Role of environmental factors on human male infertility

Poonguzali J¹, Sarala P², Usharani V², Sharmila G² and Punitha S²

¹Department of Zoology, Arignar Anna Government Arts College for Women, Walajapet, Tamil Nadu, India
²Department of Zoology, Quaid-e-Milleth College, Chennai -600 002, Tamil Nadu, India.
*Corresponding author: E-mail: prakasam_66@yahoo.co.in; Phone: +91-9443396078

Abstract

Male infertility is a social problem which is affected by various environmental factors. The main objectives of the present study are identification of abnormal semen characteristics, role of hormone levels, effect of smoking and alcohol consumption on the semen parameters, to analyse chromosomal abnormalities and the role of environmental factors on male infertility. 600 men with severe andrological abnormalities attending fertility clinic in Chennai were subjected to comprehensive questionnaire and Semen analysis was done to group the patients based on their abnormalities of the semen parameters. Karyotyping was done to analyse the chromosomal abnormalities. Student’s t-test analysis was done to analyse the relationship between the semen parameters and smoking habit in one hand and the use of alcohol and semen characteristic on the other hand. There was no significant difference (P < 0.05) in the semen parameters between non-smokers and smokers and also between alcoholic and non-alcoholic within control and other groups with abnormal semen parameters. There was no significant difference in the hormone levels, blood glucose levels and BMI between non-smokers and smokers and also between alcoholic and non-alcoholic within the control and others groups. Structural abnormality like deletion was observed in the sex chromosome Y and polymorphism in Y chromosome and autosomes were observed in the present study. Other than lifestyle, radiations and environmental pollutants do have detrimental effect on human reproduction. Prenatal or postnatal exposure to environmental agents can cause disturbances in the spermatogenesis of men. Role of environmental factors on male infertility needs more research in the animal models which can enable, to find out the impact of environmental factors on human male infertility.

Keywords: male infertility, Y-chromosome environmental factors, spermatogenesis

Introduction

The world’s population is increasing at an alarming rate and is projected to reach nine billion by 2050. Despite this, 15% of couples world-wide remain childless because of infertility (Matzuk and Lamb, 2002). Eight to ten percentage of couple’s world-wide experience some form of infertility problem in which male factors account up to 50% (WHO, 1992, 1993). Infertility is a problem faced by couples rather than the individuals. Infertility is defined as inability to conceive after one year of unprotected intercourse and thus the definition includes men with sub fertility. “The duration of the failure to conceive should be twelve or more months before an investigation is undertaken unless medical history and physical findings dictate earlier evaluation and treatment (The Practical Committee of the American Society for Reproductive Medicine, Birmingham, 2004). Azoospermic and oligoasthenoteratozoospermic (OAT) men represent 40-50% of all infertile men (Thonneau et al., 1991). Its prevalence in western countries has been estimated to be 20% (WHO, 1987). There is regional variation in the prevalence of azoospermia and oligospermia in the male partners of infertile couples from different regions of India (Mehta et al., 2006). Aetiology of male infertility includes both acquired and congenital. Acquired factors in male infertility include infection and inflammation, immuno infertility, trauma, surgical insult to the male reproductive organs and exposure to toxic chemicals or other materials (Lamb and Lipshultz, 2000). A history of urogenital inflammation is present in 5-12% of men who have attended infertility clinics. Infectious conditions degrade sperm
quality by reducing their concentration, motility and morphology. Genital tuberculosis can result in epididymal blockage or vasal obstruction. Infertility caused by ejaculatory duct obstruction is usually characterized by severe oligospermia or azoospermia (Dohle, 2003). The incidence of sperm antibodies (immune-infertility) has been shown to be higher in infertile men (8% - 21%) compared to fertile subjects (1% - 4%) (Collins et al., 1995). Several risk factors for the development of male antisperm antibodies have been identified. Vasectomy is the most common factor, associated with a post-operative presence of serum antibodies in 34% - 74% of cases (Jarow et al., 1994). Male infertility may result from exposure to a variety of gonadotoxins and other substances that have inhibitory effect on spermatogenesis. These include chemicals such as solvents and pesticides various medications such as cimetidine, testosterone replacement therapy, recreational drugs, alcohol, tobacco and radiation (Wald, 2005).

Varicocele, a surgically correctable lesion associated with male infertility was found in 40% of subfertile men with oligozoospermia (Hirsh and Pryor, 1994). Cryptorchidism (failure of testicular descent into the scrotum during development) is found in 3.4% cases (Sigman et al., 1997). It is the most common birth defect in boys, with a prevalence of 1% to 10% (Thonneau et al., 2003). Mumps orchitis occurs unilaterally in about 30% and bilaterally in 10% of patients infected with the mumps virus (Werner, 1950). Endocrine disorders of the hypothalamic-pituitary-gonadal axis are associated with male infertility. Kallmann’s syndrome, a X-linked disorder of male infertility is characterized by deficient hypothalamic secretion of gonadotropin releasing hormone (GnRH) leading to hypogonadotropin hypogonadism (Seminara et al., 1998). The most common genetic factors related to male infertility are cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation leading to congenital bilateral absence of the vas deferens (CBAVD) which is the common cause of azoospermia (Anguiano et al., 1992), chromosomal abnormalities (karyotype), Y-chromosome microdeletions in the azoospermia factor (AZF) locus and single gene defects.

Reproductive difficulties are associated intimately with cytogenetic abnormalities. Chromosomal abnormalities are common in infertile men with a prevalence of 5.8% as compared with 0.5% in fertile population (Johnson, 1998). About 4.2% of abnormalities occur on the sex chromosomes while 1.5% occurs on the autosomes. The gain or loss of an entire single chromosome results in aneuploidy whereas a polyploidy state occurs when the entire chromosomal content is multiplied. Structural abnormalities include the rearrangement or translocation of fragments of chromosomes (as in Robertsonian translocations) or deletions of single genes or portions of a chromosome. Microdeletion of the Y chromosome is the second most frequent genetic cause of spermatogenic failure in infertile men after the Klinefelter syndrome (Simoni et al., 2004). Microdeletions of the Y chromosome long arm (Yq) represent the most frequent molecular genetic cause of severe male infertility (Ferlin et al., 2006; Krause and Degl’Innocenti, 2006) represented by severe oligospermia and non-obstructive azoospermia.

**Fig 1.** Schematic diagram showing possible pathways of Male fertility due to environmental factors

Human sperm count and morphology is decreasing worldwide. Important environmental factors includes physical agents such as radiations and heat, chemical agents such as cigarette smoke, air borne pollutants and chemotherapeutic drugs, and
biological factors. Industrial and agricultural pollution are the significant concern for the male infertility (Pacey, 2010) Shepherd (2011) have identified pesticides as the most harmful environmental hazard to human reproduction. The effect of smoking and passive smoking on various semen parameters have been evaluated for the past two decades. The Practical committee of the ASRM (2004) have reported that there have been substantial harmful effects of cigarette smoke on fecundity and reproduction of both male and female and indicated that up to 13% of infertility may be attributed to cigarette smoking with reproductive hazards for both females and males. Gamete mutagenesis is one of possible mechanism where by smoking may adversely affect fecundity and reproductive performances. The results of meta-analysis examining the outcome of ART cycles indicated that smokers require nearly twice the number of IVF attempts to conceive as non-smokers. (ASRM, 2004) Sharpe and Shaekbeak (1993) suggested that the prenatal exposure of the testis to oestrogenic substances might reduce sperm count in the adult and they have pointed that the increasing sources of oestrogenic substances in our environment arising from the widespread use of oral contraceptives, oestrogens used in the livestock industry, phytosterogens in diet and variety of chemicals used in industries which can mimic oestrogenic action could reduce sperm count. The main objective of the present study are identification of abnormal semen characteristics, role of hormone levels, effect of smoking and alcohol consumption on the semen parameters, to analyse chromosomal abnormalities and the role of environmental factors on male infertility.

Materials and methods

Sampling
Six hundred male individuals (550 individuals with primary or secondary infertility and 50 proven males for control) attending a fertility clinic in the metropolitan city, Chennai, India, were subjected to comprehensive questionnaire related to their medical, surgical, family histories, life style habits (such as smoking, alcohol use and drug use) exposure to gonadotoxins (such as drugs used in cancer chemotherapy) with their informed consent. Details on age, height, weight, blood group, blood glucose, and hormone levels for all individuals were recorded. In all the 600 individuals semen analysis was done as per guidelines of WHO (1989, 1992, 1999) (under the supervision of an andrologist and with assistance of paramedical staff) in terms of volume, sperm concentration, sperm count, sperm motility and sperm morphology.

Blood Sample collection

Two to three ml of the blood samples have been collected into a sterilized container, centrifuged and the fresh serum was collected carefully from the centrifuged sample for the hormonal assay. 3 ml of the blood sample was drawn aseptically in a sterilized glass syringe, from this 3ml of the blood was transferred to a vacutainer, containing sodium heparin (100 – 200 IU) for cytogenetic analysis.

Hormonal assay

Plasma Follicle Stimulating hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T), were analysed by Enzyme Immunoassay (EIA) Kits (Omega Diagnostics) for the quantitative determination in serum. Prolactin hormone (PRL) was analysed by Immunoenzymometric assay Kit (MONOBIND, INC. Costa Mesa, CA926227) (USA).

Cytogenetic analysis

Chromosome preparations for patients were obtained from phytohaemagglutinin-M (PHA-M) stimulated peripheral blood lymphocyte following the procedure of Moorehead et al. (1960). One week old slides were subjected to Giemsa –Trypsin- Giema (GTG) banding as per the procedure of Seabright (1971). Metaphase that appeared intact with sufficiently well defined chromosome morphology and banding pattern were selected for study. Idiogram was done with an automated karyotyper machine using Cyto Vision Software.

Statistical analysis

Statistical analysis was carried out on the results of all the above experiments using SPSS
software package (SPSS). The differences between the normal and other groups with abnormal semen parameters were statistically analysed using one-way analysis of variance (ANOVA) followed by Post Hoc Tukey test at p<0.05. Chi-Square test was done to find out the significance between the different combinations of smoking and drinking habits in the different groups. Student’s t-test was applied to analyse the significance between the semen parameters and habits (Smoking and use of alcohol) with each group. Correlation coefficients were done to find out the relationship between the hormones and semen parameters of different groups.

**Results**

The patients were classified into azoospermia (no sperm in the ejaculate), Asthenozoospermia (<50% sperm with forward progression), Oligoasthenoteratozoospermia (OAT) (sperm count < 20x10^6/ml, <50% sperm with forward progression, <30% of sperms with normal morphology), Severely Oligoasthenoteratozoospermia (SOAT) (<2 ml of semen volume with disturbance of all the three variables). In the present study among the hormones FSH, LH, Prolactin and Testosterone were analysed. The mean value of FSH for Azoospermia group was 15.46 ± 10.90, which was higher than the control group indicating a significant difference at 5% level. Among the hormones, FSH showed a significant difference at 5% level between the groups and there was no significant difference in the levels of LH, prolactin and testosterone within and between the groups and the control.

Correlation coefficients (P values) for relationships between semen parameters and hormones in different groups showed a significant negative correlation was observed in OAT group between the values of FSH and sluggish motility (r = -0.358 P = 0.021), between the values FSH and sperms in normal morphology (r = -0.366 P = 0.019), and between the values of LH and sperms in normal morphology (r = -0.375 P = 0.016). Increased FSH levels were correlated with decrease in sperm motility and sperms in normal morphology. Chi-Square test was done to analyse the relationship between smoking habit and the use of alcohol based on the data collected from the individuals. In Chi-square tests, the probabilities obtained were marked more than 0.05 (P = 0.325, 0.991, and 0.468, respectively) therefore the null-hypotheses tested could not be rejected. Thus, in the subjects undergoing treatment for infertility, there is no significant association between smoking, drinking or smoking-drinking combination habit and the types of zoospermia. Student’s t-test analysis was done to analyse the relationship between the semen parameters and smoking habit in one hand and the use of alcohol and semen characteristic on the other hand within each group. There was no significant difference (P < 0.05) in the semen parameters between non-smokers and smokers and also between the non-alcoholics and alcoholics within control, AS, OAT, SOAT and AZOO groups. There was no significant difference in the hormone levels, blood glucose levels and BMI of non-smokers and smokers and the non-alcoholics and alcoholics within control, AS, OAT, SOAT, and AZOO groups.

Cytogenetic investigations revealed chromosomal anomalies in 29 out of 550 patients, while the remaining individuals were found to be having normal Karyotype 46,XY. Structural abnormality in the Sex Chromosome Y was identified, deletion in the q arm was identified in the Yq12 region [46, XY del Y (q12)] in one of the SOAT patient and 46, XY del Y (q11.2) in seven of the Asthenozoospermia patient with Yq11.2 deletion. Chromosomal polymorphism in chromosome 14, 15, 21, 22 was observed in two of the azoospermia and two of the asthenozoospermia cases showing an increase in the length of satellite on the short arm (46,XY, 14ps+), (46,XY, 15ps+), (46,XY, 21ps+), (46,XY, 22ps+) and polymorphism in Y chromosome showing and an increase in the length of long arm (46,XYq1+) was observed in 17 Asthenozoospermia cases.

**Discussion**

The past decade has witnessed significant advances in the therapeutic approaches of male infertility. The introduction of microsurgical techniques, along with significant advancements in
understanding of the genetic basis of male infertility, has revolutionized the diagnostic and therapeutic approaches to male infertility, enabled men previously thought to be hopelessly sterile to father their own biological offsprings. Smoking by males decreases the success rates of assisted reproductive procedures, not only in 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). 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. Further seven out of nine studies in fertile and six out of 19 studies in infertile men have indicated a statistically significant difference in semen quality. The largest meta-analysis study of Lewin et al. (1991) that included 662 infertile men (382 non-smokers and 280 smokers) revealed a significant difference in sperm concentration associated with cigarette smoking.

However in the present study, no significant difference was observed in semen parameters between smokers and non-smokers in the control and other groups with abnormal semen qualities, thus indicating the absence of an association between smoking habit and semen quality, in the population studied. This observation finds support from the work of Trummer et al. (2002) who screened 571 non-smokers and 478 smokers and found no difference with respect to conventional semen parameters between non-smokers and smokers. It is more relevant to suggest that several factors such as duration of smoking habit, continuous or intermittent, frequency of smoking and contents of cigarettes should be taken into consideration if the aim is to establish an association between smoking and semen characteristics. Sexual disorders have been reported in men who are long-term alcohol 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. No significant difference between non-alcoholics and alcoholics in their semen variables was observed in the present study and this is consistent with the studies of Kunzle et al. (2003). The exact mechanism of altered testicular histology in infertile males still remains evasive and needs further study.

Yq11, Yq12 deletions in the Y-Chromosome observed in the present study could be contributing factor for male infertility which has been observed by many researches from 1976. The large deletion in the Y chromosome may be a contributing factor for male infertility and, this observation is in agreement with the views of Tiepolo and Zuffardi (1976) who were the first to propose the hypothesis that there could be a correlation between Y chromosome deletions and male infertility. A similar Y chromosomal deletion (Yq12) has also been observed by Gekas et al. (2001). Miyomota et al. (2011) have explained that environment is an important factor associated with genetic polymorphism in human spermatogenesis (Fig 1). The polymorphism observed in present study is in accordance with Nagvenkar et al. (2005) and Penna et al. (2001).

In particular we are still unable to establish the precise genotype-phenotype correlation between specific chromosomal deletions and impaired spermatogenesis in infertile men. Environmental factors are the potential confounding factors on the male infertility since its exact role is a challenge for mankind. Socioeconomic status of the population in the developing countries like India would be affected by the increase of pollutants since men who want to become a biological father of a child through ART cycles will be treated with out knowing the increased risk of adverse reproductive outcomes to the future generations due to environmental factors. Parker et al. (1994) have also pointed out the adverse effects of socioeconomic status on reproduction. Inspite of advanced developments in the treatment of male infertility, still the role of unexplained factors may not be ruled out. The concept that there may be environmental factors that affect sperm count in
different regions has gained support from observations that indicators of male reproductive health show regional alterations. De Krester (1998) has pointed out the increase in regional frequency of male abnormalities over short period of time may be due 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. In particular, addition research using animal models would help better delineate the biological mechanism underpinning the associations reported in human studies. 200 cases of post neonatal mortality and 10,000 low-birth weight deliveries would have been prevented solely through reduction in air pollutants levels between 1990 and 2010 because of U.S. Clean Air Act (Wong et al., 2004). Lepecka-Klusek et al. (2011) have insisted on the continuation of studies for a larger group of males with reproductive problems to find out the role of environment.

Conclusions
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.

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