DETECTION OF CHROMOSOMAL ABNORMALITIES AND SPERM DISOMY IN IDIOPATHIC SEVERLY INFERTILE MALES DUE TO ENVIRONMENTAL FACTORS

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ABSTRACT

The association between infertility and sperm disomy is well documented. Results vary but most report that men with severely compromised semen parameters have a significantly elevated proportion of disomic sperm. Recently, many studies have found a decrease in semen quality due to occupational hazards . Generally, occupational exposures have been divided into physical exposures (heat and radiation), chemical exposures (solvents and pesticides), psychological exposures (distress), exposure to metals and welding. This study aimed to determine the incidence of chromosomal abnormalities and sperm disomy in infertile men with idiopathic severe oligoasthenoteratozoospermia (OAT) and in idiopathic infertile males with normal semen parameters who were exposed to various environmental factors . Thirty male subjects were included in this study ten infertile men with severe OAT (group 1) , ten with idiopathic infertile men with normal semen parameters (group 2) and ten fertile male as control (group 3). The participants of both groups (group 1 and group 2) were exposed to various hazardous environmental factors such as physical and chemical factors and personal habits .Through clinical examination and lab investigations semen analysis and hormonal assays were done . Cytogenetic studies were done that included FISH assessment of sperm using cocktail X,Y

prope to detect the disomic level of chromosomes X and Y .Total disomy percent showed non significant difference within the three groups. Total disomy percent showed significant positive correlation with the number of environmental factors in severe OAT patients and idiopathic infertile patients. Age showed non significant positive correlation with total disomy percent within the three groups.
Keywords: Chromosomes, FISH, male infertility, semen, spermatozoa, environment, lifestyle.

INTRODUCTION

Infertility is a common disease, affecting between 17 and 25% of all couples and is defined as the inability of a couple in reproductive age to conceive following 12 months of unprotected intercourse. (Singh and Agarwal, 2011). Several studies have shown an increased incidence of chromosomal abnormalities in infertile males (Chandley, 1979; Chandley *et al.*, 1984; Retief *et al.*, 1984; Kayed *et al.*, 2006). In patients with sperm counts below 10×10^6/ml, the rate of chromosomal aberrations is estimated to be 5–7%. The percentage of cytogenetically abnormal cases increasing up to 10–15% in patients with azoospermia. Most frequently, sex chromosome aneuploidies are reported (De Braekeleer and Dao, 1991). Nevertheless, the presence of chromosomal abnormality in the female partner could influence the outcome of intra-cytoplasmic sperm injection (ICSI) (Schreurs *et al.*, 2000; Shi and Martin, 2000; Kayed *et al.*, 2006). Semen analysis evaluates certain characteristics of a male's semen and the sperm contained therein. It is done to help the evaluation of male fertility, whether for those seeking pregnancy or verifying the success of vasectomy.

World Health Organization (WHO) has changed the reference ranges used in semen analyses. The new normal values are volume ≥1.5/ml, sperm concentration >15 million/ml, total motility >40 % progressive motility, vitality >58 % normally viable, morphology >4 % showing normal morphology (WHO, 2010). Spermatogenesis requires a normal X and Y recombination to proceed properly.

A defect in the mechanism of recombination in the XY pairing in the pseudoautosomal region could lead to defect in spermatogenesis and subsequent oligozoospermia (Myers *et al.*, 2014).

Idiopathic OAT (iOAT) is defined as defective spermatogenesis of unknown aetiology being undetectable by the common clinical, instrumental or laboratory methods. It affects approximately 30% of infertile men and is usually diagnosed by exclusion. Severe OAT cases have sperm concentration <5 millions/ ml (Cavallini, 2006; WHO, 2010). An increased rate of gonosomic aneuploidy was suggested in patients with oligozoospermia compared with the normal population (Martin *et al.*, 2003; Nagvenkar *et al.*, 2005; Mehdi *et al.*, 2006; Di Santo *et al.*, 2016).

The problem of genetic sperm defects should be seriously considered when these spermatozoa are used for assisted reproduction, due to the high risk of transmission of these genetic defects to the offspring (Collodel and Moretti, 2006).

The infertility could be attributed to poor nutrition, stress, eating disorders, intense exercise and exposure to environmental toxins. Many evidences suggested that, there are environmental reasons for deteriorating

sperm quality which cause infertility, including occupational exposure to various chemicals, heat, radiation, and heavy metals. (Lahdetie, 1995; Mieusset *et al.*, 1998; Emokpae and Uadia, 2015). In addition, exposure to environmental estrogens and pesticides has been linked to alterations in spermatogenesis.

Lifestyle risk factors are also significant, including cigarette smoking, alcohol consumption, chronic stress, and nutritional deficiencies. (Bansal *et al.,* 2015) .Cigarette smoking has been associated with decreased sperm count, alterations in motility, and an overall increase in the number of abnormal sperm. (Harlev *et al.,* 2015). Fluorescent In Situ Hybridization (FISH) is a good tool to evaluate male infertility through the assessment of the percentage aneuploid sperms in an ejaculate as a preliminary step towards understanding the association of male infertility and chromosome segregation. (Ramasamy *et al.,* 2015).

In this study we aimed to estimate the frequency and type of chromosomal aberrations in peripheral blood of patients with idiopathic severe OAT who were exposed to various environmental hazardous factors such as occupational exposure to various chemicals, heat, radiation, heavy metals, pesticides، smoking ،etc. Also to determine the incidence of sperm disomy in cases of male infertility with idiopathic severe OAT and correlate sperm analysis and clinical examination with cytogenetic results for better assessment of reproduction function of the patients with idiopathic severe OAT and to help them to get normal baby through assisted reproduction technique.

MATERIALS AND METHODS

1.Materials: This study included thirty male subjects: ten infertile men with severe OAT and ten idiopathic infertile men and ten fertile men as control . The participants of both groups (group 1 and group 2) were exposed to various hazardous environmental factors, physical factors such as heat, chemical factors such as pesticides ,paints, chemical solvents and heavy metals and personal habits such as smoking and addiction to some drugs like Tramadol.

Patients have been recruited from the Andrology outpatient clinic, National Research Center. Inclusion criteria comprised age (range 20–40 years), primary infertility for >1 year and sperm count <5 million sperm/ml for sever OAT and >5 million sperm/ml for idiopathic infertile men patients.

Patients having varicocele, malignancy, and liver or kidney diseases have been excluded. The participants have given informed written consent to participate in the study. Inclusion criteria for control group were normal semen analysis according to (WHO, 2010), free of any systemic & local diseases, fertile and having children in the last two years, their ages ranged from 20-40 years.

2.Methods:

1) Clinical and lab investigation:

a) Personal and medical history data were taken through clinical examination to exclude varicocele and other surgical causes.
b) Ultrasonography, was also done .
c) Conventional semen analysis was done, at least twice.

2) Cytogenetic evaluation :

a) Peripheral blood culture of G-banding technique .

- Peripheral blood lymphocyte micro-cultures were performed according to standard methods (Moorehead *et al.,* 1960; Hungerford, 1978).
- G-banding on metaphase chromosome was done according to (Verma and Babu, 2005).
- Twenty metaphases were analyzed for each case. Individual chromosomes were identified, arranged, and karyotyped according to International System for Human Cytogenomic Nomenclature ISCN (2016). Numerical chromosomal abnormalities include aneuploidy (monosomy, or trisomy) , polyploidy and structural chromosomal abnormalities including balanced abnormalities (inversions, translocations, or insertions), as well as unbalanced rearrangement abnormalities (deletions, duplications, marker chromosomes, ring, iso, or dicentric chromosomes) are registered.

b) Semen processing and FISH analysis

- The semen samples were prepared for FISH analysis according to Miharu *et al.* (1994) with minor modifications.
- FISH technique was done using Direct labeled cocktail X, Y probe (cytocell) (DXZ1 (spectrum green), DYZI (spectrum red)).
- Analysis of FISH was done using Ziess microscope with automated stage, couple to metasystem image analyzer .One thousand nuclei were analyzed for number of signals. Number of signals of X and Y were scored per nuclei.

STATISTICAL ANALYSIS

Numerical data were expressed as mean ± SD and range. The test of significant done by Student's t-test with ANOVA test. Correlations and there significant were tested by regression analysis. Comparisons and correlations were considered statistically significant of $P \leq 0.05$.

RESULTS

This study included thirty male subjects: ten infertile men with severe OAT(group 1) with a mean age of 29.6 ± 5.5 years (range 22–39 years) and ten idiopathic infertile men (group 2) with a mean age of 30.1 ± 3.98 years (range 26–39 years) both groups were exposed to various environmental factors like physical factors (heat) , chemical factors (pesticides ,paints and heavy metals) and personal habits like smoking and addiction to some drugs(Tramadol and alcohols). Ten normal fertile men subjects (group 3)were used as controls with a mean age of 29.1 ± 5.64 years (range 22– 40 years), as shown in table (1) .

A minimum of 1500 sperm nuclei per individual for each locus were evaluated in (group 1) and (group 2) , a minimum of 5000 sperm nuclei per individual in normozoospermic men; 165 000 sperm nuclei were evaluated in total.

Age showed non significant difference between the three groups (P = 0.95), highly significant negative correlation with ejaculated volume (r = -0.68 ,P = 2.89E-05),non significant negative correlation with sperm concentration (r = -0.20,P = 0.29) , significant negative correlation with motility (r = -0.35,P= 0.05) among the three groups .

Age showed significant positive correlation with total disomy percent between the three groups (r = 0.39, P=0.03).

Total disomy percent showed highly significant difference among the three groups (P=2.86E-09) and significant positive correlation with the number of environmental factors (r = 0.51,P=0.02) in group 1 and group 2, as shown in figure (1).

Total disomy percent showed highly significant negative correlation with ejaculated volume (r = -0.73 ,P = 5.42E-06), highly significant negative correlation with sperm concentration (r = -0.90,P = 2.50E-11) and highly significant negative correlation with motility (r = -0.89,P = 6.33E-11) among the three groups , as shown in figure (2) .

Number of environmental factors (smoking ,addiction, physical and chemical) showed highly significant negative correlation with ejaculated volume (r = -0.83 ,P = 4.84E-06),non significant negative correlation with sperm concentration (r = -0.32,P = 0.17) and non significant negative correlation with Motility (r = -0.36,P= 0.12) in group 1 and group 2, as shown in figure (1).

Cytogenetic investigations with GTG-banding technique in all subjects were 46,XY as shown in figure (3) ,except one patient of severe OAT men had structure chromosomal abnormality (translocation 9p,13q) and one patient in idiopathic infertile men had numerical sex chromosome abnormalities 46, XY/47, XXY as shown in figure (4).

DISSCUSSION

In humans, the most common chromosomal abnormality is aneuploidy. As the majority of aneuploid conceptuses die during the early stages of embryonic development, an accurate estimate of the frequency of aneuploidy at conception can only be assessed by directly studying the gametes (Templado *et al.,* 2013). Cytogenetic analyses of sperm nuclei, using FISH, are a preferred method for the evaluation of sperm chromosomal aneuploidy (Qui *et al.,* 2012). Different researchers had demonstrated that infertile men have an increased frequency of chromosome abnormalities in their spermatozoa compared with normal donors (Finkelstein *et al.,* 1998; Bernardini *et al.,* 2005; Templado *et al.,* 2005; Collodel *et al.,* 2007; Ferguson *et al.,* 2007). However, types of male infertility varied among these studies (i.e. oligo-, astheno-, oligoastheno-, oligoterato-, oligoasthenoterato-, asthenoteratozoospermia, unexplained fertility and anti-sperm antibodies) leading to different results.

It is possible that some types of infertility have an increased risk of sperm chromosome abnormalities, whereas others do not. Miharu *et al.,* (1994) and Guttenbach *et al.,* (1997) reported no difference in aneuploidy rate between fertile and infertile men. Several studies have used multiprobe FISH to investigate whether there is an association between sperm sex chromosome disomy and semen parameters.

In our work, we studied the disomic percentage of sex chromosome abnormalities in infertile severe OAT males, idiopathic infertile males and fertile men as control. Disomic percentage of sperm sex chromosomes were increased with the decrease in seminal parameters.

These results are similar to those reported by Rives *et al.,* 1999, Vegetti *et al.* 2000, Calogero *et al.,* 2001 ,they found a relationship between sex chromosome disomy and sperm concentration. Vegetti *et al.,* (2000) found a higher frequency of XY, XX and YY disomy in the spermatozoa of men with low semen quality than in those with high semen quality. In many studies about total sex chromosome disomy, a majority have shown that increase in sex chromosome disomy was associated with abnormal semen parameters when compared with normal controls (Aran *et al.,* 1999; Colombero *et al.,* 1999; Carrell *et al.,* 2003, Megan *et al.,* 2012; Younan *et al.,* 2015).

Parker *et al.,* (1994) pointed out the adverse effects of the socioeconomic status on reproduction. De Krester (1998) also noticed that the increase in regional frequency of male infertility and sperm abnormality over short period of time may be due to environmental factors including recent fashion for tight-fitting wearing.

Several reports on electronic gadgets like cellphone in the waist of men, laptops on the laps can influence abnormalities in the semen parameters. Salma *et al.* (2008) have pointed out that research field on air pollution and human reproduction needs inputs from toxicology, exposure assessment

and clinical research especially to aid in the identification and exposure of feto-toxic agents in ambient air, in the development of early markers of adverse reproductive outcomes and of relevant biological pathways (Poonguzali *et al.,* 2012).

Smoking decreases the success rates of assisted reproductive procedures, not only In vitro fertilization (IVF), but also in ICSI. Apart from putative adverse effects during fertilization, altered DNA in spermatozoa might hamper the development of the embryo. Cigarette smoking may be associated with sub-fertility in males, resulting in decreased sperm concentration, lower sperm motility, and a reduced percentage of morphologically normal sperms (Lewin *et al.,* 1991; Sofikitis *et al.,* 1995; Zinaman *et al.*, 2000; Harlev *et al.,* 2015). The meta-analysis (Vine, 1996) of 27 studies analysed the association between cigarette smoking and semen quality and have reported a significant difference in semen quality.

Sexual disorders have been reported in men who are long-term alcohol and drugs users, with a prevalence ranging from 8% to 58% (Schiavi, 1990). Lemere and Smith (1973) have reported that 8% of 17000 patients treated for alcoholism were impotent.

In our study, total disomic percent showed significant positive correlation with the number of environmental factors in group 1 and group 2.

Total disomy percent showed highly significant negative correlation with ejaculated volume, sperm concentration and motility among the three groups . The number of environmental factors showed highly significant negative correlation with ejaculated volume, non significant negative correlation with sperm concentration and motility in group 1 and group 2.

Cytogenetic investigations revealed chromosomal anomalies in 2 out of 30 patients, while the remaining individuals were found to be having normal Karyotype 46,XY. One patient in severe OAT men had structure chromosomal abnormality (translocation 9p,13q) and one patient in idiopathic

infertile men had sex chromosome abnormalities: two mosaic forms 46, XY/47, XXY.

CONCLUSION AND RECOMMENDATIONS

Several occupational and environmental exposures and toxins have known or suspected deleterious actions to male reproductive function. For some specific agents, such as smoking, addiction, heat, ionizing radiation, inorganic lead, DBCP, EDB, some ethylene glycol ethers, carbon disulfide and welding operations, the evidence is strongly supported in well-designed epidemiological studies.

Men with abnormal semen parameters should be given genetic counseling. Strategies should be developed to direct the attention of the general public

towards the possible relationship between the environmental factors and incidence of male infertility, Research on the role of environmental factors on male infertility is very young and the research field on this area is wide,

it necessitates collaboratory study from different field of science to uncover the local cause of male infertility. We should take notice of this early warning system and set about dispelling the ignorance that currently prevents us from understanding how our modern lifestyle impact on male infertility.

We explored the relationship between human sperm sex chromosome disomy, semen parameters and exposure to environmental factors or lifestyle, showing a positive correlation between total disomic percent and the number of environmental factors, a negative correlation between total disomic percent

and semen parameters and a negative correlation between the number of environmental factors and semen parameters.

The results of the present study also indicated that men with severe oligozoospermia tended to be at greater risk for XX disomy in their spermatozoa. Such an increase in XX disomy could lead to a slight increase in 47,XXX conception after ICSI. Therefore, when men with low sperm concentrations of 5 $X10^6$/ml undergo ICSI, even if they have a normal karyotype, it is important to inform them and their partners of the possible risks of aneuploidy in their fetus.

Table 1: Comparison between the studied groups regarding total disomy percent for chromosomes X, Y and semen parameters .Data are represented as mean ± SD and (range).*significant,**highly significant.

	Group 1 (n=10)	**Group 2 (n=10)**	**Group 3 (n=10)**
Age(year)[(a)]	29.6±39 (22-39)	30.1±3.98 (26-39)	29.1±5.64 (22-40)
Sperm count (10^6)[(b)]	2.02±1.01 (0.2-3)	40.3±21.76 (15-70)	68.4±10.93 (50-83)
Sperm motility (%)[(c)]	15.3±8.49 (5-30)	55.5±13.83 (5-80)	57.4±13.49 (40-73)
Ejaculate volume (ml)[(d)]	2±0.91 (0.5-3)	2.17±0.72 (1.5-4)	3.52±0.87 (2.5-5.4)
Total disomy (%)[(e)]	40±10.09 (30-58)	18.9±8.28 (6-30)	8±2.87 (5-12)
No. of envirnmental effects [(f)]	1.9±0.88 (1-3)	2±0.94 (1-4)	————

Group (1): idiopathic severe OAT, group (2):idiopathic infertile males with normal semen parameters and group (3)normal fertile males. a versus b: r = -0.20,P = 0.29 ;a versus c: r = -0.35,P= 0.05*; a versus d: r = -0.68 ,P = 2.89E-05**; a versus e: r = 0.39, P=0.03*; b versus e: r = -0.90,P = 2.50E-11**; b versus f: r = -0.32,P = 0.17; c versus f: r = -0.36,P= 0.12; c versus e: r = -0.89,P = 6.33E-11**; d versus e: r = -0.73 ,P = 5.42E-06**; d versus f :r = -0.83 ,P = 4.84E-06**; e versus f: r = 0.51,P=0.02*.

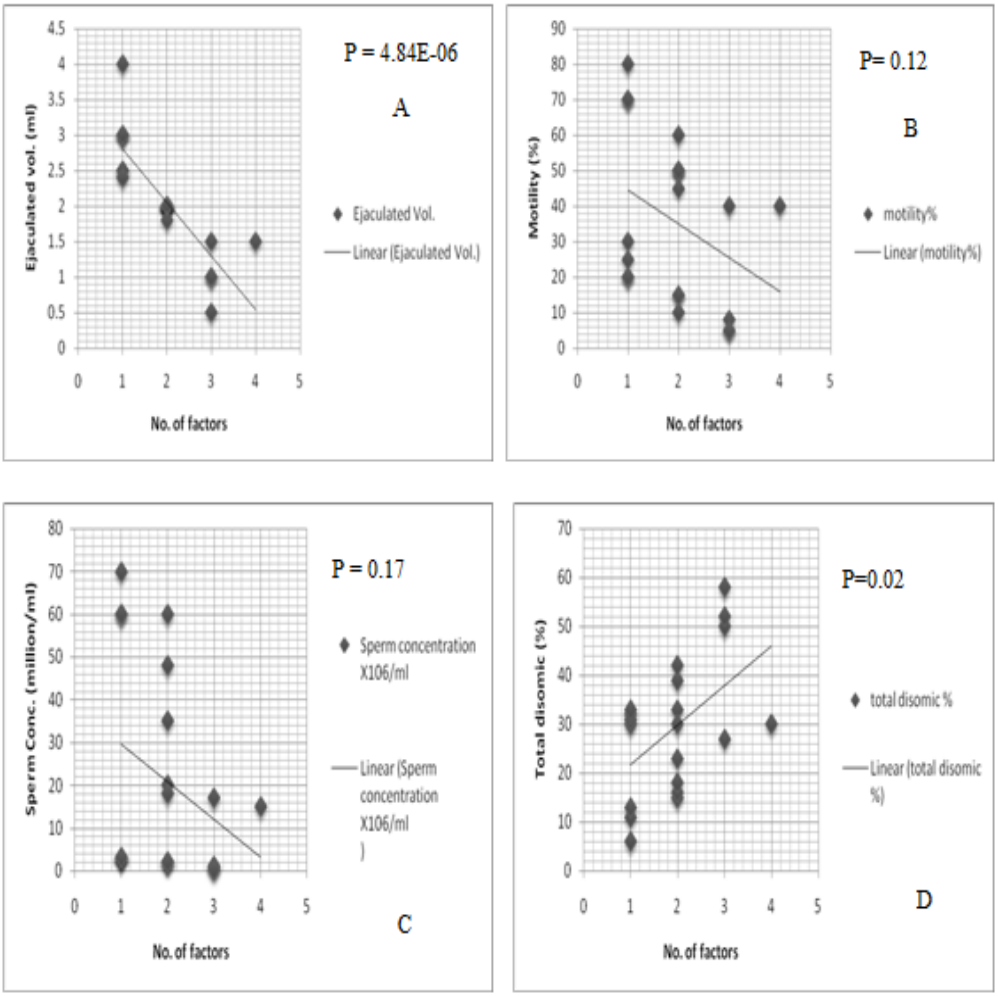


Figure 1: Correlations between number of environmental factors, semen parameters and total disomic percent. (A) represent highly significant negative,(B) represent non significant negative correlation, (C) represent non significant negative correlation and (D)represent significant positive correlation.

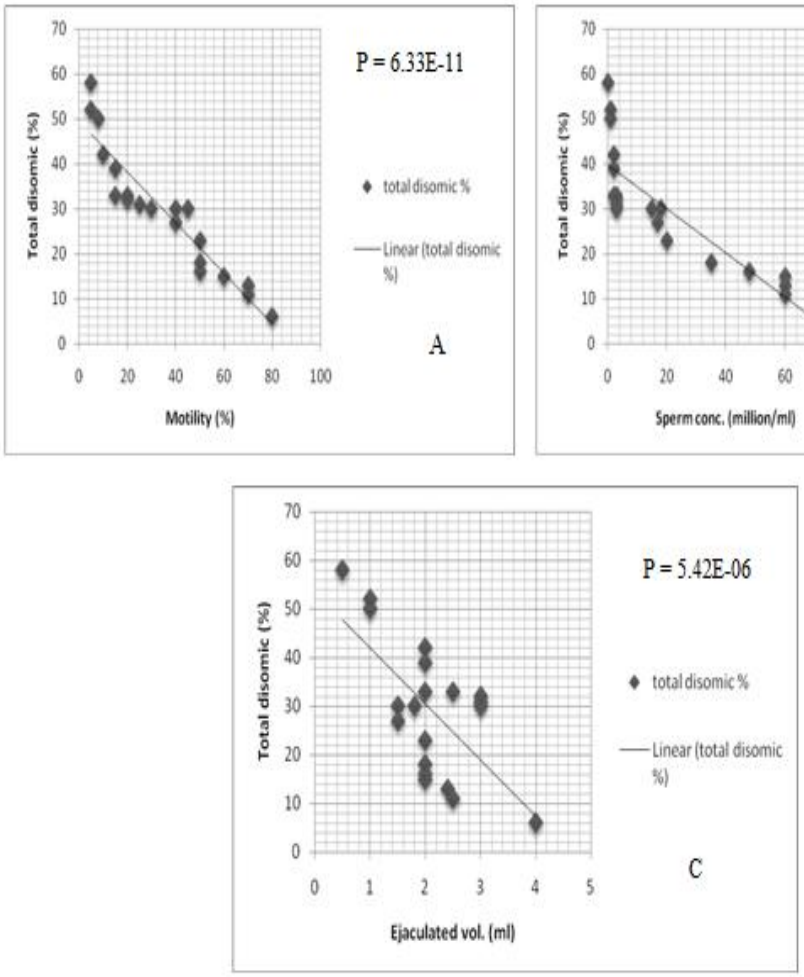
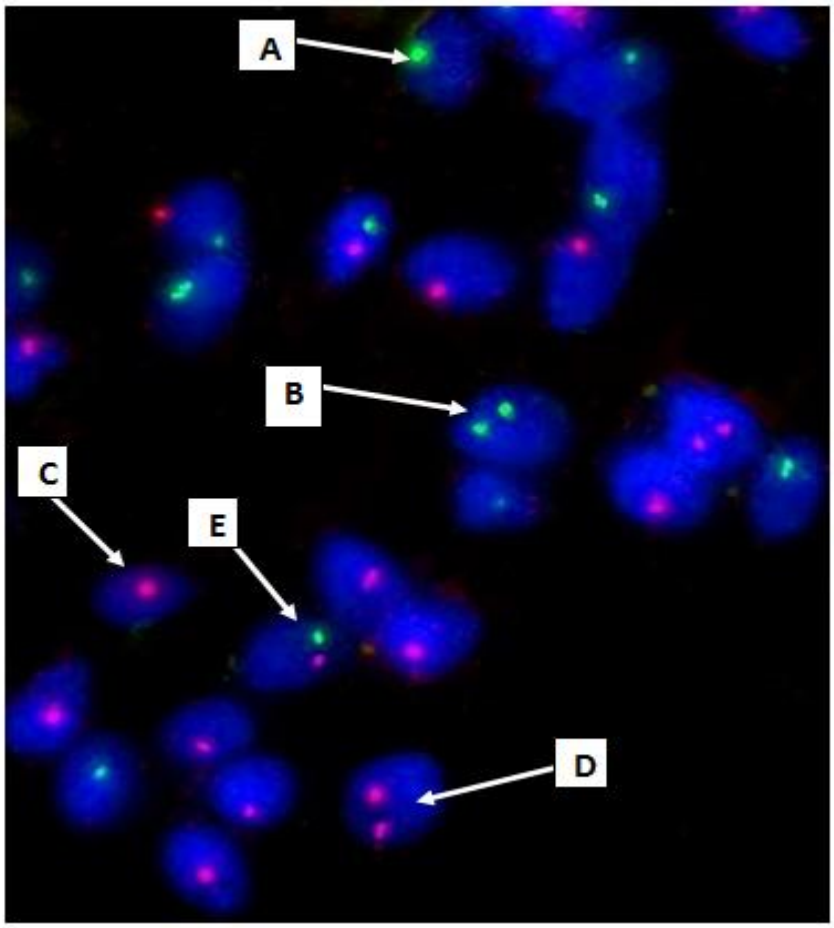


Figure 2: Correlations between total disomic percent and semen parameters. (A) represent highly significant negative correlation,(B) represent highly significant negative correlation and (C) represent highly significant negative correlation .



(*) Chromosome X labeled in green and Y labeled in red.

Figure 3: Image of sperms red showing XY(E) ,XX (B),YY (D) disomic and X(A) ,Y(C) monosomic.*

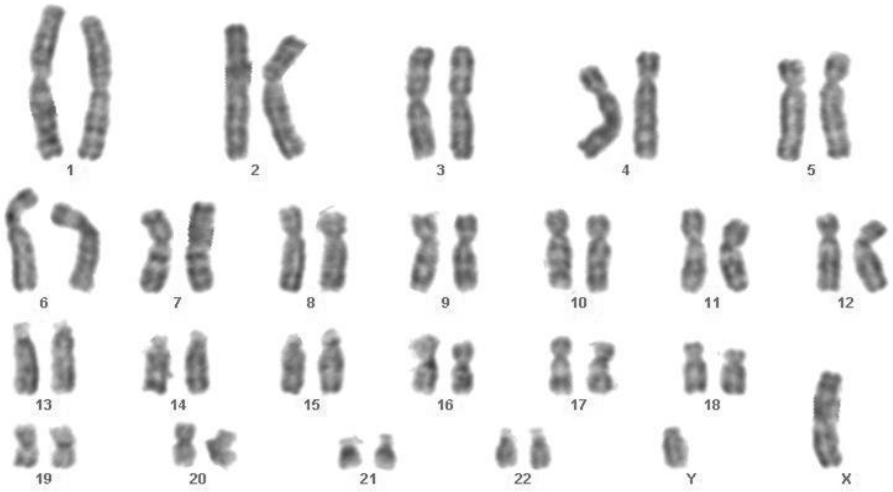


Figure 4: Karyotype showing 46,XY normal male .

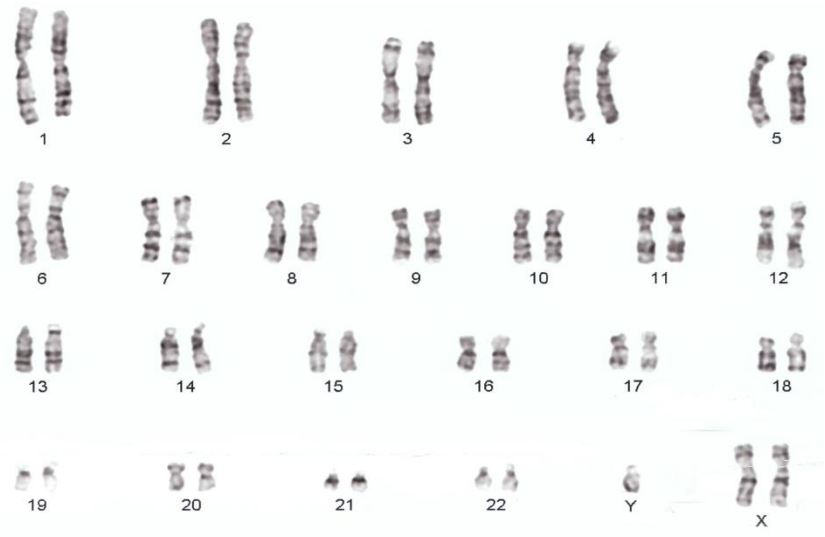


Figure 5 : Karyotype showing 47,XXY klinefelter male

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الكشف عن خلل الكر وموسومات وازدواج الحيوانات المنوية في حالات عقم الذكور مجهولة السبب نتيجة التعرض لعوامل ومخاطر بيئية

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المستخلص

لقد أصبحت العلاقة بين العقم وازدواج الحيوانات المنوية موثقة جيدا،فلقد اختلفت النتائج ولكن معظم التقارير أوضحت إن الرجال الذين يعانون من تدهور حاد في خصائص السائل المنوي والحيوانات المنوية هم الأكثر عرضه لازدواج الحيوانات المنوية ولقد أوضحت بعض الدراسات مؤخرا إن هذا التدهور يرجع إلى التعرض لبعض العوامل والمخاطر البيئية التي تؤدى إلى تدهور نوعية الحيوانات المنوية وتشوهها. ومن أمثلة ذلك التعرض للمواد الكيميائية المختلفة، والحرارة، والإشعاع، والمعادن الثقيلة.وقد أظهرت البحوث وجود علاقة بين هرمون الاستروجين البيئي ومبيدات الآفات واضطراب عملية تكوين الحيوانات المنوية. وهناك أيضا أنماط خطرة من أساليب الحياة اليومية مثل التدخين، وتعاطي الكحول والمخدرات، والإجهاد المزمن، ونقص التغذية تؤدى إلى العقم ،وارتبط التدخين بانخفاض عدد الحيوانات المنوية ، وتشوهها وقلة حركتها، وزيادة إجمالية في عدد الحيوانات المنوية غير الطبيعية.

تهدف هذه الدراسة إلى دراسة التشوهات الكروموسومية في حالات عقم الذكور مجهول السبب لحالات (OAT)والمعرضين إلى مختلف العوامل البيئية الخطرة مثل التعرض المهني للمواد الكيميائية المختلفة ، والحرارة ، والإشعاع ، والمعادن الثقيلة ،ومبيدات الآفات، والتدخين.....الخ. تحديد وجود الحيوانات المنوية الثنائية في حالات عقم الذكور المصابين بالمرض. و تم استخدام بعض الاختبارات فى هذا البحث مثل تحليل الكروموسومات ،والتهجين الموضعى الفلوروسينتى .

أوضحت نتائج البحث قوة الارتباط بين زيادة ازدواج الكر وموسومات ونقص خواص السائل المنوي وجودته نتيجة التعرض لعوامل بيئية مختلفة وبعض العادات وأنماط الحياة التى تؤدى الى هذا التدهور مما يؤدى الى ازدياد حالات عقم الذكور.

ينبغي وضع استراتيجيات لتوجيه انتباه الجمهور تجاه العلاقة المحتملة بين العوامل البيئية وحدوث العقم عند الذكور والبحوث الميدانية في هذا المجال واسع،علينا أن تنتبه لهذا الإنذار المبكر و كيفية تأثير نمط الحياة الحديثة في العقم عند الذكور.

الكلمات الدالة : الكروموسومات، التهجين الموضعي الفلوروسينتى ، العقم عند الذكور، السائل المنوي، الحيوانات المنوية، المخاطر البيئية