GENETIC FACTORS IN THE CLINICAL MANAGEMENT OF MALE INFERTILITY

Olufemi Adebari Oloyede[1,2] and Idowu Adewunmi Taiwo [2]

[1,2]Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital, Lagos, Nigeria.
[2]Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria.

ABSTRACT

Background: Infertility is a common gynecological problem. Most literatures are about female infertility with emphasis on infection sequelae and other factors such as anovulation. More recent studies reveal significant contribution from the males presenting as severe oligospermia and azoospermia.

Objective: This literature reviews the genetic factors that contribute to male infertility, in the form of genic problem in the genes on the Y chromosome (primary) or sequelae of other genetic problems (secondary). They are discussed as pre- and post–spermatogenic. The common ones such as Y-chromosome microdeletions in the azoospermia factor (*AZF*) locus, Klinifelter syndrome 47XXY and Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations leading to congenital absence of the vas deferens (CAVD) are discussed. Other less common causes include Prader Willi syndrome, Kennedy disease, Katergener syndrome and Polycystic kidney disease.

Source: Medline search of local and non local articles, as well as standard text on human genetics were used for the review

Conclusion: Genetic factors play an important role male infertility and knowledge about them forms the basis for the rational management of males with severe oligospermia and azoospermia especially in developing countries.

Keywords: Genetic, Male infertility, Clinical, Assisted Reproductive techniques, Semen

INTRODUCTION

Infertility is a common distressing gynaecological problem with the male factor as a significant contributor[1]. It is defined as the inability of a couple to achieve pregnancy within 12 months of regular unprotected sexual intercourse, with a worldwide prevalence of 10-15% among couples in the general population[2]. In Africa, a prevalence of between 15 – 40% is reported[3, 4, 5]. It is classified as either female infertility, when the cause is in the female or male infertility, when the aetiology is in the male. Primary male infertility is the condition in which the man has not been able to fertilize a normal woman, while in secondary infertility, there was a previous fertilization of an ovum by the male spermatozoa.

Male factor infertility implies a lack of sufficient numbers of competent sperms resulting in failure to fertilize the normal ovum. The normality and competence of the semen is defined by the World Health Organization criteria and include the volume of the ejaculate, the concentration of spermatozoa, motility and morphological characteristics[2]. There are concerns about the prognostic and clinical application of these criteria[1]. A significant proportion of men with normal criteria are infertile because of defects in sperm function[6,7] while a significant number of men with abnormal have been shown to possess normal sperm function[6]. In spite of the controversies about the reliability of these normal values, many researches are still based on them.

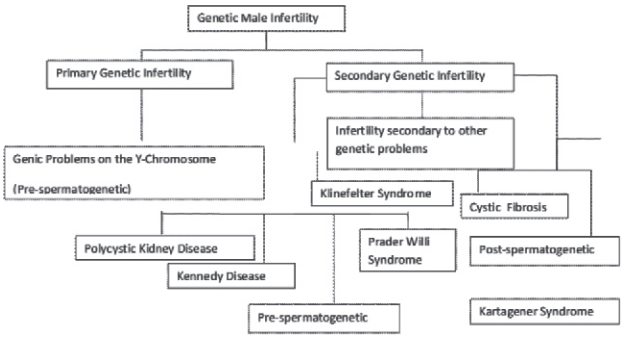
Table 1. Seminal Fluid Analysis (WHO Criteria 1999)

Parameters	Normal values
Volume	$\geq$ 2.0mls
PH	7.2-7.8
Concentration	$\geq 20x10^6$ /ml
Motility	$\geq$ 50 % (with forward progressive motility)
Morphology	15 – 30% normal forms
Viability	$\geq$75% alive
WBC	$\leq 1 \times 10^6$/ml

Correspondence: Dr Olufemi A Oloyede
P.O Box 1040, Oshodi, Lagos
Nigeria
E-Mail: oloyedeoao@yahoo.com
Tel: 2348032343130

The abnormalities of seminalysis could occur singly or in combination
Hypospermia- Semen volume less than 2mls
Oligospermia- Sperm concentration less than 20 x 10^6/ml
Azoospermia – Absence of sperm
Asthenozoospermia –Less than 50% with forward motility a,b
Teratospermia -- 15% normal forms
Teratozoosspermia – Abnormal morphology on microscopy

Classification



SCOPE OF GENETIC FACTORS IN MALE INFERTILITY

Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. There is widespread variation in the prevalence of male infertility due to difficulty in getting accurate diagnosis. It is however agreed that the male factor is the single largest cause of infertility[1, 3]. It presents either as azoospermia or significant oligospermia. There is recently a better understanding of the role of genetics in male infertility, with about 10 -15% of reproductive failure in males due to an underlying genetic factor[1, 8]. These occur as chromosomal aberrations and single gene mutations[1, 8]. There is predominance of sex chromosomal abnormalities in azoospermia males[1].

Defective genes themselves can be inherited, produced by environmental assaults (such radiation exposure), or both. The mutations have a higher possibility of being transmitted to offspring when assisted reproductive methods involve direct injection of sperm into the female egg, because it avoids the natural selective mechanism. Also there could be abnormalities in the genes that regulate male fertility or in the genetic material of sperm itself.

The genetic problems in male infertility could be classified as follows:
Primary Genetic Male Infertility – This is caused by an inherent genic abnormality especially of the genes on the Y chromosome that regulate or affect spermatogenesis. It is pre-spermatogenic problem.
Secondary Genetic Male Infertility – This refers to infertility secondary to other genetic problems. Causes in this category may be pre-spermatogenic e.g Prader Willi syndrome, Klinefelter syndrome or post- spermatogenetic e.g Cystic fibrosis (CF).

ABNORMALITIES IN THE GENES / CHROMOSOMES THAT REGULATE OR AFFECT SPERMATOGENESIS (PRE SPERMATOGENESIS)

Sperm cells are produced in the process of spermatogenesis. Spermatogenesis is in turn regulated by genes on the Y chromosome. The region involved in this regulation and fine tuning of spermatogenesis is located on the long arm (Yq11.21)

Structure of the Y chromosome

Y chromosome is made up of the short (p) and long arms (q) connected by a centriomere. The most distal part of the Y chromosome referred to as the pseudoautosomal regions occurs terminally on both arms and participates in the exchange of genetic materials with X chromosome during male meiosis. Next to the pseudoautosomal region is the genetically inert heterochromatic region that retains records of mutations occurring to the chromosome over the years. Sons inherit their father's portion during meiosis.
The Euchromatic region is the most vital region on the long arm necessary for spermatogenesis. Complete spermatogenesis is postulated to reside in the functionality of the locus referred to as Azoospermic factor (AZF) on the long arm of the chromosome [9]. The AZF is divided into 3 different non overlapping sub regions (AZFa, AZFb, and AZFc), with each having important role in spermatogenesis. A fourth sub region, AZFd was recently identified [10].
AZFa sub region contains the *Ubiquitin-Specific peptidase 9* (*USP9Y*) and the *DBY or Dead Box 3 gene* (DDX3Y). The *USP9Y is involved in the production,* **fine tuning** and **improvement of efficiency** of sperm cells, while the DDX3Y, Y RNA helicase is responsible for maturation and spermatogenesis [11].The AZFb sub region has 5

identifiable genes of which 3 are male fertility related. These are the RNA Binding Motif gene (RBM gene), which encodes proteins involved in spermatogenesis, Chromodomain Y (CDY) gene, a chromatin binding and XK related (XKRY) gene (X - linked Kell blood group), involved in spermatogenesis [12]. The genes in the **AZFc sub** region include the PTP-BL Related Y (PRY) gene, Basic Protein Y2 (BPY2) gene, Testis Transcript Y2 (TTY2) gene and the Deleted in Azoospermia (DAZ) gene.

Y chromosome abnormality

Structural abnormality involving the Y chromosome is the most important cause of impaired spermatogenesis[11]. The most frequent of these is the microdeletion and occurs in about 10-20% of azoospermia or severe oligospermia[10]. The microdeletions of AZF (a, b and c) locus occur during DNA replication. It has been mapped to deletion interval 5 – 7 in band q11.21- q11.23, although most deletions occur in deletion interval 5 and 6. It may be singly or in combinations (AZFa, AZFb, AZFc, AZFb+c, AZFa+b+c). While the AZFa is rarely involved (2%), the AZFb is found to be recurrent deleted in infertile males and the AZFc is the most common complete deletion and known genetic cause of testicular dysfunction and male infertility. In some cases, Y microdeletion is compatible with formation of small numbers of normal spermatozoa. The affected males usually are perfectly healthy man apart from been infertile.

Duplications can also involve the sub regions because the loci are prone to rearrangements causing azoospermia or oligozoospermia[13]. The AZFc region is one of the least stable regions in the human genome, consisting almost entirely of very long repeats and highly prone to rearrangement.

Translocation involving the Y chromosome is another mechanism of male infertility. It is established that translocation between the autosome and Y chromosome producing a translocation chromosome Y causes pairing problems during male meiosis between the translocation Y and the normal X chromosome. The pairing abnormality leads to spermatogenic failure (degeneration of spermatocytes after pachytene stage) and is best observed at pachytene stage It is theorized that translocation causes disruption of gene responsible for replacement of histones involved in the packaging of DNA into the sperm head[14].

Klinefelter Syndrome

Klinefelter syndrome is the commonest chromosomal abnormality associated with male hypogonadism and infertility[14]. **The incidence** is between 1 in 600-1000 male live-births while it occurs in about 7-13% of azoospermic men. Available statistics about the prevalence especially in developing countries are extrapolated. In the USA, about 250,000 men are affected. The condition has no known racial predisposition.

Klinefelter syndrome is a numerical abnormality of the sex chromosome, in which an extra X is added to the normal XY due to meiotic nondisjunction. In the majority (90%) of affected males, the chromosomal constitution is 47 XXY, while mosaics (46XY/47XXY chromosome constitution) are found in about 10%. The features of this syndrome were first observed in 1942 and its association with abnormalities of spermatogenesis was confirmed by Klinefelter in 1959[15]. Affected males are normal in appearance before puberty. After puberty, the classic features include small firm testes, gynecomastia, azoospermia, disproportion in body size, abnormal social behaviour and below average IQ (depends on number of X chromosome). Infertility in Klinefelter syndrome results from the severe histological changes in the testicles hyalinization/destruction of the lining of the seminiferous tubules. Generally, affected men are infertile. Some can have healthy children, but also has increased chance to have a child with a sex chromosome abnormality.

ABNORMALITIES IN THE GENES OR CHROMOSOME THAT AFFECT THE TRANSPORT OR CONDUCTION CHANNEL OF SPERM CELLS (POST SPERMATOGENESIS)

Sperm cells after spermatogenesis are transported through the seminiferous tubules.

Cystic Fibrosis

Cystic Fibrosis is the most common autosomal recessive disease with highest prevalence in the Caucasians[14]. The prevalence is similar in both the United Kingdom and the United States of America, being 0.737 and 0.797 per 10,000 respectively[16]. It is generally speculated that the prevalence is low in African countries (except North Africa), probably due to under reporting or misdiagnosis of condition as chronic pulmonary infection and gastrointestinal infection, malnutrition, tuberculosis, or high infant mortality rate [17]. The predicted incidence is between 1 in 784 and 1 in 13 924 births[17].

The gene responsible for CF, also called cystic

fibrosis transmembrane regulator (CFTR) is located on chromosome 7. More than 150 identifiable mutations are reported, with the common ones being D 508 (70%), 5T, R117H, R75Q, G542X, N1303, W1282X, G551A, and R347H. They affect the processing of the gene products. **Affected individuals have defects in** ion transport across cell membranes that cause abnormal sticky viscous secretions from various body organs. CF is usually diagnosed in infancy and early childhood as a fatal condition.

Above 95% of men with CF have fertility problem characterized by absence of **p**ortion or all of the reproductive ducts (including vas and seminal vesicles). When the 2 sides are involved, it is called congenital bilateral absence of the vas defence (CBAVD) while involvement of one side is referred to as congenital unilateral absence of the vas deferens (CUAVD). In both situation, there is trapping or obstruction to the passage of sperm and individual presents with azoospermia or oligospermia associated with low semen volume and acidic pH. Spermatogenesis is intact. CBAVD may be associated with several diseases, including **cystic fibrosis (CF).** Detectable genetic mutation of one of the cystic fibrosis genes is found in 65 - 80% of men with CBAVD [18]. Among infertile males CBAVD is found in 2%. CUAVD affects 0.5-1% of the general population and **rarely** presents with infertility, because the individual have a normal side and is not azoospermic.

Other syndromes associated with male infertility.

Prader Willi Syndrome (PWS)

The phenotype of PWS was initially described by Langdon Down [19] and named after Prader Willi in 1956[20]. It is as a rare condition that occurs sporadically. True population prevalence is difficult to determine. This condition is regarded as the first clinico-genetic disorder in humans caused by genomic imprinting. In about 75% of cases, the genomic imprinting is in the SNRPN gene located on the long arm of paternally derived chromosome 15 (15q11-q13) during oogenesis and its concurrent deletion. Consequently, the affected individual does not have a normal copy of this gene. In about 20% of cases the condition arises from maternal uniparental disomy caused by chromosomal nondisjunction, while mutational error in the imprinting centre is responsible in about 2% of individuals [21].

Infertility associated in Prader Willi syndrome is attributable to dysfunctional hypothalamic-pituitary-gonadal axis, which manifests as retarded or incomplete sexual development (hypogonadotropic hypogonadism) [12, 19]. In some cases, hypothalamic-pituitary-gonadal is normal [22]**.** Puberty is usually delayed in children with Prader-Willi syndrome. The histology of biopsies shows few germ cells, hyalinization and thickening of tubular basement membranes and atrophy of seminiferous tubules. The Leydig cells are largely normal[23]. It is as an autosomal dominant condition, with a 50% chance of transmission of the trait to the sons of affected father.

Kennedy disease

Kennedy disease v rris an inherited X linked disorder, characterized by late onset (about 30 years), progressive neurone degeneration and muscular weakness. It was named after Williams Kennedy who first described it in 1968. This condition is also referred to as Spinobulbar muscular atrophy (SBMA).

It is caused by mutation in a gene encoding a protein known as the androgen receptor (AR). Affected males have impaired AR that reduces the effect of male sex hormones. It is associated with reduced sex drive, infertility, impotence, and shrunken testicles. In some males, impaired sperm production follows testicular atrophy, while in a few cases, sexual function and fertility are normal. It is regarded as a rare genetic disease, with a higher prevalence reported in Western Finland.

Kartagener syndrome

The classical Kartagener syndrome describes the occurrence of situs inversus, sinusitis, bronchiectasis and occasionally sterility in males[24,25]. It is also called immotile cilia syndrome or primary ciliary dyskinesia because of its association with partial or total dysfunction of ciliary or flagellated cells. Most of the symptomatology is from the respiratory tract because of the predominance of ciliated cells in this organ. It is however, known that there are immotile spermatozoa with anoxemal structures found under EM studies in infertile males and this is suggested as the basis for including male sterility as an additional component of this syndrome [26].

The syndrome is an autosomal recessive disorder caused by a mutation in the DNAI1 gene, which produces a cellular protein required for normal function of the cilia and sperm motility. It occurs in 1/6000 and 1/40000 of male infertility[25]. Infertility

results from impaired sperm motility.

Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease is a systemic disorder with extrarenal organs such as the liver, pancreas and reproductive tract involved[27].

It is caused by a mutation affecting one out of three genes: PKD1 (on the 16th chromosome), PKD2 (on the 4th chromosome) and PKD3 (still unmapped) [28].This condition is associated with male infertility[27, 28].

It is theorized that imbalance between cell growth/proliferation inhibitors and stimulators leads to cyst formation that occupies the seminal vesicles, prostate and testes causing ejaculatory obstruction and azoospermia [27,28] [29]. Other less frequently reported abnormality include axonemal 9 + 0 defect, severe congestion of the epididymis and marked pituitary dysfunction, which is not reversed by dialysis[28] [9]. Occasionally, there are structural abnormalities of the sperm such as asthenozoospermia and oligoteratospermia related to abnormal function of polycystin [27].

Clinical Considerations

In clinical practice in many developing countries, the role of genetic factors in severe oligospermia and azoospermia has not been a major consideration. **In the evaluation of male factor infertility due to genetic disorder, the following are important. Accurate diagnosis of the genetic factor is based on the history and physical examination. Counselling about all aspects of the condition including risk of transmission and finally management goals based on the diagnosis and therapeutic options available in the environment, are discussed with the couple.**

A. DIAGNOSIS

The appropriate management of genetic male infertility starts from making the diagnosis of the precise underlying genetic defect. It determines the prognosis, the treatment modality and ultimately the outcome of treatment.

HISTORY

This is the first step. CF is suggested by history of bulky stools, persistent coughing with accompanying phlegm, wheezing or shortness of breath. Abnormal social behavior in association with below average intelligence quotient may be the first symptom of Klinefelter syndrome. PWS is suggested by a previous history of genital hypoplasia, cryptochidism and delayed testicular descent even if there was a premature growth of pubic and axillary hair[29].

Pedigree analysis is recommended when the above features are found in other members of the family or recur in same family. It reveals the mode of inheritance. Vertical transmission is characteristic of autosomal dominant conditions, horizontal transmission of autosomal recessive disorders and absence of male to male transmission characteristic of X linked disorders. It should however be noted that in a number of cases, transmission is sporadic, with no delineable pattern of inheritance. For example, less than 10% of cases of Polycystic kidney disease are sporadic, and in the remaining 90%, the disease has been inherited independently from the mother or the father[27].

EXAMINATION

Physical examination follows history. It includes assessment of breast development, body stature, height and proportions of body parts. Klinefelter syndrome is characterized by tallness, asthenic stature with disproportionately long extremities, while short stature and robust built are more common in PWS. Assessment of the mental level of the individual is also important. Lower than average IQ may point to conditions such as PWS, while in combination with hyperactivity may suggest KLinefelter syndrome. Special emphasis should be given to the examination of the genitalia. Small firm testicles are found in Kennedy disease and Klinefelter syndrome. The pennis is small in CF and PWS.

INVESTIGATIONS

The seminal fluid analysis is usually the first and perhaps the most important investigation carried out during infertility evaluation. It is also the most rewarding. Semen should be produced by masturbation and the parameters individually interpreted in relation to the history and examination findings.

Reduced volume with a low pH is characteristic of CBAVD. Azoospermia with near absence of fructose is associated with Polycystic disease of the kidney, while azoospermia alone is found in Klinefelter syndrome. Impaired motility is characteristic of Katargener syndrome. In all the

conditions, there may be severe oligospermia depending on the severity of the pathology.
Hormonal studies may reveal a pattern of hypogonadotrophic hypogonadism found in PWS.

Ultrasound studies are vital investigative tool in male infertility. It could be by both transabdominal and transrectal approach. Roser et. al (2008)[27], defined normal and abnormal parameters. Epididymal cysts are defined as well-defined anechoic areas with no internal echoes and with posterior acoustic enhancement. Seminal vesicle cysts were defined as discrete anechoic areas (simple cysts) or hypoechoic areas containing internal echoes (hypoechoic cysts) $\geq$ 5 mm in diameter. Presence of isolated anechoic and isoechoic areas, is used to differentiate between seminal vesicle cysts and dilation of seminal vesicles. Prostate cysts were defined as discrete anechoic areas characteristically thin walled, well defined, homogeneous, and with posterior acoustic enhancement. Transrectal ultrasonography revealed severe congestion and extensive cystic changes in the seminal vesicles.

Karyoptyping is considered appropriate when there is a high index of suspicion for Klinefelter syndrome. In the absence of any other significant aetiology, all men with severe oligospermia and azoospermia, should be offered Y chromosome microdeletion analysis. Complete Cystic Fibrosis mutation studies should be done in azoospermia or oligospermia associated with acidic pH, because of the high chance of a mutation in at least one CF gene.
Testicular biopsy is rarely indicated in routine modern management of male infertility as most diagnosis can be concluded without it. It may however be used to further confirm diagnosis in some genetic conditions.

COUNSELING

Counselling should be individualized using either directive or non directive counselling option. Caution must also be exercised.
Y deletions are considered to be random/sporadic. It is expected that all sons inherit their fathers' Y chromosomes, and therefore all sons of a father with an AZF deletion would also inherit the deletion and are at high risk of presenting with infertility. **However**, brothers of affected individual usually have normal seminal fluid analysis and fertility. **Klinefelter syndrome following m**eiotic non disjunction is a random event. The affected individuals can have healthy children, but also has increased chance to have a child with a sex chromosome abnormality. **Counselling in CF, should emphasize the fact that the identification of** one or even two cystic fibrosis (CF) mutations/variants does not imply that the individual is affected by the disease. When the wife is the carrier, each pregnancy/child has a CF risk of 25% (95% with CF have fertility problem) and a carrier risk of 50%. If spouse is negative for the common mutation, her CF carrier risk is about 0.4% and the risk for this couple to have a child with CF genotype is no more than 0.2% (95% will present with infertility). In Kennedy disease it is important to inform the individual that it is an X linked recessive condition that is found in men, whose mother is a carrier of the mutation. Such males have a 50% risk of inheriting the disorder. In Kartagener syndrome, counselling dwells more on the difficulty in getting live semen for ICSI.

MANAGEMENT

The management of male infertility due to genetic factors is influenced by the technology available for investigation and treatment. While the investigative procedures are much available in many advanced countries, they are scarce in most developing countries.
The treatment options available include but not limited to the following:
1. Assisted reproductive techniques (ART)
2. Adoption

The Assisted reproductive options used in male infertility are
In vitro fertilization and embryo transfer IVF-ET
Intracytoplasmic Sperm Injection (ICSI)
Micromanipulation techniques
Microsurgical Epididymal Sperm Aspiration
Percutaneous Epididymal Sperm Aspiration

In obstructive conditions, such as CBAVD and Polycystic kidney disease, the sperm is entrapped and the recommended option is to retrieve semen by a suitable sperm aspiration technique such as PESA or MESA. The aspirated semen is genetically examined before fertilization through methods such as IVF-ET or ICSI. Complete spousal CF mutation analysis to define the risk of passing along CF or CBAVD to the offspring, is recommended when the

males has CBAVD and are scheduled for microsurgical epididymal sperm aspiration and subsequent ICSI.

Males affected by Polycystic kidney disease and has normal seminalysis, should be offered the opportunity for sperm cryopreservation, while those with oligateratospermia and normal testicular biopsy could be considered for early microscopic epididymal sperm aspiration for *in vitro* fertilization.

CONCLUSION

Genetic factors are gradually becoming a crucial issue in the management of male infertility. It is important for clinicians to be aware of some of the common ones discussed in this review in order to optimize the care of affected couples.

.

References

1. **Irvine, D. S. Epidemiology and aetiology of male infertility.** *Human Reproduction* **1998;** 13:1
2. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 1999; 4th edition. Cambridge University Press. Cambridge.
3. Okonofua I. F. Prenatal Diagnosis and Therapy. In: Okonofua F, Odunsi K.(editors). Contemporary Obstetrics and Gynaecology for Developing Countries. 1st ed. Woman's Health and Action. Research Center. Benin City Nigeria. 2004; 387–409pp.
4. Basu J K. Service, training and research into infertility in public hospitals in South Africa. *S. Afr. Med. J.* 2007; 97(11): 1018.
5. Ulla Larsen. **Trends in Infertility in Cameroon and Nigeria.** *International Family Planning Perspectives*. 1995; **21:**138–142 & 166.
6. Aitken, R.J., Best, F.S.M., Richardson, D.W. *et al.* An analysis of sperm function in cases of unexplained infertility: conventional criteria, movement characteristics, and fertilizing capacity. *Fertil. Steril.,* 1982; 38: 212-221.
7. Aitken, R.J., Irvine, D.S. and Wu, F.C. Prospective analysis of sperm - Oocyte Fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Am. J. Obstet. Gynecol.,* 1991;**164:** 542-551.
8. Ferlin, A., Arredi, B., Foresta., C. Genetic causes of Male Infertility. *Reproductive Toxicology.* 2006; 22 (2): 133-41.
9. **Vogt, P.H.** *AZF* deletions and Y chromosomal haplogroups: history and update based on sequence. *Human Reproduction Update*. **2005;** 11(4):319-336.
10. Poongothai J, Gopenath, T. S., Manonayaki, S. Genetics of Human Male Infertility. *Singapore Medical Journal*. 2009; **50**(4):336-47.
11. Lardone, M.C., Parodi, D.A., Valdevenito, R., Ebensperga, M., Plottante, A., Madariaga, M., Smith, R., Pommer, R., Zambrano, N., Castro, A. Quantification of DDX3Y, RBMY1, DAZ and TSPY mRNAs in testes of patients with impairment of spermatogenesis. *Molecular Human Reproduction*. 2007; 13 (9): 705-712.
12. **Bray G.A, Dahms W.T, Swerdloff R.S, Fiser R.H, Atkinson R.L, Carrel R.E**. The Prader-Willi syndrome. A study of 40 patients and a review of the literature. *Medicine*. 1983; 62:59–80.
13. **Zhang, Jun, Pei-qiong Li, Qi-hong Yu, Hua-yun Chen, Juan Li and Yun-shao He.** Development of a multiplex quantitative fluorescent PCR assay for identification of rearrangements in the AZFb and AZFc regions. *Molecular Human Reproduction*. **2008;** 14:371-376
14. Gelehrter T.D. and Collins S F. Principles of Medical Genetics. 1st Edition Williams and Wilkins. United States of America. 1990; Pp. 299
15. Klinefelter H F Jr, Reifenstein E C Jr, Albright F. Syndrome characterized by of follicle-stimulating hormone. *Journal of Clinical Endocr Metabl.15;* **2**:615-624
16. Farrel P.M.The prevalence of cystic fibrosis in the European Union. *J. Cystic Fibrosis.* 2008;7(5):450-3.
17. Carolyn Padoa, Andrea Goldman, Trefor Jenkins, Michele Ramsay. Cystic fibrosis carrier frequencies in populations of African origin. *Journal of Medical Genetics. 1999: 36:41-44*
18. Casal, T., Bassas, L., Ruiz-Romero, J., Chillon, M., Gimenez, J., Ramos, M.D. Tapia, G., Narvaez, H., Nunes, V., Estivill, X. Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one CFTR allele could be detected. *Human Genetics*. 2004; 95 (2): 205-11.
19. Down J. L. *Mental Affections of Childhood and Youth*. 1887:172.
20. Prader A, Labhart A, Willi H. Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchismus und

Oligophrenie nach Myatonieartigem Zustand im Neugeborenenalter. *Schweiz Med Wschr.* 1956; 86:1260-1.

21. Buiting K, Gross S, Lich C, et al. Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *American Journal of Human Genetics*. 2003; 72 (3):571-7.

22. Ritzén E.M, Bolme P, Hall K. Endocrine physiology and therapy in Prader- Willi syndrome. *NATO ASI Series* H61: 1992; 153–169.

23. Katcher M.L, Bargman G.J, Gilert E.F, Opitz J.M. (1977). Absence of spermatogonia in the Prader-Willi syndrome. Eur J Pediatr. 1933; 124:257–260.

24. Kartagener M. Zur Pathogenese der Bronchiektasien bei Situs viscerum inversus *Beitr. Klin. Tuberk,* 1933; 83: 489-501.

25. Ceccaldi PF, Carré-Pigeon F, Youinou Y, Delépine B, Bryckaert PE, Harika G, Quéreux C, Gaillard D. Kartagener's syndrome and infertility: observation, diagnosis and treatment. *J. Gynecol Obstet Biol Reprod*. 2004; 33(3):192-4.

26. Afzelius B.A. A human syndrome caused by immotile cilia. *Science 1976;* 193: 317-319.

27. Roser Torra, Joaquim Sarquella, Jordi Calabia, Jordi Martı, Elisabet Ars, Patricia Ferna´ndez-Llama, and Jose Ballarin. **Prevalence of Cysts in Seminal Tract and Abnormal Semen Parameters in Patients with Autosomal Dominant Polycystic Kidney Disease.** *Clin. J. Am. Soc. Nephrol.* 2003; 3: 790-793.

28. Manno M, Marchesan E, Tomei F, Cicutto D, Maruzzi D, Maieron A, Turco A. Polycystic kidney disease and infertility: case report and literature review. *Arch. Ital Urol Androl*. (2005); 77(1):25-8.

29. Lee P.D. Endocrine and metabolic aspects of Prader-willi syndrome. In: Greenswag L.R, Alexander R.C, (eds). *Management of Prader-willi Syndrome*. 1995; 2nd ed. New York, NY: Springer-Verlag., pp32-57.