and associated in one case and of chromosome 22 was found isolated in 2 cases and associated in one case. Twenty men presented beyond chromosomal variations factors that couldn't be discharged as cause of infertility as orchites and criptorchidia.

**Conclusions:** The incidence of heterochromatin polymorphism was high in infertile men, as observed in the present work. This increased rate in infertile males seems to be more than an incidental finding, and must be considered an important factor contributing to male infertility.

**Keywords:** Chromosomal variations; Male factor infertility; Cytogenetic screening; Heterochromatin

## Introduction

Polymorphic variations are known to occur in the general population. They include varying sizes of heterochromatin blocks, satellite or repeat sequence regions and inversions [1,2].

These polymorphisms have been observed from the early studies of cytogenetics and are believed to have no impact on phenotype [3]. However, higher frequencies of these variants have recently been reported in infertile and subfertile individuals, Madon *et al.* [4] & Sahin *et al.* [5], compared with population cytogenetic data obtained mainly from newborn screening surveys [6].

Increased rates of chromosomal polymorphic variants have been shown to be associated with poor spermatogenesis [7]. These chromosomal variations associated with male infertility, including structural or numerical chromosomal abnormalities and quantitative or positional modifications of the constitutive heterochromatin, have been shown to affect male gamete formation and function possibly due to the silencing effect of these heterochromatic variations on otherwise normally expressed genes [8].

The aim of this study was to determine the frequency of chromosomal variants in a group of Brazilian infertile men attending an infertility service.

## Materials and Methods

### Study Group

This study made a retrospective assessment of the data of 392 infertile men (age range 22 to 60 years, mean: 36,6 ± 6,8 years), recruited consecutively between June 2006 to September 2010 at the Andrology Outpatient Clinic of the Human Reproduction Service of the Faculdade de Medicina do ABC, Santo André/SP, Brazil. We also included, as control group 63 semen donors (age range 20 to 43, mean 29,12±7,07), recruited in the same period of time in the Human Reproduction Service of FMABC.

All patients underwent an andrological work-up, which included routine clinical and laboratory tests, including analysis of semen and karyotype. For semen donors analysis of semen and karyotype were performed. Semen analysis was performed according to the guidelines of the World Health Organization [9]. Microdeletion of 5 regions of Y chromosome was performed to all infertile men.

There were 111 men with azoospermia and 160 with oligozoospermia with a sperm count of $<5 \times 10^6$/ml. All participants gave informed

consent, according to the protocol approved by the local ethics committee (FMABC No. 237/2008). All semen donors presented as an inclusion criteria normal seminal volume and concentration.

## Karyotyping

Chromosome investigations were performed on cultures of peripheral blood lymphocytes bystandard protocols. From each patient, 40 well spread metaphases were analyzed by G-banding. The metaphases were karyotyped using a Zeiss Axioskop microscope (Carl Zeiss Light Microscopy, Göttingen, Germany) and MetaSystems Ikaros software (MetaSystems, Altlussheim, Germany). To characterize the polymorphisms, specific techniques such as C-banding and NOR staining were additionally applied. All chromosomal abnormalities have been reported in accordance with the current international standard nomenclature [2].

## Classification of polymorphic Variations

Polymorphic variations in the length of the centromeric heterochromatin on the long arms of chromosomes 1, 9, 16 are designated as 1qh+, 9qh+, 16qh+ (increased heterochromatin). Heterochromatin can also be reduced in these chromosomes, such as 1qh-, 9qh- and 16qh-. The pericentric inversion of chromosome 9, inv(9)(p11q13), was also considered as a heteromorphism. For the Y chromosome increase of heterochromatin (Yqh+) was considered when it was larger than chromosome 18, and decrease of heterochromatin (Yqh-) when the Y chromosome was smaller than the G-group chromosome [10].

Increase in length of the short arm satellites (ps+) and stalks (pstk+) of the acrocentric chromosomes (13, 14, 15, 21, 22) were also recorded [2]. The alteration was classified as a variant, when it has at least twice the size of the corresponding region on the other homologue [11]. Double satellites can also be observed and are designated as PSS [2]. All karyotypes were examined by three independent laboratory technicians to avoid uncertainty and variable results.

## Statistical analysis

Fisher's exact test was applied to compare data between cases and

controls. Statistical analysis was performed by SPSS Windows 18.0 (SPSS, Inc., Chicago, IL) and a $p < 0.05$ was considered significant.

## Results

The prevalence of chromosomal polymorphic variation on our sample is shown in Table 1. From the 393 male patients evaluated, 47 patients of the sample showed chromosomal variants (12% of the sample). Considering these men, 8 presented idiopathic infertility, where 19 presented severe oligozoospermia, 18 had azoospermia, 2 presenter recurrent gestational loss.

The three most common variants observed were 9qh+ observed in 9 men (19.15%), 9ph present in 9 men (19.15%) and 16qh+ also found in 9 cases (19.15%). In one case 9qh+ was associated to 21ps+. In two cases 9ph was associated to other variant. In three cases 16qh+ was associated to other chromosomal variants. Chromosome Y increased heterochromatin was found in 5 cases (10.64%), being associated to other variation in 2 cases and Yqh- was observed in one man (2.13%). Chromosome 1 increased heterochromatin was found only associated to other variations (Table 1).

Concerning satellites' variation, 13ps+ was found associated to other variation (9qh+, 13ps+). 14ps+ was found in one case (2.13%). 14pstk+ was associated to 22ps+ in one case. 15ps+ was found in 2 cases (4.25%). 21ps+ was found in 6 cases (12.77%), being associated to other variation in 2 cases. 22ps+ was found in 6 cases (12.77%), 3 of them associated to other variation (Table 1).

Twenty man presented beyond chromosome variant factors that couldn't be discharged as cause of infertility as orchite and criptorchidia. One patient with azoospermia presented Y chromosome microdeletion beyond the chromosome variant (16qh+).

In comparison to control group we found no difference in the incidence of chromosomal variants (Table 2).

## Discussion

Chromosomal variations have been associated to male infertility,

| Karyotype | Number of affected and frequency | Cause of Infertility | | | |
|---|---|---|---|---|---|
| | | azoospermia | oligospermia | idiopathic infertility | recurrent pregnancy loss |
| 46,XY,1qh+,Yqh+ | 1 (2.13%) | 1 | - | - | - |
| 46,XY,16qh+ | 6 (12.77%) | 3 | 2 | 1 | - |
| 46,XY,1qh+,16qh+ | 2 (4.25%) | 1 | - | 1 | - |
| 46,XY,9ph | 7 (14.9%) | 3 | 1 | 2 | 1 |
| 46,XY,9ph,9qh+ | 1 (2,13%) | 1 | - | - | - |
| 46,XY,9ph+,21ps+ | 1 (2,13%) | - | - | 1 | - |
| 46,XY,9qh+ | 8 (17,02%) | 3 | 4 | 1 | - |
| 46,XY,9qh+,13ps+ | 1 (2,13%) | - | - | 1 | - |
| 46,XY,Yqh+ | 5 (10,64%) | - | 5 | - | - |
| 46,XY,Yqh- | 1 (2,13%) | 1 | - | - | - |
| 46,XY,14ps+ | 1 (2,13%) | - | 1 | - | - |
| 46,XY,14pstk+,22ps+ | 1 (2,13%) | 1 | - | - | - |
| 46,XY,15ps+ | 2 (4,26%) | 1 | - | - | 1 |
| 46,XY,21ps+ | 4 (8,51%) | 2 | 1 | 1 | - |
| 46,XY,21pstk+,22ps+ | 1 (2,13%) | 1 | - | - | - |
| 46,XY,22ps+ | 3 (6,38%) | 1 | 2 | - | - |
| 46,XY,22ps+,Yqh+ | 1 (2,13%) | - | 1 | - | - |
| 45,X,16qh+[10]/46,XY,delY (q11.9-19.2),16qh+[30] | 1 (2,13%) | - | 1 | - | - |
| total | | 19 | 18 | 8 | 2 |

**Table 1:** Chromosomal variants found in infertile man with idiopathic infertility, distributed according to the cause of infertility.

| Karyotypes | | Patients with heteromophism (total sample=392) | Controls with heteromophism (total sample=63) | p |
|---|---|---|---|---|
| **1, 9, 16qh+** | | | | |
| | 46,XY,9qh+ | 8 | 1 | 0.41 |
| | 46,XY,16qh+ | 6 | 0 | |
| **13, 14, 15, 21, 22, ps+, pstk+** | | | | |
| | 46,XY,14ps+ | 1 | 0 | 0.64 |
| | 46,XY,15ps+ | 2 | 0 | |
| | 46,XY,21ps+ | 4 | 0 | |
| | 46,XY,22ps+ | 3 | 1 | |
| **9 ph (inv 9)** | | | | |
| | 46,XY,9ph | 7 | 0 | 0.28 |
| **Y variation** | | | | |
| | 46,XY,Yqh+ | 5 | 0 | 0.32 |
| | 46,XY,Yqh- | 1 | 0 | |
| **Multiple variation** | | | | |
| | 46,XY,1qh+,Yqh+ | 1 | 1 | |
| | 46,XY,1qh+,16qh+ | 2 | 0 | |
| | 46,XY,9ph,9qh+ | 1 | 0 | |
| | 46,XY,9ph+,21ps+ | 1 | 0 | |
| | 46,XY,9qh+,13ps+ | 1 | 0 | |
| | 46,XY,14pstk+,22ps+ | 1 | 0 | 0.77 |
| | 46,XY,15pstk+,16qh+ | 0 | 0 | |
| | 46,XY,21pstk+,22ps+ | 1 | 1 | |
| | 46,XY,22ps+,Yqh+ | 1 | 0 | |
| | 45,X,16qh+[10]/46,XY,delY(q11.9-19.2),16qh+[30] | 1 | 0 | |
| **Total** | | 47 | 4 | 0.19 |

**Table 2:**

including structural or numerical chromosomal abnormalities and quantitative or positional modifications of the constitutive heterochromatin, possible affecting male gamete formation and function [12].

The role of chromosome heteromorphisms in infertility has been studied previously for many authors and despite of being overrepresented in infertile couples, no consistent data was found to correlate these variations with infertility. This subject continues to be an intriguing question.

Madon *et al.* [4] evaluated 842 individuals attending an IVF clinic with primary infertility or repeated miscarriages, showed polymorphic variants in 28.82% of males and 17.19% of females. Hong *et al.* [11] studied the effect of polymorphic variants in the outcome of *in vitro* fertilization. 1978 couples were evaluated and 182 males presented chromosomal variations (9.2%). No differences among implantation rates were observed but there was a trend toward higher first trimester pregnancy loss rates, compared with normal karyotype couples.

In the present study we evaluated 392 infertile patients and found 47 patients of with chromosomal variants (12% of the sample). Considering these men, 8 presented idiopathic infertility, where 19 presented severe oligozoospermia, 18 had non-obstructive azoospermia, 2 presented recurring pregnancy loss. The prevalence here observed is in accordance to previous works of male infertility.

Between all chromosomes, chromosome 9 variations seem to be overrepresented on infertile couples (18 patients – p=0.08). All variation analysis will be discussed in a separate session.

### Chromosome 9 variations and infertility

DNA sequence and analysis of human chromosome 9 revealed that it is highly structurally polymorphic and contains the largest autosomal block of heterochromatin, which is heteromorphic in 6–8% of humans, while pericentric inversions occur in more than 1% of the population [13]. Chromosome 9, has been described as being especially prone

to breaks that produce asymmetric bivalents in meiotic metaphase I spreads (Sarrate *et al*. [14]) and structural chromosome aberrations in sperm studies [15].

Pericentric inversion was determined on chromosome 9 in 2.3% of the sample (9 patients). In the general population, the frequency of this inversion is as low as 1–1.65%. Even though it is known that these inversions on chromosome 9 do not have a phenotypic effect, by means of previous studies, inversions on chromosome 9 and men infertility were thought to be related [16-18]. In our sample, 4 patients presented oligospermia, 3 patients idiopathic infertility, 1 patient azoospermia and one patient presented recurrent gestational loss.

Pericentric inversions are structural chromosomal abnormalities resulting from two breaks, one on either side of the centromere, within the same chromosome, followed by 180° rotation and reunion of the inverted segment. They can perturb spermatogenesis and lead to the production of unbalanced gametes through the formation of an inversion loop, even if the inverted region includes only heterochromatin [19].

If one chromosome is inversed, the mechanics and time constraints imposed by the meiotic machinery on the formation of a pairing loop can delay meiosis. Recombination is reduced within the pairing loop, and this also leads to a breakdown of meiosis [20-22].

Lissitsina *et al*. [23] in their study of 90 infertile men observed inv(9)(p11q13) three times more often than controls. Collodel *et al*. [24] studied the sperm quality of 18 male carriers of a chromosome 9 inversion and found a variety of effects, from azoospermia to severely altered sperm morphology, motility and meiotic segregation.

Enlarged chromosome 9 heterochromatin (9qh+) is considered a polymorphism, and its incidence is estimated between 6 and 8% of general population. This heteromorphism possibly makes synapse difficult due to morphological differences between the two homologous and, as a consequence, may delay or prevent it [25]. In our sample we found an incidence of 2.55% (10 patients), representing 21.3% of variants. Four of them presented oligospermia, four of them azoospermia, and 2 of them presented recurrent pregnancy loss.

In our study we have one patient who presented oligoasthenozoospermia had both the 9ph and the 9qh+ variants. This case was reported previously (Belangero *et al*. [26]), and we believe that this morphological difference between the homologous chromosomes 9 could have led to an error in crossing-over and, as a consequence, produced aberrant gametes.

## Chromosome y and infertility

The high incidence of chromosomal variants in the infertile male population can also be attributed to polymorphic variations on the Y-chromosome, contributing to male infertility or subfertility possibly due to the silencing effect of these heterochromatic variations on otherwise normally expressed genes [27].

In the normal male population, Hou and Wang reported a 3.6% prevalence of Yq+ in a genetic survey of 6286 males [28]. Nagvenkar *et al*. [29] reported 3.4% of Yq+ in an Indian population. In our study population, there were seven patients who presented the variant Yqh+ karyotype, representing 1.78% of the sample and 14.9% of variants. Interestingly, six of them presented azoospermia.

Increased size and occurrence of the variant Yqh+ could possibly be associated with the inhibition of gene transcription due to the silencing effect on the genes/gene promoters in close proximity [8].

Heterochromatic variations on the Y chromosome could also be associated with severe male factor infertility by inducing epigenetic alterations/modifications [30,31].

The occurrence of decreased heterochromatin (Yqh-) was short in our sample present only in 1 patient of our sample. This patient presented no microdeletion of Y chromosome. In the presence of a microdeletion the decrease of heterochromatin was justified and consequently, the decrease of heterochromatin was not considered. A possible association of heterochromatic blocks with the silencing of gene expression, particularly genes associated with spermatogenesis and other fertility/infertility associated genes, should not be ignored.

## Chromossome 1 and 16 and infertility

Nakamura *et al*. [31], investigating 1790 infertile Japanese man found that 46,XY,1qh(+) was the most common autosomal anomaly, observed in 30 cases and its incidence was significantly higher than in normal controls. Nine of this patients presented azoospermia. In our sample the variant 1qh+ was associated with other variations (Yqh+, 16qh+). Patients presented oligozoospermia and idiopathic infertility. Nagvenkar *et al*. [29] in an Indian sample of 88 infertile men found one patient with azoospermia and 16qh+ variant and one with 1qh+.

## Polymorphic variations in acrocentric chromosomes

On acrocentric chromosomes, the NOR region on the stalks (pstk) of satellites consists of rRNA, while the short arm (p) and satellites (ps) consist of heterochromatin. Chromatin modification, covalent modifications of histone core proteins, noncoding small interfering RNA (siRNA) – related silencing of gene expression, and reversible methylation of DNA all form part of epigenetic alterations that affect gene expression [32].

## The impact on gametes

Similar to chromosomal rearrangements such as translocations, heterochromatin-related defects in centromere function, and kinetochore assembly, chromosomal segregation may be impaired. This may lead to aneuploidy in spermatozoa involving not only the chromosomes affected but also the other chromosomes through interchromosomal effects [33].

Polymorphic heterochromatic regions were found to alter the synapsis of homologous chromosomes during meiosis. These regions are the last to enter synapse, changing the timing of the whole division and leading first to probable meiotic defects, eventually to infertility [25].

One other interesting point about heterochromatin is its effect on genes located close to it. In a variety of organisms, euchromatic genes brought into juxtaposition with pericentric heterochromatin show position-effect variegation, a silencing of gene expression in a subset of the cells in which the gene is normally expressed leading to a mosaic phenotype [34]. This phenomenon was observed in drosophila, yeast and mouse models [35-37].

Yakin *et al*. [7] revealed a high rate of aneuploidy in spermatozoa obtained from men with heterochromatin polymorphism. This study detected the heteromorphisms mainly in autosomal chromosomes, and a significant aneuploidy was detected on the sex chromosomes. The association of heterochromatin variants and special forms of severe sperm head and neck abnormalities were also observed. These severe morphological defects were associated with poor results in assisted reproductive techniques.

Mau *et al.* [38] reported chromosomal polymorphism in 13 out of 150 infertile male (8.7%). Yakin *et al.* [7] reported a 10.9% incidence of polymorphic variants in the infertile male group. In agreement with these studies, we found a large frequency of chromosomal variants in the infertile men (12%) and an incidence of 6.35% in controls. However, no statistical difference was found between the incidence in cases and controls (p=0.19).

Hong *et al.* [11] investigated the effect of chromosomal polymorphic variations on the outcome of IVF and embryo transfer in 1978 for infertile couples, 8.05% with polymorphic variation and found no statistically significant differences in implantation rates between groups. Although, there was a trend toward higher first trimester pregnancy loss rates in the group of male with chromosomal polymorphic variations compared with normal karyotype couples, with no statistical significance (p>0.05).

The high frequency of chromosomal variants in infertile men could support the opinion that the large heterochromatic blocks on the polymorphisms of heterochromatic regions may destabilize the pairing of chromosomes and cause meiotic arrest, resulting in infertility [23]. Therefore, the possible association of heterochromatic blocks with the silencing of gene expression, particularly genes associated with spermatogenesis and other fertility/infertility-associated genes, should not be ignored [8]. Complementary investigation with high performance molecular techniques (e.g. microarray) could identify gains and losses associated to the heteromorphisms, and point to new sequences associated to infertility and pregnancy losses [39].

In our study, despite of we didn't find differences in the incidence of chromosomal variation compared to controls, the high incidence of variants found in infertile men, including azoospermic ones, can point to the presence of other chromosomal aberrations unidentified by karyotype. More sensitive techniques applied to a larger sample of cases and controls could help us to identify the aberrations associated to male infertility. Variants should be well described and associated with infertility and the cytogeneticists and clinicians should not ignore the high frequency of polymorphic chromosome variants in infertile males.

## References

1. Borgaonkar DS (1997) Chromosomal variation in man: a catalogue of chromosomal variants and anomalies. (8th edn) Wiley-Liss, New York.

2. Shaffer LG, Slovak ML, Campbel LJ (2009) ISCN 2009: an international system for human cytogenetic nomenclature. S Karger Publishing, Basel, Switzerland, 53-54.

3. Brothman AR, Schneider NR, Saikevych I, Cooley LD, Butler MG, et al. (2006) Cytogenetics Resource Committee, College of American Pathologists/American College of Medical Genetics. Cytogenetic heteromorphisms: Survey results and reporting practices of Giemsa-band regions that we have pondered for years. Arch Pathol Lab Med 130: 947-949.

4. Madon PF, Athalye AS, Parikh FR (2005) Polymorphic variants on chromosomes probably play a significant role in infertility. Reprod Biomed Online 11: 726-732.

5. Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, et al. (2008) Chromosome heteromorphisms: an impact on infertility. J Assist Reprod Genet 25: 191-195.

6. Bhasin MK (2005) Human population cytogenetics: a review. Int J Hum Genet 5: 83-152.

7. Yakin K, Balaban B, Urman B (2005) Is there a possible correlation between chromosomal variants and spermatogenesis? Int J Urol 12: 984-989.

8. Minocherhomji S, Athalye AS, Madon PF, Kulkarni D, Uttamchandani SA, et al. (2009) A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. Fertil Steril 92: 88-95.

9. World Health Organization (1999) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. (4th edn), Cambridge University Press, Cambridge.

10. Hsu LY, Benn PA, Tannenbaum HL, Perlis TE, Carlson AD (1987) Chromosomal polymorphisms of 1, 9, 16, and Y in 4 major ethnic groups: a large prenatal study. Am J Med Genet 26:95-101.

11. Hong Y, Zhou YW, Tao J, Wang SX, Zhao XM (2011) Do polymorphic variants of chromosomes affect the outcome of in vitro fertilization and embryo transfer treatment? Hum Reprod 26: 933-940.

12. Antonelli A, Gandini L, Petrinelli P, Marcucci L, Elli R, et al. (2000) Chromosomal alterations and male infertility. J Endocrinol Invest 23: 677-683.

13. Humphray SJ, Oliver K, Hunt AR, Plumb RW, Loveland JE, et al. (2004) DNA sequence and analysis of human chromosome 9. Nature 429: 369-374.

14. Sarrate Z, Blanco J, Egozcue S, Vidal F, Egozcue J (2004) Identification of meiotic anomalies with multiplex fluorescence in situ hybridization: preliminary results. Fertil Steril 82: 712-717.

15. Templado C, Bosch M and Benet J (2005) Frequency and distribution of chromosome abnormalities in human spermatozoa. Cytogenet Genome Res, 111: 199-205.

16. Tomaru M, Sasagawa I, Ishigooka M, Hashimoto T, Izumiya K, et al. (1994) Pericentric inversion of chromosome 9 associated with Sertoli-cell-only tubule. Urol Int 52: 118-120.

17. Sasagawa I, Ishigooka M, Kubota Y, Tomaru M, Hashimoto T, et al. (1998) Pericentric inversion of chromosome 9 in infertile men. Int Urol Nephrol 30: 203-207.

18. Davalos IP, Rivas F, Ramos AL, Galaviz C, Sandoval L, et al. (2000) inv (9) (p24q13) in three sterile brothers. Ann Genet 43: 51-54.

19. Morel F, Laudier B, Guérif F, Couet ML, Royère D, et al. (2007) Meiotic segregation analysis in spermatozoa of pericentric inversion carriers using fluorescence in-situ hybridization. Hum Reprod 22: 136-141.

20. Brown GM, Leversha M, Hulten M, Ferguson-Smith MA, Affara NA, et al. (1998) Genetic analysis of meiotic recombination in humans by use of sperm typing: reduced recombination within a heterozygous paracentric inversion of chromosome 9q32-q34.3. Am J Hum Genet 62: 1484-1492.

21. Ichioka K, Yoshimura K, Honda T, Takahashi A, Terai A (2005) Paracentric inversion of chromosome 7(q22-31) associated with nonobstructive azoospermia. Fertil Steril 83: 455-456.

22. Anton E, Blanco J, Egozcue J, Vidal F (2002) Risk assessmen t and segregation analysis in a pericentric inversion inv6p23q25 carrier using FISH on decondensed sperm nuclei. Cytogenet Genome Res 97: 149-154.

23. Lissitsina J, Mikelsaar R, Varb K, Punab M (2003) Cytogenetic study in infertile men. Ann Genetic 46: 185.

24. Collodel G, Moretti E, Capitani S, Piomboni P, Anichini C, et al. (2006) TEM, FISH and molecular studies in infertile men with pericentric inversion of chromosome 9. Andrologia. 38: 122-127.

25. Codina-Pascual M, Navarro J, Oliver-Bonet M, Kraus J, Speicher MR, et al. (2006) Behaviour of human heterochromatic regions during the synapsis of homologous chromosomes. Hum Reprod 21: 1490-1497.

26. Belangero SI, Christofolini DM, Bianco B, Gava MM, Wroclawski ER, et al. (2009) Male infertility related to an aberrant karyotype, 46,XY,9ph,9qh+. Fertil Steril 91: 2732.e1-3.

27. Hemming L, Burns C (1979) Heterochromatic polymorphism in spontaneous abortions. J Med Genet 16: 358-362.

28. Hou JW, Wang TR (1999) Study of human Y chromosome polymorphism in Taiwan. Acta Paediatr Taiwan 40: 302-304.

29. Nagvenkar P, Desai K, Hinduja I, Zaveri K (2005) Chromosomal studies in infertile men with oligozoospermia & non-obstructive azoospermia. Indian J Med Res 122: 34-42.

30. Eiben B, Leiopoldt M, Rammelsberg O, Krause W, Engel W (1987) High incidence of minor chromosomal variants in teratozoospermic males. Andrologia 19: 684-687.

31. Nakamura Y, Kitamura M, Nishimura K, Koga M, Kondoh N, et al. (2001) Chromosomal variants among 1790 infertile men. Int J Urol 8: 49-52.

32. Liu L, Li Y, Tollefsbol TO (2008) Gene-environment interactions and epigenetic basis of human diseases. Curr Issues Mol Biol 10: 25-36.

33. Pellestor F, Imbert I, Andreo B, Lefort G (2001) Study of the occurrence of interchromosomal effect in spermatozoa of chromosomal rearrangement carriers by fluorescence in-situ hybridization and primed in-situ labelling techniques. Hum Reprod 16: 1155-1164.

34. Cryderman DE, Cuaycong MH, Elgin SC, Wallrath LL (1998) Characterization of sequences associated with position-effect variegation at pericentric sites in Drosophila heterochromatin. Chromosoma 107: 277-285.

35. Weiler KS, Wakimoto BT (1995) Heterochromatin and gene expression in Drosophila. Annu Rev Genet 29: 577-605.

36. Allshire RC, Javerzat JP, Redhead NJ, Cranston G (1994) Position effect variegation at fission yeast centromeres. Cell 76: 157-169.

37. Kioussis D, Festenstein R (1997) Locus control regions: overcoming heterochromatin-induced gene inactivation in mammals. Curr Opin Genet Dev 7: 614-619.

38. Mau UA, Backert IT, Kaiser P, Kiesel L (1997) Chromosomal findings in 150 couple referred for genetic counseling prior to intracytoplasmic sperm injection. Hum Reprod 12: 930-937.

39. Campanho Cde L, Henrich JK, Couto E, Barini R (2011) Subfertility phenotype, chromosome polymorphism and conception failures. Rev Bras Ginecol Obstet 33: 246-251.

40. Cortés Gutiérrez EI, Cerda-Flores RM, Dávila-Rodríguez MI, Hernández-Herrera R, Vargas-Villarreal J, et al. (2004) Chromosomal abnormalities and polymorphisms in Mexican infertile men. Arch Androl 50: 261-265.

41. Eiben B, Leiopoldt M, Rammelsberg O, Krause W, Engel W (1987) High incidence of minor chromosomal variants in teratozoospermic males. Andrologia 19: 684-687.

42. Gardner RJM, Sutherland GR (2004) Chromosome abnormalities and genetic counseling. (3rd edn), Oxford University Press, New York, USA.

43. Giglio S, Broman KW, Mastumoto N, Calvari V, Gimelli G, et al. (2001) Olfactory receptor-gene clusters, genomic-inversion polymorphisms and common chromosome rearrangements. Am J Hum Genet 68: 874-883.

44. Rusché LN, Rine J (2001) Conversion of a gene-specific repressor to a regional silencer. Genes Dev 15: 955-967.