Chromosomal abnormalities associated with male infertility in Baghdad Iraq
علاقة التشوهات الكروموسومية بعقم الرجال في بغداد، العراق

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Abstract

In the current study, 142 patients (109 azoospermia and 33 severoligospermia) were studied in order to explore the cytogenetic cause's background and the hormonal study of male infertility in Baghdad Strip of Iraq. Of the 142 infertile males,14 patients showed abnormal chromosomal karyotypes corresponding to a frequency of 9.85% (14/142), the occurrence of chromosomal abnormalities was only confined to the azoospermia patient group. Patients with abnormal karyotypes represented 12.84% (14/109) of the azoospermia patients. Nine had a 47, XXY karyotype of Klinefelter's syndrome [47XY;64.28% (9/14)], [47XY+mar;14.28% (2/14)], [46XY,del13(p12) and (del21(p12);7.14% (1/14)], [46XY,tra(5;12, -6); 7.14% (1/14)] and one with chromosomal instability that showed multiple mosaic karyotypes 7.14% (1/14). The hormonal levels of nine patients with Klinefelter's syndrome (KFS) were diagnosed in the present study, and the results showed elevated FSH and LH concentrations compared with control. While serum testosterone values were below the control.

Key words: male infertility, cytogenetic, abnormal chromosom

الملخص

استخدمت في الدراسة الحالية 142 مريض (109 يعانون انعدام الحيامن و33 قلة الحيامن الشديدة) لتوضيح التغيرات الوراثية الخلوية في بغداد/العراق، من بين 142 مريض 14 ذكر غير خصب يعانون من تشوهات كروموسومية بنسبة 9,85 %، اقتصر حدوث التشوهات الكروموسومية على المرضى الذين يعانون من انعدام الحيامن. نسبة المرضى الذين يعانون من التشوهات الكروموسومية 12,84 % من مرضى انعدام الحيامن وتسعة مرضى لديهم متلازمة كلنفلتر 47XXY : [47XY;64.28% (9/14)], [47XY+mar;14.28% (2/14)], [46XY,del13(p12) and (del21(p12);7.14% (1/14)], [46XY,tra(5;12, -6); 7.14% (1/14)] ومريض واحد يعاني من عدم الاستقرار الكروموسومي الذي اظهره التصنيف المزائيكي المتعدد. كما تم دراسة مستوى الهرمونات في المرضى الذين يعانون من متلازمة كلنفلتر والبالغ عددهم (9). اظهرت النتائج ارتفاع في مستوى الهرمون المحفز لنمو الجريبات والهرمون اللوتيني مقارنة مع مجموعة السيطرة, بينما اظهرت النتائج انخفاض في مستوى هرمون التيستوستيرون اقل من مجموعة السيطرة.

الكلمات الدالة: عقم الرجال، التغيرات الوراثية الخلوية، التشوهات الكروموسومية

Introduction

Male causes for infertility are found in about 50% of infertile couples [1,2]. Reduced male fertility can be a result of congenital and/or acquired abnormalities. They include infections of the genital tract, varicocele, developmental and anatomical abnormalities, endocrinopathies, immunological factors, environmental exposures, and genetic abnormalities. Frequently, however, male infertility is difficult to diagnose, and about 60-75% of cases remain idiopathic. These idiopathic cases present with no previous history associated with fertility problems and have normal findings on physical examination [3].

Congenital abnormalities include a history of testicular maldescent, karyotype abnormalities, and azoospermia (sperm concentration is 0×10^6/ml) due to congenital agenesis of the vasa deferentia [4]. Karyotype abnormalities like in Klinefelter's syndrome that characterized by the presence of one or a number of extra X chromosomes, and in Down syndrome that associated with moderate to severe reduction in sperm production, also a number of rare complex genetic syndromes can affect fertility in men [5]. In case of Y-chromosome gene deletion, micro deletion are more prevalent in infertile individuals, and deletions can cause severe spermatogenic defects ranging from non-obstructive azoospermia to oligozoospermia [6].

Aneuploidies can be easily diagnosed after performing G banding using trypsin and Giemsa (GTG) karyotyping. Therefore, GTG karyotyping is certainly a mandatory test in the diagnostic workup of any infertile man.

Klinefelter's syndrome is the most frequent sex chromosome abnormality [7,8]. Adult men with Klinefelter's syndrome have small firm testicles devoid of germ cells. The phenotype can vary from a normally virilised man

to one with androgen deficiency, including female hair distribution, scanty body hair and long arms and legs because of late epiphyseal closure. Leydig cell function is commonly impaired in men with Klinefelter's syndrome [9].

The hypothalamus-pituitary endocrine system regulate the hormonal events that required to the normal testicular function. Hypothalamus stimulated the pituitary gonadotropins which are : Luteinizing Hormone (LH) stimulate the production of testosterone , and Follicle-Stimulating. Hormone (FSH) which stimulate the production of seminiferous fluid [5]. Normal levels of LH and FSH are necessary for maintenance of spermatogenesis, disorders of the pituitary or hypothalamus will cause inadequate gonadotropin stimulation of the testis and th at will lead to problems with fertility [5].

Normal FSH concentration may indicate obstruction of sperm transport. Elevated FSH concentration may suggest severe defects in spermatogenesis, but in men with reduced testicular volume and signs of hypoandrogenism with the presence of high FSH level may indicate primary testicular failure, but if FSH is not elevated in these men that may due to failure of the hypothalamo-pituitary function or to pituitary tumor [10].

Materials and Methods

In the present study we used 142 patients (109 azoospermia and 33 severoligospermia) and 15 fertile male as control group were studied in order to explore the cytogenetic cause's background and hormonal levels of male infertility in Baghdad Strip of Iraq.

Blood Sampling

Five ml of blood was collected by vein puncture obtained from some Baghdad Hospitals and clinics doctors from February 2013 till May 2014. Each collected blood sample was dispensed into tubes Heparinzed tubes for cytogenetic studies and obtained plasma for hormonal studies.

Cytogenetic Analysis

Lymphocyte cultures were set up in the laboratory by adding 0.5 ml of heparinized blood to 4.5 ml of modified RPMI-1640 medium Quantum PBL(Proplem-based learing) supplemented with L-Glutamine, Fetal calf serum and penicillin and streptomycin (10000 I.U), and phytohemaglutinin (PAA, Austria). Cells were incubated for 70 h in a 5% CO2 incubator. Colcemide (PAA, Austria) at concentration 1 µg/ml was added to the cultures and incubated at 37ºC inside water path for 35-40 min. The cultures were then centrifuged at 10000 rpm for 10 min. The pellet was resuspended in hypotonic solution (KCl, 0.075M, PAA, Austeria) and immediately centrifuged at 10000 rpm for 10 min, and resuspended in freshly prepared, ice-cold fixative containing methanol: acetic acid (3:1) (Merck, Darmstadt, Germany), left for 20 min at room temperature. The solution was then centrifuged at 10000 rpm for 10 min, and the pellet was resuspended in freshly prepared ice-cold fixative containing methanol:acetic acid (3:1). If the solution was not clear after additional centrifugation, the last step was repeated until a clear solution was obtained. After decantation to reduce the volume to about 1 ml, the pellet was mixed with the remaining fixative and dropped from about 30-80cm with a Pasteur pipette onto an ethanol washed slide; the fixative was removed by slight blowing, decantation and air-drying. Subsequently, the slides were stained in 5% Giemsa solution for 10 min. [11]

Karyotyping

GTG (Giemsa-Trypsin) banding technique was performed. When the banding of the chromosomes was not successful, the protocol was repeated. After staining, at least 20 metaphase plaques were analysed for each sample by using cytovision.

Hormonal Assay

Testosteron , FSH , LH , and prolactin were determined by using miniVIDAS Bio merieux/ Italia.

Statistical Analysis

The statistical SPSS version (13) program was used to analyze the level of hormones Significant differences were obtained according to ANOVA.

Results and discussion

Cytogenetic analysis by GTG banding

Among the 142 infertile patients included in this study, 14 patients showed abnormal chromosomal karyotypes which represent 9.85% (Table.1and Figure.1) The occurrence of chromosomal abnormalities was only confined to the azoospermia patients group. Patients with abnormal karyotypes represented 12.84% (14/109) of the azoospermia patients. Nine had a 47, XXY karyotype (Klinefelter's syndrome) and represented 6.33%,

64.28% (9/14) of the chromosomal abnormalities patient group, and 8.25% (9/109) of the azoospermia patient group Figure (2). Two patient (case 53,126) had 47,XY, +mar karyotype, and represented 1.40% (2/142) of the patient study population, 14.28% (2/14) of the chromosomal abnormalities patient group, and 1.83% (2/109) of the azoospermia patient group Figure (3). One patient (case 93) had [46,XY,del13(p12) and del21(p12)] karyotype, Figure (4) and One patient (case 87) had 46,XY, tra(5;12, -6) karyotype, Figure (5) and One patient had a chromosomal instability with a heterogeneous scale of mosaic aneuploides, Figure (6). represented 0.7% (1/142) of the patient study groups, 7.14% (1/14) of the chromosomal abnormalities patient group, and 0.91% (1/109) of the azoospermia patient group.

Table (1): Abnormal cytogenetic results among azoospermia patient

Case	Karyotype
11	**47,XXY**
31	**47,XXY**
54	**47,XXY**
63	**47,XXY**
69	**47,XXY**
77	**47,XXY**
96	**47,XXY**
121	**47,XXY**
128	**47,XXY**
53	**47,XY,+mar**
126	**47,XY,+mar**
72	**Chromosomal instability (multiple karyotypes)**
93	**46,XY,del13(p12) and del21(p12)**
87	**46,XY,tra(5;12)**

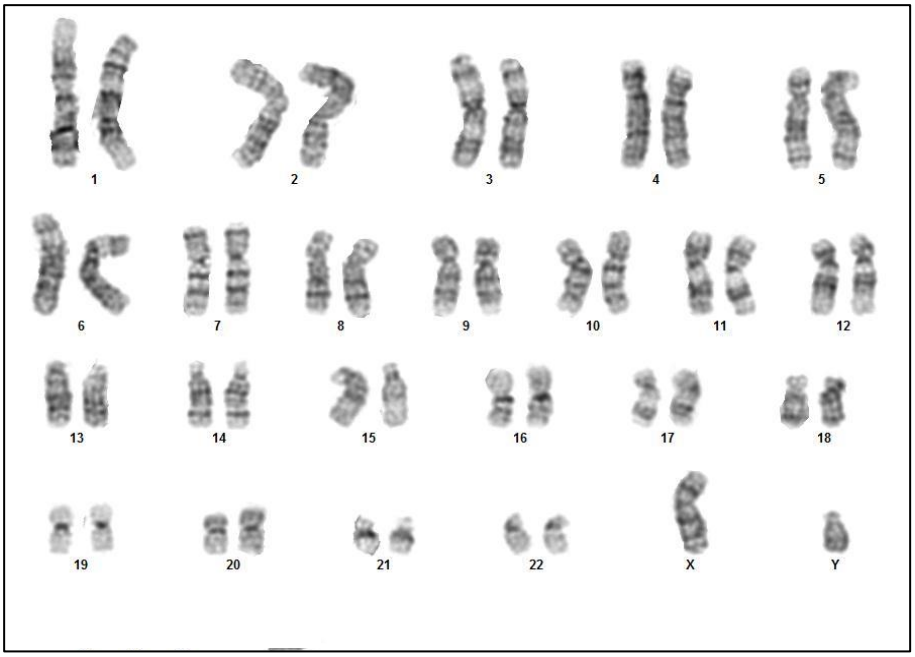

Fig. (1): Showing one X and one Y chromosome in addition to 22 pairs of somatic chromosomes (case 3)

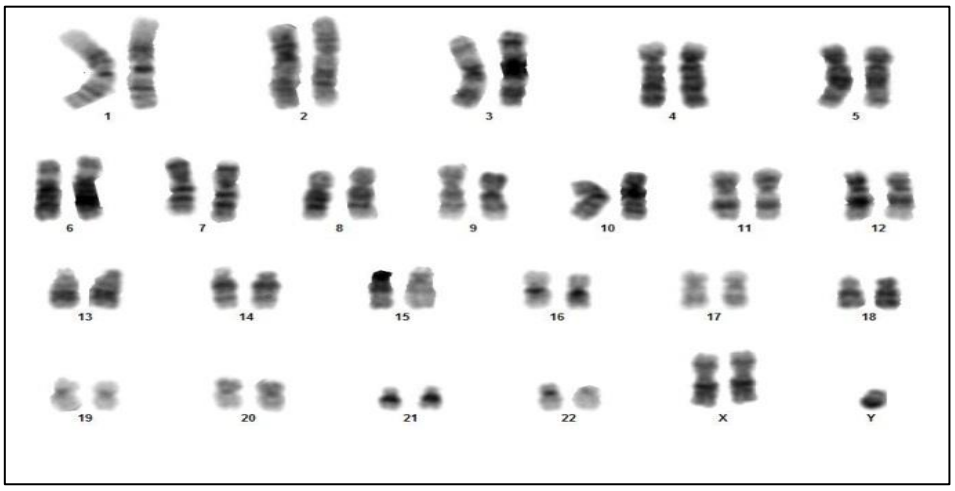

Fig. (2): Karyotype of one Klinefelter's syndrome patient showing 47,XXY (case 31)

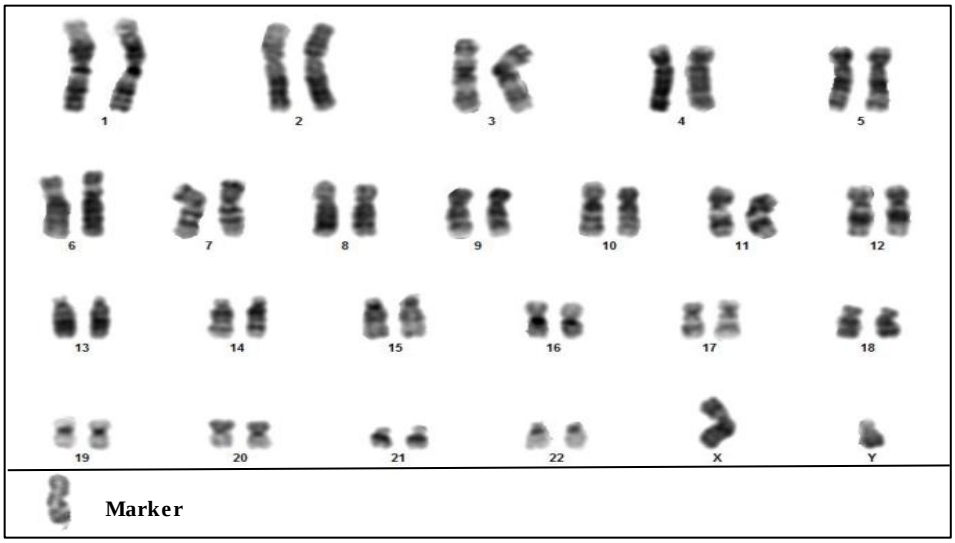

Fig. (3): 47,XY,+mar karyotype showing extra marker chromosome (case 126)

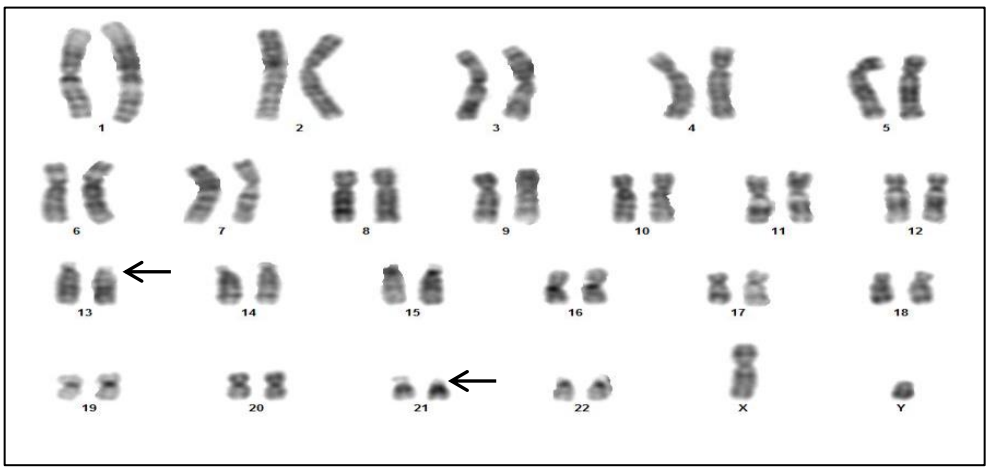

Fig. (4): 46,XY, del(13)(p12) and del(21)(p12) karyotype (case 93)

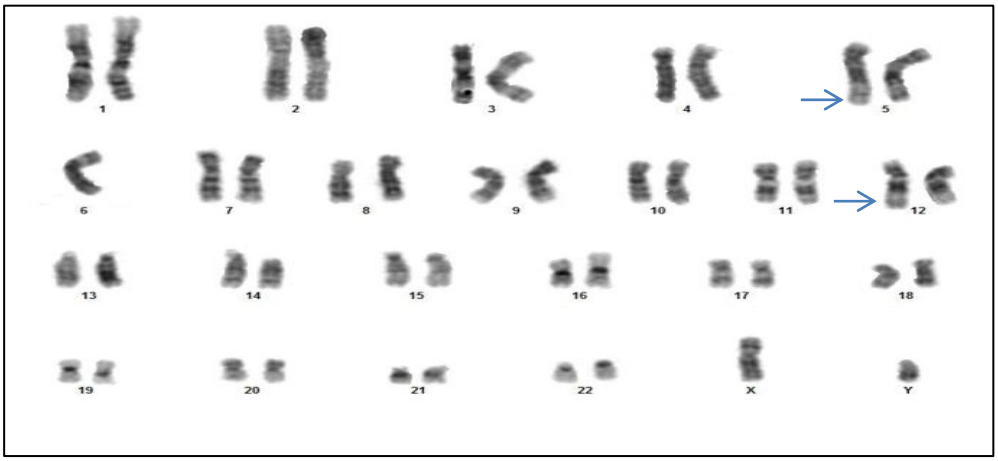

Fig. (5): 45,XY, tra(5;12) , -6 chromosome karyotype (case 87)

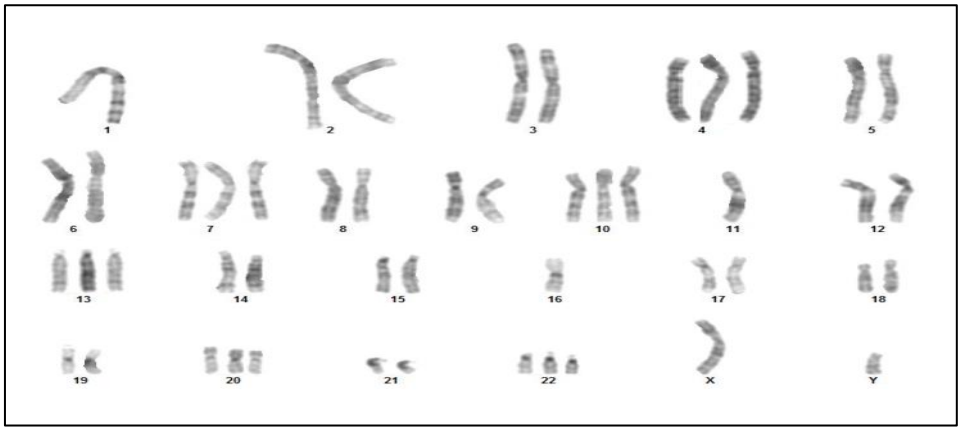

Fig. (6): Hyperdiploid cell karyotype: 49,XY,-1,+4,+7,+10,-11,+13,-16,+20,+22 (case 72)

Klinefelter's Syndrome Patients (KFS)

Nine patients with Klinefelter's syndrome (KFS) were diagnosed in the present study all showed elevated FSH and LH concentrations and means (32.14±3.84 mIU/ml) , (20.32±2.38 mIU/ml) compared with control (5.73±1.85 mIU/ml) , (4.28±1.03 mIU/ml), respectively Figure (7). Serum testosterone values mean was (3.21±1.15 ng/ml) all were below the control (13.25±3.16 ng/ml) Figure (8).

All of the patients were azoospermic, and all had small testes

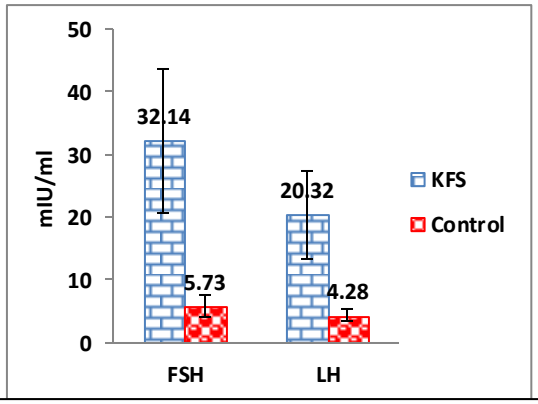

Fig. (7): FSH and LH levels in KF patients and control

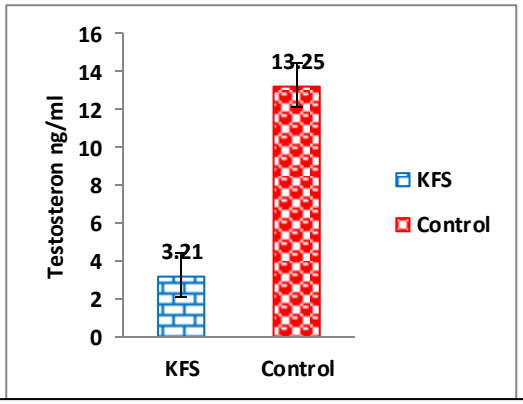

Fig (8): Testosteron level in KF patients and control

No signs of hypergonadotropism were detectable in the KFS boys during prepuberty or early puberty. After midpuberty, however, concomitantly with elevations in basal FSH and LH levels, their response to GnRH stimulation became abnormal [12].

These observations are in agreement with earlier findings [13,14] and suggest diminished testicular inhibition of gonadotropin secretion. In addition, the boys with KFS developed after midpuberty low T/LH ratios. This timing is in agreement with gradual appearance of Leydig cell hyperplasia during midpuberty [15]. Such changes in activity of the hypothalamicpituitary-testicular axis thus probably represent a state of compensated hypergonadotropic hypogonadism attributed to diminished responsiveness of Leydig cells to LH with advancing puberty.

In the present study, both numerical and structural chromosomal aberrations were found in our patient study population. The occurrence of the chromosomal abnormalities was only confined to the azoospermia patient group; this could be the effect of the small size and slightly unbalanced nature of our patient study population (azoospermics 109 and severely oligozoospermia 33). The prevalence of chromosomal anomalies among the studied infertile men was found to be 9.85%. This lies within the previously published range (3.6-22.6%), as shown in Table (2). Association between human male infertility and chromosomal anomalies has been known for a long time [16,17].

Chromosomal abnormalities are more frequently observed in the population of azoo/oligozoospermic males than in the general population [18]. Thus, it would not be unusual to find chromosomal abnormalities in men attending infertility clinics.

Table (2): Literature reporting chromosomal abnormalities in infertile men

Source	Study subjects (n)	Chromosomal abnormalities (%)
Kondoh *et al.*, 1992	**130**	**13.8**
Pandiyan and Jequier, 1996	**1210**	**3.6**
Gündüz *et al.*, 1998	**102**	**15.7**
Kleiman *et al.*, 1999	**72**	**16.6**
Akeel *et al.*, 2001	**64**	**12.5**
Penna Videa. *et al.*,2001	**84**	**22.6**
Nagavenker *et al.*, 2005	**88**	**10.2**
Pina-Neto *et al.*, 2006	**165**	**9.6**
Hellani *et al.*, 2006	**257**	**3.9**
Mohammed *et al.*, 2007	**289**	**8.0**
Shaqalah, 2007	**85**	**9.4**
Samir *et al.*, 2011	**217 azoospermia**	**16.28**
	92 oligospermia	**5.56**
This study, 2014	**142**	**9.85**

The distribution of chromosomal abnormalities detected in the present study showed that Klinefelter's syndrome (47,XXY) was the most prevalent abnormality, representing 64.28% (9/14) of our positive cases. This result was in agreement with several previously published studies [19,20]. Our result was not unexpected since Klinefelter's syndrome was described as the most frequent genetic cause of male infertility [21,22].

The exact mechanism by which chromosomal anomalies induce infertility is not clear. It is likely that the presence of abnormally distributed chromatin may interfere with meiotic division, therefore, reduces sperm production [23]. Testicular histology in such patients may reveal areas of atrophy and hyalinization of the seminiferous tubules as well as some areas with tubules of normal appearance that contain a reduced number of mature spermatozoa [24].

Spermatozoa bearing abnormal chromosomes may cause abnormal embryonic development that can in turn cause early pregnancy loss [25]. Moreover, these chromosomal aberrations may have serious implications for infertile males who seek the help of intra-cytoplasmic sperm injection (ICSI) due to the possibility of transmission of these abnormalities to the offspring [26,27].

To conclude, chromosomal abnormalities found with relatively high prevalence in our infertile males are the major cause of their male infertility, and justify the requirement of cytogenetic analysis for every infertile male, particularly azoospermics, seeking children.

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