Infertility

Human Reproduction Archives

ISSN 2595-0711

RESEARCH ARTICLE

Karyotype Analysis in Infertile Men from the Center Western Brazil

Roberta Machado de Oliveira Frota Curado[1, 2, 3]*, Bruno Faulin Gamba[2], Lucilene Arilho Ribeiro Bicudo[2], Manoel de Araújo Rocha Filho[3], Mário Silva Approbato[3], Waldemar Naves do Amaral[3], Nádia Aparecida Bérgamo[2]

[1]Programa de Pós-graduação em Ciências da Saúde, Faculdade de Medicina da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
[2]Laboratório de Genética Molecular e Citogenética da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
[3]Laboratório de Reprodução Humana do Hospital das Clínicas da Universidade Federal de Goiás, Goiânia, Goiás, Brasil

Abstract

Objective: To investigate the presence of chromosomal abnormalities in infertile men with azoospermia or oligozoospermia evaluated at the Human Reproduction Laboratory of the Hospital das Clinicas (LabRep -HC) of the Federal University of Goias (UFG), Goiania, Goias, Brazil, in the period 2012-2014. Methods: Metaphases were analyzed by GTG banding obtained from the culture of lymphocytes of 33 men with idiopathic infertility. Results: The patients' ages ranged from 24-59 years and they had been attempting to conceive for an average of 5 (± 4,59) years. In 4 cases (12%) have been Klinefelter syndrome (KS) karyotype 47,XXY, was observed in three of these subjects and the fourth showed mosaicism 47,XXY[9]/46,XY[2]. The remaining 29/33 had normal karyotype. Conclusions: KS affects approximately 5 to 10% of infertile men, according to the literature. In this study, 12% of subjects were observed to have chromosomal abnormalities and sex chromosome aneuploidies were the only abnormality to be observed in the samples. Genetic testing can help identify which patients would benefit from technical reproduction. As well as contributing to unraveling the etiology of male infertility in patients treated at LabRep-HC-UFG, this study is relevant because assisted reproduction techniques ignore the process of natural selection and some classic chromosomal abnormalities or deleterious mutations could be inherited. Thus, counseling based on genetic assessments may prevent the inheritance of primary and secondary congenital defects in the offspring of patients with male infertility.

Keywords: infertility; male; karyotype; klinefelter syndrome; x chromosome

INTRODUCTION

Male infertility is a public health problem affecting 2,5% a 12% of the world's population.[1] Male infertility has a multifactorial etiology, with 30% being considered idiopathic and with a significant proportion of underlying genetic variations.[2,3] Chromosomal abnormalities are one of the genetics causes of human infertility which interfere with spermatogenesis. Potentials chromosomal analysis is fundamental in the diagnosis, prognosis and monitoring of chromosomal abnormalities.[2,4] Semen analysis is the first step in the evaluation of men with difficulties conceiving. Although abnormal semen parameters are not a definitive indicator of male infertility, they are correlated with a low probability of pregnancy; the most common factors are seminal abnormalities, azoospermia, and oligozoospermia.[2,5] It is known that patients with oligozoospermia or azoospermia have a higher risk of chromosomal abnormalities than the general population.[6] Thus, individuals with abnormal semen analysis results should be evaluated first by classical cytogenetics.[5,7]

Among the most frequent chromosomal abnormalities is the presence of an extra chromosome carrying the sex karyotype 47, XXY, a condition known as Klinefelter's Syndrome (KS). KS is present in 1/600 males from birth, only 10% are diagnosed prenatally, with another 25% diagnosed during childhood or adulthood. Underdiagnoses occurs due to the fact that not all individuals with KS have the characteristic clinical signs but only mild symptoms.[8,9] KS affects approximately 5 to 10% of infertile men.[10,11]

Conflicts of interests: The authors declare no conflicts of interest.
Submitted: September 01, 2018.
Accepted: February 08, 2022
The study was carried out at Universidade Federal de Goiás (UFG), Goiânia, Goiás, Brasil

Here we investigate the presence of chromosomal abnormalities in infertile men with azoospermia or oligozoospermia evaluated at the Human Reproduction Laboratory of the Hospital das Clinicas (LabRep -HC) of the Federal University of Goias in the period 2012-2014.

METHODS

Thirty-three infertile men, who are undergoing treatment in LabRep - HC, were selected to participate in this study. Written informed consent for publication was obtained from Patient The Ethics Committee of the Hospital das Clinicas and of the Federal University of Goias. The results of semen analysis indicated oligozoospermia or non obstructive azoospermia was a criterion of inclusion.

Peripheral blood samples were collected from these individuals and cultured by blood lymphocytes for 72 hours, the cells were harvested, hipotonised and fixed using 3:1 methanol: acetic acid.[12] GTG-Band chromosomal technique was performed and metaphases were spread on slides.[13] At least 11 metaphases of each case were analyzed and chromosomal abnormalities were described according to the International System for Chromosome Nomenclature (ISCN).[14]

RESULTS

Of the 33 selected infertile men, it was found that 48,48% were azoospermic (n = 16) and 51,51% were oligozoospermic (n = 17). The patients' ages ranged from 24-59 years and they had been attempting to conceive for an average of 5 (± 4,59) years.

The results of cytogenetic analysis of the all individuals revealed sex chromosome aneuploidy to be the only constitutional abnormality. Four cases were shown to have karyotype 47,XXY (Figure 1) and in one of these cases was observed a low level of mosaicism 47,XXY[9]/46,XY[2], and one case was polymorphic variants 46,XY,22ps+ (Table 1).

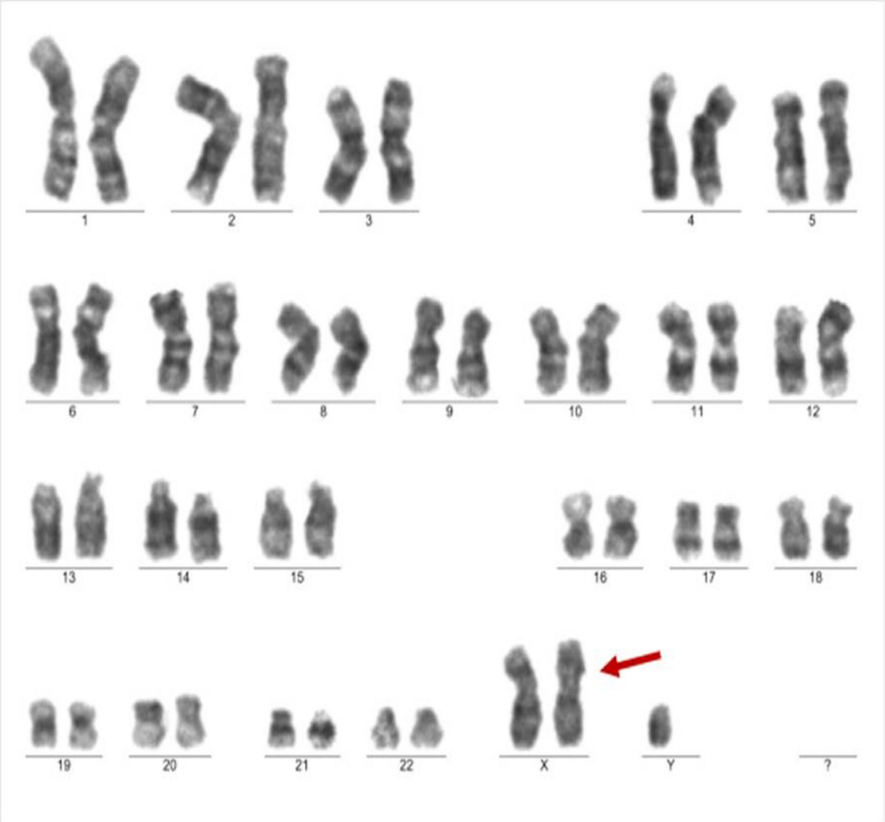

Figure 1. Metaphase with 47, XXY Case No. 10, indicating the extra X chromosome.

Table 1. Distribution of patients with azoospermia or oligozoospermia and the results of the evaluation karyotype.

Chromosomal abnomalies	Results	Karyotype	Azoospermia Frequency (N=16)	Oligozoospermia Frequency (N=17)
	Normal	46,XY	11	17
Numerical abnormalities	Klinefelter Syndrome	47,XXY	3	0
	Klinefelter Syndrome	47,XXY/46,XY	1	0
Structural abnormalities	Pseudo satellite	46,XY,22ps+	1	0

DISCUSSION

Lack of knowledge of the etiology of male infertility complicates the clinical diagnosis and the targeting for the best treatment.[15] It is known that men affected by infertility are at a high risk of carrying constitutional chromosomal abnormalities.[7] About 3 to 11,7% of male infertility can be explained by somatic chromosomal abnormalities.[6] Between 6 and 15% by microdeletions in Yq.[16] In the literature, approximately 80% of patients presenting with KS were found to have karyotype 47,XXY, while 20% showed some degree of chromosomal mosaicism, including 47, XXY/46,XX, or structural abnormalities of chromosome X.[17]

Polymorphic variations occur in the general population and are typical. They appear in varying sizes of heterochromatin blocks, inversions, satellite duplication or repeat sequence regions. Although they have not altered the phenotype, but the highest frequencies of these variants have been reported in infertile individuals. In the literature of infertile men presents a variation between 2.2 and 14.4% of variants of short arms of acrocentric chromosomes.[6,18,19] Chromosomal polymorphism was observed in one case (3%) which a double satellite in chromosome 22.

Christofolini et al.,[18] observed as polymorphic variants although they were overrepresented in some patients, no consistent data were found to correlate them with infertility. This subject remains intriguing need more study.

This study was conducted to explore the implications of chromosomal abnormalities in male infertility. Sex chromosome aneuploidies were the only abnormalities observed in the samples. Of the 33 men studied, 12% (4/33 subjects) were diagnosed with KS, which is reported as the most common cause of infertility in men.

KS affects approximately 5 to 10% of infertile men.[10,11] Ferlin et al.,[20] observed a similar frequency in azoospermic Klinefelter's individuals and in individuals with severe oligozoospermia the rate was slightly higher (5%). In our study, no individuals in the oligozoospermic group were found to have KS. All four KS cases were in the azoospermic group, which corresponds to 25% (4/16) of the azoospermic men. In the present study, 12% were diagnosed three with karyotype 47,XXY and one had amosaic 47XXY/46,XY karyotype.

In general, mosaics for KS are less severely affected and the chance of finding sperm in the ejaculate of these individuals is significantly higher than in non-mosaics.[21] Although this study identified one case with the mosaic karyotype 47,XXY[9]/46,XY[2], this patient did not show any presence of sperm in their ejaculate. Perhaps the absence of sperm in this case is due to the low level of mosaicism, since only two cells are normal with the predominance of 47,XXY. Furthermore, mosaicism in peripheral blood lymphocytes in the germinal may not reflect the state of the tissue. Individuals with KS are generally underdiagnosed, only 10% are diagnosed prenatally, with another 25% diagnosed during childhood or adulthood.[9] The condition results in a heterogeneous phenotype of variable severity normally are relatively slight, which also explains why many individuals do not receive medical attention until adulthood, when seeking medical advice about infertility or small testicles. Other signals such as hormone failure, sexual dysfunction, endocrine and metabolic syndrome, cardiovascular diseases, osteoporosis, and autoimmune diseases can also lead to a diagnosis.[22,23] When a diagnosis is made in adulthood, you lose the opportunity to intervene in body and cognitive development, and also to prevent comorbidities earlier, since KS is associated with an increased risk of comorbidities, resulting in a reduction of two to six years' life expectancy, compared to men who are karyotype 46,XY.[24] The phenotypic abnormalities more frequent in men with KS are small testicular volume, hormonal dysfunction, gynecomastia, decreased libido, and sexual or ejaculatory dysfunction and lack of sperm in the adult individual,[25,26] In this study, we observed some of these characteristics, such as ejaculatory decrease, erectile difficulty, and reduced testicular volume, however all these subjects had a normal karyotype. Of the four men with KS, only one individual presented with a smaller right testis volume.

Infertility due to obstructive azoospermia is not a major concern for men with KS,[27] since the majority of individuals affected by this syndrome are azoospermic and unable to conceive by natural methods.[28] There are isolated pockets

of spermatogenesis in the testes of individuals with KS.[29] Using microsurgical testicular sperm extraction (microTESE) it is possible to uniquely identify the tubules where spermatogenesis occurs in patients with KS, enabling the recovery of sperm so they may be a candidate for ICSI.[15,29,30] The initial success rate for this observed in subjects with KS was 40 to 50%,[17] whereas with the use of microTESE, the success rate was 70%, which is more favorable for karyotype 47 individuals, with XXY.[31]

Some spermatogonia in individuals with KS are able to complete the process of spermatogenesis, leading to the formation of mature sperm, but with increased risk of common genetic imbalance.[32] Consequently, there is a risk of producing a chromosomal abnormality in the offspring, either involving the sex chromosomes or autosomes (mainly chromosomes 18:21).[32,33]

Although there is a risk of the transmission of genetic imbalance, more than 100 healthy children have been born by TESE after ICSI to parents with KS.[17] Pre-implantation genetic diagnosis (PGD) – chromosomal analysis using the embryo - allows the transfer of healthy embryos and greater deployment potential.[8]

Men diagnosed with changes in the semen should undergo pre-treatment research, as we found a high prevalence of cases with chromosomal alteration in a small sample size.[5] Even individuals with normal karyotype must undergo molecular tests to investigate the genes responsible for spermatogenesis, so that the children produced using assisted reproduction techniques are not affected. This investigation is required primarily when the man produces few sperm, for oligozoospermia may be a consequence of genetic alteration, which can be masked by fertilization procedures.[6] Chromosomal and molecular analysis of these men assists in the selection process of the ideal technique for fertilization and pregnancy success.[34]

It is very important to establish the causes of infertility as early as possible, to provide an explanation for any failure in the reproductive success, avoid the physical, psychological, and financial distress, and guide the treatment of infertility by *in vitro* fertilization with donation semen or oocytes.[35] Individuals with unbalanced chromosomal aberrations require genetic counseling with specific information about the type of abnormality, its clinical relevance, the genetic risk of having affected offspring, and prenatal diagnosis possibilities. In addition, it should be possible to clarify whether other family members also carry the same abnormality.[36]

CONCLUSION

Our results showed a high frequency of individuals with KS diagnosed only after investigation into causes of infertility. Genetic tests are very important because assisted reproduction techniques ignore the process of natural selection and some chromosomal abnormalities or deleterious mutations can be passed to future generations. Although some patients have been not diagnostic with chromosomal abnomalis, karyotype analysis showed be an efficient method to investigate chromosomal abnomalies in a infertile man group. This finding suggests the need for cytogenetic analysis prior to the application of assisted reproduction techniques. As well as helping to unravel the etiology of infertility patients treated at LRH-HC UFG, this evaluation may lead to genetic counseling and hence the primary and secondary prevention of birth defects in offspring.

REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37. **http://dx.doi.org/10.1186/s12958-015-0032-1**.
2. Pizzol D, Bertoldo A, Foresta C. Male infertility: biomolecular aspects. Biomol Concepts. 2014;5(6):449-56. **http://dx.doi.org/10.1515/bmc-2014-0031**. PMid:25390025.
3. Gunes S, Agarwal A, Ersoy A, Henkel R. Genetic variations and male infertility. In: Horcajadas JA, Gosálvez J, editors. Reproductomics. London: Academic Press; 2018. Chapter 2; p. 21-45. **http://dx.doi.org/10.1016/B978-0-12-812571-7.00003-4**.
4. Mierla D, Jardan D, Stoian V. Chromosomal abnormality in men with impaired spermatogenisis. Int J Fertil Steril. 2014;8(1):35-42. PMid:24696767.
5. Khatun A, Rahman MS, Pang MG. Clinical assessment of the male fertility. Obstet Gynecol Sci. 2018;61(2):179-91. **http://dx.doi.org/10.5468/ogs.2018.61.2.179**. PMid:29564308.
6. Suganya J, Kujur SB, Selvaraj K, Suruli MS, Haripriya G, Samuel CR. Chromosomal abnormalities in infertile men from southern India. J Clin Diagn Res. 2015;9(7):GC05-10. **http://dx.doi.org/10.7860/JCDR/2015/14429.6247**. PMid:26393143.
7. Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, et al. European academy of andrology guideline management of oligo-astheno-teratozoospermia. Andrology. 2018;6(4):513-24. **http://dx.doi.org/10.1111/andr.12502**. PMid:30134082.
8. Amouri A, Hammami W, Kilani O, Bouzouita A, Ayed W, Ben Meftah M, et al. Chromosomal evaluation in a group of Tunisian patients with non-obstructive azoospermia and severe oligozoospermia attending a Tunisian cytogenetic department. C R Biol. 2014;337(4):223-8. **http://dx.doi.org/10.1016/j.crvi.2014.02.006**. PMid:24702890.

9. Bojesen A, Gravholt CH. Klinefelter syndrome in clinical practice. Nat Clin Pract Urol. 2007;4(4):192-204. **http://dx.doi.org/10.1038/ncpuro0775**. PMid:17415352.
10. Hammami W, Kilani O, Khelifa MB, Ayed W, Bouzouita A, Zhioua F, et al. Genetic diagnosis in non-obstructive azoospermic tunisian men. Austin J Reprod Med Infertil. 2015;2(2):1012.
11. Mackic-Djurovic M, Hasic S, Kiseljakovic E, Rukavina D, Ibrulj S. Cytogenetic abnormalities found in patients with reproductive problems. Med Arh. 2017;71(6):396-9. **http://dx.doi.org/10.5455/medarh.2017.71.396-399**. PMid:29416198.
12. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocites cultured from human peripheral blood. Exp Cell Res. 1960;20(3):613-6. **http://dx.doi.org/10.1016/0014-4827(60)90138-5**. PMid:13772379.
13. Scheres JM. Identification of two Robertisonian translocation with a Giemsa technique. Humangenetik. 1972;15(3):253-6. PMid:4117149.
14. Simons A, Shaffer LG, Hastings RJ. Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res. 2013;141(1):1-6. **http://dx.doi.org/10.1159/000353118**. PMid:23817294.
15. Krausz C. Male infertility: pathogenesis and clinical diagnosis. Best Pract Res Clin Endocrinol Metab. 2011;25(2):271-85. **http://dx.doi.org/10.1016/j.beem.2010.08.006**. PMid:21397198.
16. Walsh TJ, Pera RR, Turek PJ. The genetics of male infertility. Semin Reprod Med. 2009;27(2):124-36. **http://dx.doi.org/10.1055/s-0029-1202301**. PMid:19247914.
17. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet. 2004;364(9430):273-83. **http://dx.doi.org/10.1016/S0140-6736(04)16678-6** PMid:15262106.
18. Christofolini DM, Mafra FA, Pedrenho No R, Barros RASA, Santos AA, Peluso C, et al. Correlation between Chromosomal Variants and Male Infertility in a Population of Brazilian Infertile Men. Reprod Syst Sex Disord. 2012;1(01):1. **http://dx.doi.org/10.4172/2161-038X.1000105**.
19. Purandare H, Fernandes NV, Deshmukh SV, Chavan S. Heterochomatic variations and losses in Humans. Int J Hum Genet. 2011;11(3):167-75. **http://dx.doi.org/10.1080/09723757.2011.11886139**.
20. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab. 2007;92(3):762-70. **http://dx.doi.org/10.1210/jc.2006-1981**. PMid:17213277.
21. Aksglaede L, Juul A. Testicular function and fertility in men with Klinefelter syndrome: a review. Eur J Endocrinol. 2013;168(4):R67-76. **http://dx.doi.org/10.1530/EJE-12-0934** PMid:23504510.
22. Wistuba J, Brand C, Zitzmann M, Damm OS. Genetics of klinefelter syndrome: experimental exploration. In: Vogt PH, editor. Genetics of human infertility. Basel: Karger; 2017. Vol. 21, p. 40-56. **http://dx.doi.org/10.1159/000477277**.
23. Wikström AM, Dunkel L. Klinefelter syndrome. Best Pract Res Clin Endocrinol Metab. 2011;25(2):239-50. **http://dx.doi.org/10.1016/j.beem.2010.09.006**. PMid:21397196.
24. Bojesen A, Høst C, Gravholt CH. Klinefelter's syndrome, type 2 diabetes and the metabolic syndrome: the impact of body composition. Mol Hum Reprod. 2010;16(6):396-401. **http://dx.doi.org/10.1093/molehr/gaq016**. PMid:20231162.
25. Yatsenko AN, Yatsenko SA, Weedin J, Lawrence AE, Patel A, Peacock S, et al. Comprehensive 5-Year study of cytogenetic aberrations in 668 infertile men. J Urol. 2010;183(4):1636-42. **http://dx.doi.org/10.1016/j.juro.2009.12.004**. PMid:20172548.
26. Tincani BJ, Mascagni BR, Pinto RD, Guaragna-Filho G, Castro CC, Sewaybricker LE, et al. Klinefelter syndrome: an unusual diagnosis in pediatric patients. J Pediatr (Rio J). 2012;88(4):323-7. **http://dx.doi.org/10.2223/JPED.2208**. PMid:22915094.
27. Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, et al. Ageas a limiting factor for successful sperm retrieval in patients with non mosaic Klinefelter's syndrome. Fertil Steril. 2005;84(6):1662-4. **http://dx.doi.org/10.1016/j.fertnstert.2005.05.053**. PMid:16359961.
28. Forti G, Corona G, Vignozzi L, Krausz C, Maggi M. Klinefelter's syndrome: a clinical and therapeutical update. Sex Dev. 2010;4(4-5):249-58. **http://dx.doi.org/10.1159/000316604**. PMid:20664188.
29. Schlegel PN. Non obstructive azoospermia: a revolutionary surgical approach and results. Semin Reprod Med. 2009;27(2):165-70. **http://dx.doi.org/10.1055/s-0029-1202305**. PMid:19247918.
30. Franik S, Smeets D, Van Zande G, Gomes I, D'Hauwers K, Braat DDM, et al. Klinefelter syndrome and fertility-Impact of X- chromosomal inheritance on spermatogenesis. Andrologia. 2018;50(5):e13004. **http://dx.doi.org/10.1111/and.13004**. PMid:29512178.
31. Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab. 2005;90(11):6263-7. **http://dx.doi.org/10.1210/jc.2004-2322**. PMid:16131585.
32. Staessen C, Tournaye H, Van Assche E, Michiels A, Van Landuyt L, Devroey P, et al. PGD in 47,XXY Klinefelter's syndrome patients. Hum Reprod Update. 2003;9(4):319-30. **http://dx.doi.org/10.1093/humupd/dmg029**. PMid:12926526.
33. Denschlag D, Tempfer C, Kunze M, Wolff G, Keck C. Assisted reproductive techniques in patients with Klinefelter syndrome: a critical review. Fertil Steril. 2004;82(4):775-9. **http://dx.doi.org/10.1016/j.fertnstert.2003.09.085**. PMid:15482743.
34. Vogt PH, Fernandes S. Polymorphic DAZ gene family in polymorphic structure of AZFc locus: artwork or functional for human spermatogenesis? APMIS. 2003;111(1):115-26. **http://dx.doi.org/10.1034/j.1600-0463.2003.11101161.x**. PMid:12752250.

35. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. Clinics (São Paulo). 2011;66(4):691-700. **http://dx.doi.org/10.1590/S1807-59322011000400026**. PMid:21655766.

36. Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018;62:2-10. **http://dx.doi.org/10.1016/j.clinbiochem.2018.03.012**. PMid:29555319.

***Correspondence**

Roberta Machado de Oliveira Frota Curado
Universidade Federal de Goiás (UFG), Programa de Pós-graduação em Ciências da Saúde, Faculdade de Medicina
Address: Rua J-35 qd. 67 lt.22 nº 231, Setor Jaó, zip code: 74673-530, Goiânia, Goiás, Brasil.
Phone number: +55 (62) 98120-2018
E- mail: robertafrota@hotmail.com

Authors information

RMOFC- Biomedical, Master in Health Sciences, UFG.

BFG- Biologist, PhD in Genetics and Researcher CNPq at the Biological Sciences Institute, UFG.

LARB- Biomedical, PhD in Genetics, Professor of the Institute of Biological Sciences, UFG.

MARF- Urologist, Professor of the Medical School, UFG.

MSA- Gynecologist and Obstetrician, PhD in Tocoginecologia, Professor of the Medical School and professor of the post-graduate health sciences program, Coordinator of the Laboratory of Human Reproduction, UFG.

WNA- Gynecologist and Obstetrician, PhD in Infectious and Parasitic Diseases, Professor of the Medical School and professor of the post-graduate health sciences program, UFG. NAB-. Biologist, PhD in Genetics, Professor of the Institute of Biological Sciences, UFG.

Authors contribution

All authors participated in each step of research and revising it critically for important intellectual content. All authors gave final approval for final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

RMOFC- Development of the research project, reading related literature, processing of samples, execution of techniques, analysis and interpretation of data for the work.

BFG- Correction and interpretation of data for the work, discussion and writing of the text.

LARB- Substantial contributions to design of the work, collaborated with the training of techniques, discussion and writing of the text.

MARF- Clinical evaluation and selection of patients for the study, discussion and writing of the text.

MSA- Orientation of the study, selection of patients for the study and collaborated with information male infertility, discussion and writing of the text.

WNA- Substantial contributions to the conception the work, correction and interpretation of data for the work, discussion and writing of the text.

NAB- Coordinator of the project, participated in the experimental design, collaborated in the development of experiments and written work, correction and interpretation of data for the work, discussion and writing of the text.