GENETIC DETERMINANTS OF MALE INFERTILITY IN THE SELECTED AFFECTED FAMILIES OF KPK PROVINCE

Researcher

Ali Nawaz

169-FBAS/MSBT/F-14

Supervisor

Dr. Muhammad Arshad Malik

Assistant Professor

Department of Bioinformatics and Biotechnology

Faculty of Basic and Applied Sciences

International Islamic University, Islamabad

(2014- 2016)

Human reproduction

Hormonal analysis

Gene mutation

(Begin with the name of ALLAH in Beneficent and Merciful)His

(ALLAH) Dignity, when He intends anything, is only to say

To it, "Be", so it is (Quran: Sura 36; Verse 82)

(Begin with the name of ALLAH in Beneficent and Merciful)His

(ALLAH) Dignity, when He intends anything, is only to say

To it, "Be", so it is (Quran: Surah 36; Verse 82)

Department of Bioinformatics and Biotechnology

International Islamic University, Islamabad

Dated: _____

FINAL APPROVAL

It is certified that we have read the final project report submitted by **Mr. Ali Nawaz** and it is our Judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS Degree in Biotechnology.

COMMETTEE

External Examiner I

Dr. Mazhar Qayyum
Professor and Chairman
Department of Zoology
PMAS-AAU, Rawalpindi

Internal Examiner II

Dr. Jabar Zaman Khan Khattak
Assistant Professor
Department of Bioinformatics & Biotechnology
International Islamic University, Islamabad

Supervisor

Dr. Muhammad Arshad Malik
Assistant Professor
Department of Bioinformatics & Biotechnology
International Islamic University, Islamabad

Department of Bioinformatics and Biotechnology

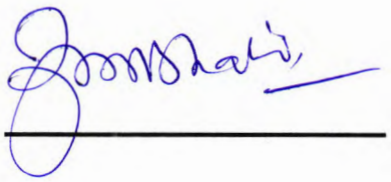
International Islamic University, Islamabad

Chairman

Prof. Dr. Zafar Mahmood Khalid

Department of Bioinformatics and Biotechnology

International Islamic University, Islamabad



Dean, FBAS

Professor Dr. Muhammad Sher

International Islamic University, Islamabad

A thesis submitted to Department of Bioinformatics and Biotechnology,
International Islamic University, Islamabad as a partial Fulfillment of requirement of the award of the
Master in Sciences of Biotechnology (MSBT)

DEDICATION

I dedicated this Research to my beloved Parents and family who sacrificed their wills on my wills and encouraged me during the course of this research work.

DECLARATION

I hereby solemnly declare that the work "GENETIC DETERMINANTS OF MALE INFERTILITY IN THE SELECTED AFFECTED FAMILIES OF KPK PROVINCE" presented in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other Degree.

Dated: 20/10/2016

Ali Nawaz

169-FBAS/MSBT/F-14

LIST OF CONTENTS

ACKNOWLEDGMENTS

First of all I bow down my head to the omnipotent, omnipresent and omniscient ALMIGHTY ALLAH who provided me an opportunity of exploring texture of His natural beauties at the molecular level, as ALLAH says:

"Universe is full of resources now it is your duty to explore it"

I have been very fortunate working with my co-operative and benign supervisor Dr. Muhammad Arshad Malik. He has been very helping and generous in delivering all his knowledge. Throughout my thesis-writing period, he provided encouragement, sound advice, good teaching, good company, and lots of good and innovative ideas. Dr Muhammad Arshad Malik provided me a refreshing insight and cynical common sense that was vitalizing and electric I am especially indebted to Dr. Muhammad Jamil Chairman department of Biotechnology and genetic Engineering KUST and also to Dr. Saad Ullah Khan and Dr. Noor Muhammad who were very supportive.

A Special thanks is addressed to Dr. Zafar Mahmood Khalid Chairman Department of Bioinformatics and Biotechnology Iiui for his excellent attention toward students time and research problems. I am great full to Ph.D students Mr. Shakir Ullah Khan, Mr. Pirzada Khan, Mr.Anwar Kamal and Waqar Amad for their support whenever I needed help. I would also like to thanks Adil Rehman, Hamid Rehman Khattak, Hazrat Usman, Afrasiab, Amir Atlas, Muhammad Sufyan and Mr. Jawwad Butt for helping and guiding me throughout my research work. I value my friends and laboratory fellows who always found time for me on their calendar. I would also like to acknowledge my friends and laboratory fellows, Hanif Ullah, Fiaz Ahmad , Muhammad Faheem and Muhammad Farhat Ullah for all the moral support and motivation during various stages of my research work.

I feel pride to acknowledge my family members Malik Asmatullah Khan, Zulfiqar Nawaz Khan, Abdullah Khan, Nargis Nawaz and Engineer Asad Khan Sikandri who supported me financially throughout my study years and will acknowledge Dr. Saima Sadiq (KRL HOSPITAL) and Muhammad Shoaib Nawaz PhD Scholar at QAU who helped me out while understanding this topic throughout the entire process, by helping me in knowing various things in my research work.

ALI NAWAZ

ABBREVIATIONS

Abbreviation	Definition
Apical ES	Apical ecto Plasmic Specialization
AZF	Azoospermia Factor
BP	Base Pair
Bcrp	Breast Cancer Protein
BTB	Blood-Testis Barrier
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Receptor Gene
°C	Degree Celsius
DAZ	Deleted in Azoospermia
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleoside Triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
FSH	Follicle Stimulating Hormones
ICSI	Intracytoplasmic Sperm Injection
IVF	In Vitro Fertilization
KPK	Khyber Pakhtunkhwa
LH	Luteinizing Hormones
M	Molar
MgCl	Magnesium Chloride
Mb	Mega Basis
mM	Mili Molar
NGS	Next Generation Sequencing
NaCl	Sodium Chloride
pH	Potential Of Hydrogen Ions
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SC	Sertoli Cells
STD	Sexually Transmited Disease
STS	Sequence Tagged Site
SNP	Single Nucleotide Polymorphism
TJs	Tight Junctions
TBE	Tris borate EDTA
TEDTA	Tris ethylene Diamine Tetra Acetic Acid
tRNA	Transfer Ribonucleic Acid
TSH	Thyroid Stimulating Hormone
UV	Ultra Violet

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Infertility refers to the abnormal condition to conceive even if a person has regular non protected intercourse for more than a year. Significant numbers of people are being affected with this disorder worldwide. On the bases of different morphological conditions infertility is grouped into several sub types. It is affecting both male and female in the similar ratio. It has many types due to morphology like oligospermic and azoospermic condition which are denoted on the basis of sperm count and sperm mobility/locomotion. There are many factors which are possible reasons of infertility including both physiology and genetics of the person. Environmental conditions sometimes play a key role in affecting fertility but it is very rare known cause. A regular exposure to radiations may affect the process of spermatogenesis too but it is also a rare case. Sometimes, infertility is caused due to a regular smoking by a person, which disrupts the process of sperm formation. Obesity is also reported by some researchers that affects fertility. Besides all these causes, the major cause is the genetic level variation which takes place in familial conditions. In male, Y chromosome is a region which is important for the male characters carrying large number of genes and various regions having group of genes which play a vital role in the human male character. The Y sex chromosome contains a region called AZF which has genes for fertility and has further sub regions AZFa, AZFc and AZFc. In current study, all markers of these regions were genotyped to check for any mutation in all three families that were collected from KPK, Pakistan but no microdeletion was detected. In these experiments, two types of controls, positive and negative, were used against affected individuals. Positive control was normal male while negative was composed of normal female, PCR amplification bands were to be observed for normal male and for negative control female but in case of microdeletions, there should be no bands in abnormal male individuals. For further analysis, different hormonal studies were carried out to see whether some other genes may be involved e.g., like testosterone, LH, FSH and also prolactin. Our study has shown that some individuals were having high level of testosterone and FSH variation suggesting involvement of some other genes.

INTRODUCTION

1. INTRODUCTION

1. Genetics

Among the several branches of science, Genetics is one its most important branch. It is specifically that branch of biology that gives the identification of how a particular characteristic will transfer from parents to the offspring. It is because human contain a genetic material known as DNA. The DNA is then further containing genes and each and every gene has got its own specific structural and functional importance that actually determine how the traits does the shifting from parents to their young ones. Any sort of disruption in these genes may lead to some unusual abnormal functioning of a body part which produces disorder so called genetic disorder. Up till now approximately 4000 different types of genetic disorders have been identified. These 4000 different types of disorders have harsh effect on individual's health and also on society. These afore mentioned disorders are basically inborn and it also can be a cause of an extreme illness and then ultimately results to death (Kaku and Freire, 1992). Approximately 3 in 100 of the globally pregnant women give birth to an abnormal individual which are affected by genetic disorder. A human body consists of approximately 35000-40000 genes and each gene is transcribed into a functional RNA. This functional RNA encodes for protein production or for other RNA types that is rRNA and tRNA which helps in different cellular processes at protein levels.

1.2 Human Molecular Genetics

Human molecular genetics is that branch of genetics which is in relevance to the studies of all characteristics including both mental and physical aspects which can be transmitted from parents to their children's. Human molecular genetics includes studies of genetic disorder such as autosomal dominant, autosomal recessive and X-linked (Tomlinson and Bodmer, 1995).

1.3 Genetic Disorder Classification

Disorders at gene level occur due to many possibilities which also includes mutation in particular gene or disruption in the pathways of these genes. It then leads into truncated product productions (Sertié *et al.*, 2000). Genetic disorders are classified into different types on the bases of their functions and origin such as chromosomal, single gene disorder and polygenic.

1.3.1 Chromosomal Disorder

Chromosomal abnormalities occurs due to change in chromosomal structure and also sometime in chromosome numbers during the process of cell division. During cell division chromosomes changes their chromatids at the time of crossing over in which some of the mismatching parts of chromosomes are attached to one another. This sort of attachment then causes abnormal functioning of chromosomes and sometime it is also because of chromosomes numbers. One cell may get one chromosome extra or less such as trisomy and monosomy which results in loss of normal function. This loss or gain of the chromosome leads to incompetency of survival or production of disorder (Scriver *et al.*, 1973).

1.3.2 Single Gene Disorder

Single gene disorder is another important type of disorder which comes in occurrence due to defect in a gene of the cell. Single gene disorder is because of gene product truncation which usually happens because of single base pair mutation in gene or sometimes because of deletion of insertion in certain portion of gene. Due to this deletion of insertion the gene cannot perform its normal function. Single gene disorder are further more classified into three types which are autosomal dominant, autosomal recessive and X-linked which are named because of its mode of inheritance (Sriram *et al.*, 2005).

1.3.3 Enzymatic Defects

Mutation in genes of enzymes which controls certain cellular pathways can also be a cause of genetic illness. Although it is indirectly regulating cellular activities but have very worst effects on total cell functions. These defect results in metabolic pathways irregulation, and also when the substrate unusually converts into product that causes accumulation of substrate.

1.4 Polygenic Disorder

The word polygenic is combination of two words that is poly and genic. Poly means many or more in number while genic refers to genes. Therefore the word Polygenic is self evident and self explanatory that those disorder which are controlled by many genes which are more than adequate and which are multifactorial disorders (Purcell *et al.*, 2009).

1.5 Somatic Cell Genetic Disorders

Somatic cell disorders are those types of disorders which are caused in mutation in other than germ cells as it is caused due to mutation in gene which is present in other than germ cells (Park

et al., 2008). In cases of cancer their mutation occurrence in the somatic cell of body in which cell division becomes uncontrolled.

1.6 Multifactorial Disorders

Multifactorial are two disorders in which certain and environmental conditions interacting with each other and results in causing disorder. Neural tube and heart disease are some example of multifactorial diseases (Neal and Kendler, 1995).

1.6.1 Mitochondrial Disorders

The general division of disorder is mainly based on as where does it occur or because of gene due to mutation in that gene it is caused. Mitochondrial disorders are two in number which are caused due to non-genomic DNA that is mutation in mitochondrial (Schaefer *et al.*, 2004).

1.7 Causes of Genetic Disorders

1.7.1 Mutation

The change in structure and function of gene that is caused by single base pair change which is either deleted or inserted resulting in inappropriate functioning of that gene is known as called mutation and it is transmitted from generation to generation (Bamford *et al.*, 2004). Most information on human mutation rate comes from studies of rare autosomal dominant traits for which it is much easier to estimate mutation other than to estimate this for recessive train (Edwards, 1992).

1.7.2 Small Population

In Human civilization the populations are based on the fact of religion and geography etc. Population is sub-divided which form genetic isolates. This population may be the cause of a carrier for the mutant gene and that is why it becomes a reason for genetic disorder. This genetic disorder varies in size as in large population the total percentage of disorder appears to be higher and vice versa (Lichtenstein *et al.*, 2009).

1.8 Infertility

The failure to conceive in a couple struggling to reproduce for a period of two years without any sort of regard for conception is known as infertility. There is the possibility that 15 out of 100 couples may be childless. The ratio of such infertility between male and female is almost equal, i.e. male factor infertility accounts for approximately 50% of causes and likewise

in female (poongothai J *et al.,* 2009). Infertility is further classified into two categories i.e primary infertility and secondary infertility.

1.8.1 Primary Infertility

A couple which fail to get pregnant after two years of unprotected intercourse is called primary infertility. The reasons responsible for primary infertility are immunological, anatomical, endocrinological and genetic problems which resulted to the reproduction failures.

1.8.2 Secondary Infertility

When a couple has experienced to have one or more successful pregnancies but afterwards getting difficulty in conceiving again is known as Secondary Infertility. The causes aggravating the secondary infertility are exposure to different toxic materials, poor diet, UTI (urinary tract infection), socio-cultural norms such as marriages between relatives (endogamous marriage) and maiming of female genetalia. The major consequence of secondary infertility in a couple is childlessness but it is easy to avoid it (Evens E. M, 2004).

1.9 Types of Infertility

There are two types of infertility i.e. female infertility and male infertility.

1.9.1 Female Infertility

In women, ovulation disorder is one of the prominent reasons of infertility which is responsible for 50 per cent of cases. There are some other reasons which also contribute in female infertility such as reproductive tract disease, endometriosis and hyperprolactinemia but the main components responsible for infertility are damaged fallopian tubes. Sometimes infertility inducing microorganisms such as Chlamydia trachomatis and Neisseria gonorrheae are also the reason for female infertility. Pelvic surgery in female also increases the chance of infertility (Mueller and Daling, 1989). Other type of female infertility includes ectopic pregnancy and huge number of unsafe miscarriages in a society. Furthermore, chain smoker and cervical mucous malfunctioning are also considered responsible for female infertility especially childhood cancer (Zenzes, 2000). Developing countries are more prominent in terms of female infertility as compared to the developed countries.

1.9.2 Male Infertility

Spermatogenic disorders are considered to have a significant genetic component and are also the main cause of male infertility. Several types of genetic abnormalities have been

connected to impaired spermatogenesis in men, also consisting of multifactorial diseases where infertility is associated with a syndrome, chromosome abnormalities, and single gene disorders (Griffin D K and Finch K A, 2005).

In 30–40% of infertile couples, the reason comes out to be the male infertility. From 10 to 30 per cent cases are either unrevealed or because of both male and female infertilities (Zegers-Hochschild F, 2010). In the male the fertility criteria include normal spermatogenesis, complete maturation of the spermatozoa during the passage through the accessory organs of the reproductive system, accessory organs' patency, production of an adequate volume of seminal fluid, capability to deliver the semen into the female genitalia, mobilization of the spermatozoa sufficient to reach the oocyte in the uterine tubes and penetrate it (Travaglini *et al.*, 2006).

Male infertility is a multidimensional aliment encircling a huge range of malfunctioning. About 50 per cent of male infertility is either un-identified or congenital or may be acquired. The infertile male may show many abnormal conditions such as Azoospermia, Oligozoospermia, Teratozoospermia, Asthenozoospermia, Necrospermia and Pyospermia (Poongothai *et al.*, 2009). The decrease in male fertility may be as a result of acquired abnormalities or congenital abnormalities. The main causes for such abnormalities are developmental, genetic and anatomical abnormalities, varicocele, endocrinopathies, genital tract infections and immunological and environmental factors. However, 60 to 75 per cent of male infertility causes are un-identified and is hard to counter it. Among those unrevealed cases, majority of them have no past records correlated with problems of fertility and show normal identification by examining them physically (Jungwirth A *et al.*, 2004).

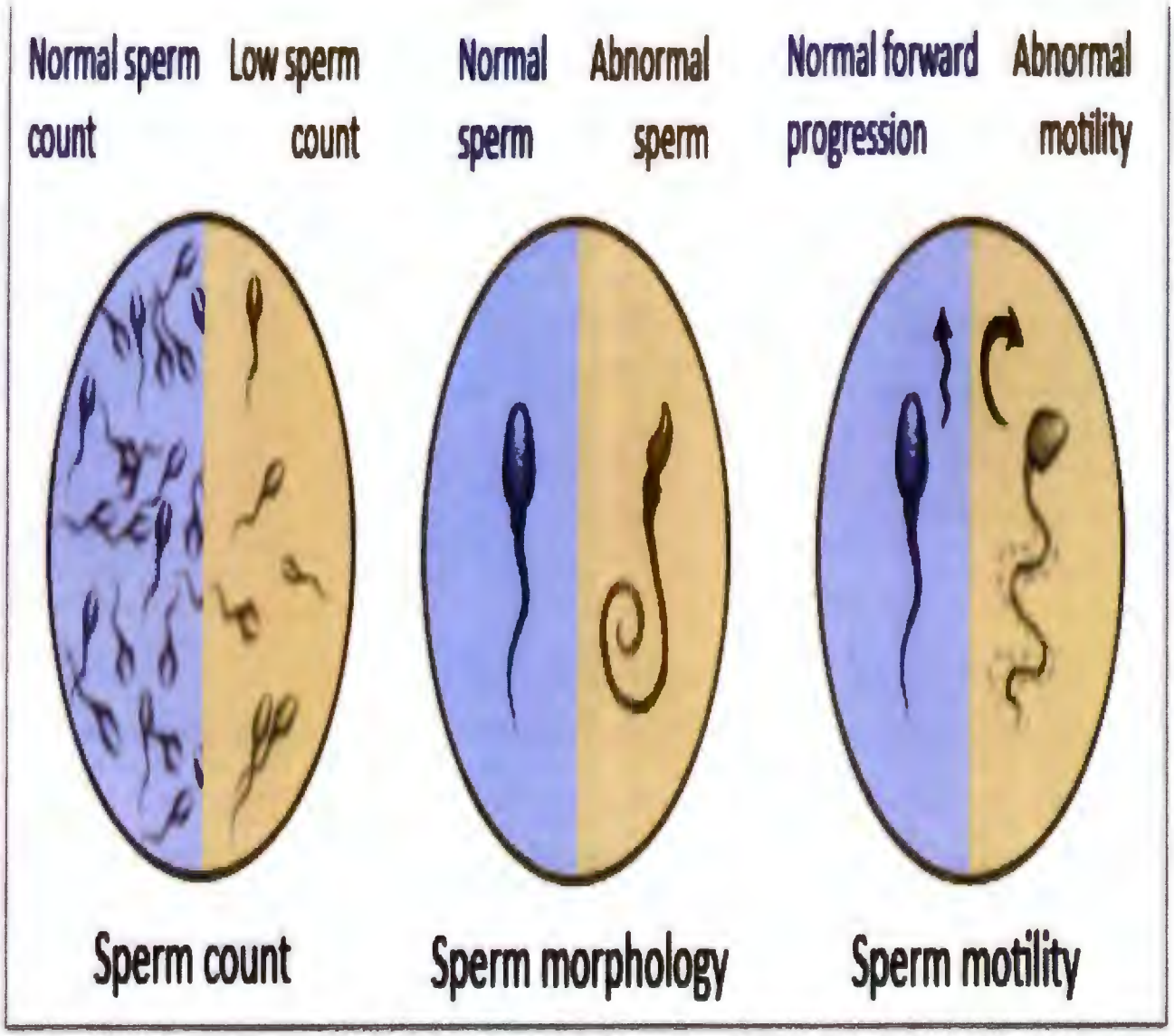


Figure1: Normal and abnormal sperm count, morphology and motility.

1.10 Prevalence of Male Infertility

It is predicated worldwide that about 60 to 80 million of couples are affected from the experiences of childlessness per year, out of which presumably 15–20 million are in India alone (Poongothai *et al.*, 2009) the collective data of demographic survey reveals that there is no differentiation between involuntary and voluntary fertility regardless of gender and human developments e.g. 10 to 17 per cent in Finland and 18 to 21 per cent in Switzerland while such

prevalence is 5 to 8 per cent in women of Ukraine, Czech republic, Poland, Romania and Norway and less than 10 per cent in Canada and Sweden. The huge deviation in between countries is due to extractable to huge variation in voluntary infertility (Lunenfeld and van Steirteghem, 2004). China is one of the top of list among those countries which possesses low primary infertility rate i.e. 1.3 per cent. The prevalence rate within china is also different, some provinces shows higher prevalence rate like Qinghai 2.3 per cent, Tibet 3.7 per cent and xinjiang 3.7 per cent as compare to other localities in china (Liu *et al.*, 2005). Interestingly Pakistan, the only muslim country with high population growth rate and also has high infertility rate of 15 to 22 per cent (Hardee K, Leahy E., 2008). The regions have high prevalence of infertility are south Asia 29 per cent and sub Saharan 22 per cent (RHO, 2004).

1.11 Genetic or Molecular Causes of Male Infertility

A chromosomal abnormality is one of the vital reason of genetic male mutation of the cystic fibrosis transmembrane receptor gene (CFTR) and Y chromosome microdeletion (Tuttelmann and Simoni, 2008). According to some approximation 4000 genes are responsible for the functioning of human spermatogenesis (Gianotten *et al.*, 2004).

1.11.1 Chromosomal Abnormalities

The prevalence of chromosomal abnormalities is estimated as15 per cent in azoospermic male population (Ferlin *et al.*, 2007). The main reason for azoospermia is the abnormality found on Y chromosome especially microdeletion i.e. the complete absence of sperm or oligospermia i.e. less than 20 million sperm /mL (Ferlin *et al.*, 2007). Aneuploidy or incorrect chromosomes number in infertile men is also occurred due to chromosomal abnormalities (Emery and Carrell, 2006). Non obstructive azoospermia men usually have maximum possibility of aneuploid (Palermo *et al.*, 2002), especially in their sex chromosomes (Mateizel *et al.*, 2002). However aneuoplid sperm has a changed number of genetic materials which in rare cases can effectively fertilize the oocyte and pass on an abnormal chromosomal sequence to their offspring (Carrell DT., 2008).

1.11.2 Klinefelter Syndrome

Klinefelter syndrome, which is the most common chromosomal abnormality caused by aneuploidy, has got a prevalence of 5% in men with severe oligozoospermia and 10% in azoospermic men (Foresta *et al.*, 2005). The syndrome usually is responsible for the arrest of

spermatogenesis at the primary spermatocyte stage, but occasionally in the later stages of sperm development are observed (Georgiou I *et al.,* 2006). There are two types of Klinefelter syndrome: nonmosaic, 47, XXY; and mosaic, 47, XXY/ 46, XY. Although previously believed to be sterile, it has been calculated that 25% of nonmosaic Klinefelter syndrome patients have sperm in their ejaculate (Ferlin *et al.,* 2007). Men with the mosaic form of the disease may have residual spermatogenesis in their seminiferous tubules and study has given the estimated number that 74% of the men were azoospermic (Foresta *et al.,* 2005). Klinefelter syndrome patients may try to achieve pregnancy using ICSI, but they risk producing offspring with chromosomal abnormalities (Ron-El *et al.,* 2000). Some research shows fruitful results of having nonmosaic men and ICSI (Yamamoto *et al.,* 2002).

1.11.3 Chromosomal Translocations

An additional source of aneuploidy is chromosomal translocation (Gianaroli *et al.*, 2002). Translocations can result into the loss of genetic material at the break points of genes, which can disrupt the genetic message (Carrell, 2008). The possibility of autosomal translocation is 4-10 time less in normal males than in infertile males (Elliott and Cooke, 1997). The fusion of two acrocentric chromosomes caused Rebertsonian translocation, which is a common chromosomal abnormality found in human i.e. 1 out of 1000 men (Ferlin *et al.,* 2007). However, Robertsonian translocation is 9 times less in general population as compared to infertile males which is 0.8 per cent (De Braekeleer and Dao TN, 1991). The translocation is responsible for variation in sperm production phenotype i.e. from regular spermatogenesis to an irregular spermetogonia (Georgiou *et al.,* 2006). Oligospermic and azoospermic men are exposed to Robertsonian translocation i.e. 1.6 per cent (oligospermic men) and 0.09 per cent (azoospermic men) (Johnson, 1998). Carriers of Robertsonian translocations may demonstrate a normal phenotype but could be infertile because of a lack of gamete production (Ferlin *et al.,* 2007). To avoid the threat of passing translocation to offspring, recommendation of fluorescent in situ hybridization along with probes added for common translocation is suggested in order for the determination of chromosomal composition of the sperm. (Georgiou *et al.,* 2006).

1.11.4 CFTR Gene Mutation

The gene responsible for autosomal genetic disorder is mutation in CFTR gene, consequently disturbing the reproduction capabilities (Visser and Repping, 2010). Such mutation

results in cystic fibrosis disorder or other related phenotype i.e. CFTR- related disorder (CFTR-RD) infertility, especially clinically manifested disorder of CF is enable to hit both sexes with or without clinically apparent disease. CFTR disorder is very rarely caused by CFTR-RD malfunctional situation, just like CBAVD, chronic pancreatitis, dissemenated brochiectesis or chronic rhinosinusitis. Commonly present CFTR-RD is because of CBAVD and is held responsible for 1 to 2 per cent of sterile community, however, they are healthy. The possibility of CFTR-RD in obstructive azoospermia is about 25 per cent in males (Yu J *et al.*, 2012). Common CFTR mutations causing CBVAD are, C.1210-121 (5), C. 1521-1523de ICTT (F508del) and C.350G. A (R117H) (Claustres, (2005); Dork *et al.*, (1997); Casals *et al.*, 2000). Late study exhibit a proof for inclusion of CFTR protein in few pathways during the time spent spermatogenesis (Ruan *et al.*, 2012). Another study demonstrated that expanded frequencies of CFTR changes in barren patients with non obstructive azoospermia, disabled spermetogenesis and low sperm quality (Yu *et al.*, 2011). However, another study shows no association of CFTR mutations with idiopathic male infertility (Mak *et al.*, 2000). Hence, bigger investigation of CFTR transformation screening among unselected infertile guys with various sperm numbers may add to the clarification of the part of CFTR quality in the weakened spermatogenesis and male barrenness.

1.11.5 Y Chromosome Microdeletion

The role of Y chromosome in male infertility was first explicated in 1976 when Tiepolo and Zuffardi have proposed the presence of a key factor which controls spermatogenesis encoded by a gene that is localized within the euchromatic region of the Y chromosome long arm (Yq11), which was called the azoospermia factor (*AZF*), because the first six men observed with microscopic terminal deletions in Yq, by routine karyotyping, were azoospermic (Tiepolo and Zuffardi, 1976). The vital area of interest for male factor infertility is Y chromosome because it possesses certain genes which are very crucial for spermetogenesis and puberty. The complexety of Y chromosome and the position of deletion of huge segments of the chromosome relates multiple genes, which is then hard to examine the real reason for infertile phenotype (Reynolds N and Cooke HJ., 2005). An addition, such phenotype is also caused by certain other deletions or

mutations. Such factors make it difficult to show a relationship of mutations with infertile phenotype.

Y chromosome microdeletion ranked second after the klinefelter syndrome which is responsible for genetic male infertility. Last decade witnessed a huge number of infertile patients which certainly attracted the attention of researchers toward the diagnostic workup of male infertility (Simoni *et al.,* 2008).

Y chromosome micro deletion screening provides diagnostic, prognostic, and preventive impacts. In addition, it can improve genetic counseling. Moreover, infertile men are known to be at increased risk of androgen deficiency and testicular neoplasia (Giannouli *et al.,* 2004; Nathanson *et al.,* 2005); however, whether the patients with Yq deletions have a greater risk also requires careful monitoring. Deletions of the long arm of the Y chromosome (Yq) are the most frequently recognized genetic cause of male infertility, with a prevalence of approximately 5% in azoospermic or severely oligospermic men (Krausz and Giachini, 2007). The azoospermia factor region (AZF region) is a specific zone of Yq which keeps genes that are involved in the growth and development of sperm. The AZF region has also three sub regions i.e. AZFa, AZFb and AZFc. Researcher, research is an attempt to specify deletion process in the particular region of AZF in order to unfold the hurdles came in the way of determining the diagnosis of infertile males (Vogt, 2005).

1.12.6 AZFa REGION

The two main genes located in the AZFa region are USP9Yand DBY (also called DDX3Y). Deletions in the AZFa region that remove both of these genes cause Sertoli cell–only syndrome, a condition characterized by the presence of complete Sertoli cells in the testes but a lack of spermatozoa in the ejaculate (Nuti and Krausz, 2008). The major gene on AZFa region is DBY gene. It has a prominent role in infertility due to its location in testis and its involvement in the premeiotic germ cells (Vogt PH., 2005). Previously conducted research on transcriptional activity of several AZF region genes shows that all other genes examined were transcribed normally espect men with Sertoli cell–only syndrome had reduced level of DBY transcripts. Such results reflex the importance of DBY gene in spermatogenesis, however further studies would be conducted to replicate such findings (Lardone *et al.,* 2007). The USP9Y gene is also involved in spermatogenesis; however it seems that this gene has the capacity to govern spermatogenesis as well as to pass to the next generation (Tyler-Smith, 2008). Shortening or

deletion of the USP9Y gene causes azoospermia (Galan *et al.,* 2007), oligozoospermia or oligoasthenozoospermia. Additional research work is required for the determination of exact role of such particular gene in the development of fertility and to diagnose the infertile males through the targeted Y chromosome screening method (Krausz *et al.,* 2006).

1.11.7 AZFb REGION

Deletions of the AZFb region cause arrest of spermatogenesis at the primary spermatocyte stage (Vogt PH., 2005), indicating that the region is essential for fertility (Nuti and Krausz, 2008). The most prominent gene in the AZFb region is RBMY. There are six different RBMY copies of the gene present on the Y chromosome (Skaletsky H *et al.,* 2003).

1.11.8 AZFc REGION

The AZFc is a 4.5-Mb region of the euchromatin and its complete deletion is one of the most frequent causes of male infertility (Zhang *et al.,* 2007). The AZFc region is prone to many smaller subdeletions that are thought to be caused by intrachromosomal recombination's (Ferlin *et al.,* 2007). Three most vital sub deletions on the AZFc region of the Y chromosome are gr/gr, b1/b3 and g1/g3 (b2/b3) (Lin YW *et al.,* 2007). The gr/gr subdeletions after removing its major content in the AZFc region shows the harsh effects of the ethnic background specially on gene functioning. Many research works specify the deletion as a risk factor for the loss of spermetogenesis but still other did not specify any relation among them (Zhang *et al.,* 2007). Approximately 80% of AZF microdeletions occur in AZFc region and most of them result in entire DAZ (deleted in azoospermia) gene deletion (Li *et al.,* 2013). AZFc region has the 'deleted in azoospermia (DAZ)' gene cluster as an important candidate gene (Foresta *et al.,* 2001). DAZ genes are assumed to have a huge range of roles in the development of spermetogenic activities because they are very prominent in all developmental stages of germ cells (Reynolds and Cooke, 2005). Deletions of such DAZ genes show a spectrum of phenotypes. These phenotypes have its specific range i.e. from oligospermia to azoospermia (Reijo *et al.,* 1995). The Y chromosome contains 300 sequence tagged sites (STS), which correspond to the AZF regions and could be exploited for easier characterization of micro deletions (Foresta *et al.,* 2001). It is needed to define a specific STS for different ethnic populations Mitra *et al.,* 2008).

1.12 OBJECTIVES OF THE PROPOSED RESEARCH

So far, no genetic study has been conducted regarding male infertility in population of KPK province; hence, following study will mainly focus on,

- Identification of families with male infertility in KPK Province.
- Screening of selected genes in the collected families.
- Analysis of identified mutation and its impact on the genes.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Vogt P. H., (2004) was doing experiment on fertility factor in human being and he focused experimentation on AZF region. AZF is specifically located at Y chromosome at sub band 11. He also divided the bands into other three sub bands that are AZFa, AZFb and AZFc. The sub grouping of the band into three different bands occurs because of crossing over and some other genetic processes like genetic recombination during cell cycles. Researchers believe that when infertility it was investigated that few deletion of AZFc regions were detected that was considered as a cause of infertility. Deletion in the genetic sequences occurs because of cellular division processes like homologous recombination between two chromatids in repetitive sequences. AZFc deletions are known as a putative cause of genetic cause of infertility in human especially in human males. However, some heterogeneities are identifies in the genomic region of Y region in fertile male and in cases of testicular pathology AZFa and AZFb deletion association also founded. Partial AZF deletions are associated with variable pathologies and partial AZFc deletions may even have no impact on male fertility. The study suggests various genes that are present on AZFa and AZFb which are examined and found to have a clear relationship between the occurrence of first micro deletions then macro deletions in the repetitive structure of Yq11 where large palindromes are probably promoting multiple gene conversions and AZF rearrangements.

Carvalho *et al.,* (2004) inspected causes of male infertility in human being and he also reported some previously studied reports and also some of his new findings about male infertility factors. He also reported few base pairs deletion on Y chromosome as a causative agent of azoopermia and also that of oligospermia. Investigation based on experimental work shows that approximately 7-15% of the affected individuals were having missing few base pairs on the long arm of Y chromosome that has been called AZF (azoospermia factor). The frequency of these micro deletion changes with various factors like area, races and individuals. For this purpose a relatively large population was studied to know whether there is a relation between the frequency and the population variations. For this a research sample of 51 infertile individuals of Israeli background were investigated in which 9 of these were having micro deletion on Y chromosome region. After micro deletion detection next was to haplotype analysis using hierarchical system which was having markers upto eight with two alleles. Deletion was also investigated 50f2/C and

the results obtained showed that seven patients were not having AZFc deletion but only one patient was detected with AZFb micro deletion so indicating that these micro deletion overlap with one another. To scan Y chromosomes micro deletion some other genetic tests that are necessary are azoospermic and oligospermic diagnostics. Since 1999, the European molecular hereditary qualities and European foundation of Andrology have been effectively involved in supporting the change of the quality demonstrative measures by distribution of the lab quid line for atomic analysis of Y chromosome microdeletion and by offering outer quality assessment trials.

Krausz *et al.,* (2004) also studied some other necessary and helping aspects in studying these Y chromosomes micro deletions that is proper laboratory guidelines for the clinical investigation of Y chromosome microdeletion, gene specific micro deletion and also provide an inform on the results of the quality control program. This research pointed out some other factors that are gr/gr deletion is also known to be the biggest cause of the male infertility that is investigated in the last decade. The protocol that was developed for the detection as based on PCR and also on the investigation of AZF region missing base pairs and it requires a minor modification in populaces with a specific Y chromosome foundation. Be that as it may, in regards of newly discovered data and also the phenotype/ genotype relationship it is highly recommended to investigate the two regions of AZF which are thought to be a hotspot regions having microdeletion and those regions are AZFa and AZFb. Some other improvements were made in the investigation process that's novel strategies and units with exorbitantly high number of markers but sensitivity of the investigation was not enhanced with all these progresses.

Galan J *et al.,* (2005) analyzed different genes that are involved in human male infertility and also investigated polymorphism within these genes like ESR1, FSHR, ESR2, CYP19A1, and NRIP1. All these genes are identified as a factor for human male idiopathic infertility and to test while combine effects of these genes on function of male reproductive system. Studies were continued to investigate various genetic association and its relation with male infertility using both single gene and multi locus experimentation. Different private and public sectors were engaged for medical investigation of male fertility factor and approximately one hundred Spanish male were analyzed with azoospermia, oligozoospermia and 95 unselected race-matched healthy controls from the same geographic region. The research also covers homozygous infertility in males on ESR1 g.938TC marker. Different loci of different gene were analyzed and

their genetic interaction among five different gene markers that are influential over male infertility. Additionally five different loci were identified with different frequency of 9.4% in controls but absent in infertile men. The results are in favor of relevant role for estrogenic pathways. Notably the ESR1 gene, in human male reproductive function and advocate a complex trait model for male infertility.

Krausz *et al.*, (2007) investigated molecular investigation of chromosome which was considered as a hotspot in the infertility factor of male especially Y chromosome. This infertility occurs due to few alterations on Y chromosome that sometimes deletion of few base pairs that is an ultimate cause of azoospermia a condition in infertility in human males. Different techniques and mechanisms were used to improve quality of laboratory some times by publication of proper laboratory guidelines by European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) for molecular diagnosis of Y-chromosomal micro deletions and by offering external quality assessment trials. Recently in 2004 a laboratory guidelines were published to identify novelties in Y chromosome (classic, partial and gene-specific deletions, genotype–phenotype correlations, methodological issues). In the last decade few other reasons were find out as a cause of infertility in human males such as gr/gr deletion which is considered as an impaired sperm formation. Be that as it may, the screening for this erasure sort in the routine symptomatic setting is still a wrangled about issue among specialists. The road map that was developed for analysis of Y chromosome micro deletion is consisting of PCR remains completely legitimate and fitting for exact investigation of azoospermia factor deletions and it needs some specific alteration in a large population with Y chromosomes micro deletion. After all these millstones achievements some newly find data on the bases of genotype and phenotype analysis AZFa and AZFb microdeletion investigation is recommended. Some other modern techniques do not improves the sensitive of this test either by using kit method with large number of markers. Although the external quality control programme is highly cherished by the participant.

Zuccarello *et al.*, (2008) identified that asthenozoospermia (AZS) is a common cause of male infertility characterized by reduced forward motility (WHO grade A1B sperm motility <50% or A < 25%) or absent sperm motility in fresh ejaculate. AZS can occur separately without an association with other disorders like some other syndromes and sperm abnormalities. Some genes that help in cilia/flagella structure which are associated AZS and some other genes are also

reported which have contribution in AZS abnormalities, including primary ciliary dyskinesia (PCD) and Kartagener syndrome (KS). Axonemal ultra structural abnormalities like missing or shorting of working arms of dyneins are reported to be involved in approximately 50% of PCD/KS patients. More than 90 % of patients with KS who are male having an abnormality of AZS. Dynein genes mutation are found in mostly KS patients further screening of genes like DNAI1, DNAH5 and DNAH 11 are studied in more than 85% students who were having AZS non syndromic phenotype and 200 controls. This research reported some novel results that are three new alteration in that single gene that is in correlation with azoospermia in approximately seven patients (7.8%). Mutations are inherited from the mothers and may be found in familial clusters. No ultra-structural axonemal anomaly was detected in sperm.

Lourenço *et al.,* (2009) was doing research on genetic causes of ovarian insufficiency which is non syndromic in nature. A nuclear receptor, NR5A1 (likewise called steroidogenic factor 1) a key transcriptional component that enacts translation of quality that are included in the hypothalamic pituitary steroidogenic axis. Alteration of NR5A1 causes disorders that are associated sex chromosome and non sex chromosomes that are actually 46 in numbers, these chromosomes are involved in sex development in one way or other way, with or without adrenal failure. NR5A1 was hypothesized that it is involved in ovarian failure and also considered that it have a function of ovary development. Certain families were examined in this research project and it was concluded from the population examination that each of the four families and the in other 25 families 2 were investigated with isolated ovarian abnormalities which is having alteration in NR5A1 and these alteration were of various type like frame shift deletion and missense mutation. When the gene functional analysis was carried out indicates that alternation in gene does not directly affect the gene but it affects the transcriptional factors that further activates gene for regulating cellular processes.

Kee *et al.,* (2009) was doing experiment on different factors of infertility and he noticed some solid reasons behind infertility in human male and female. He also identified that it is caused due to qualitative and quantitative defects in the cells which are related to sex like germ cells (oocyte and sperm) development. The clear understanding of these germ cells is not very effective because it developmental stages at fetal level could not easily be traced so scientist are not sure about their formation and differentiation. But with development of animal biotechnology it has become possible to know that germ cells differentiated from human and

mouse cells particularly embryonic cells. To isolate primordial germ cells from both male and female some of the germ cells reporter was used for its further quantification of these germ cells. Human germ cells formation and development progression some of the important protein that specifically binds to RNA was inhibited in other words their expression was silenced. Some of the following observations were made after this study like human DAZL (Deleted in Azoospermia-Like) functions in primordial germ cell formation, whereas closely-related genes, and some other genes like DAZ and BOULE have an important function the gametes formation and also in progression of meiosis.

Gülşah *et al.,* (2010) was investigating infertility and defined infertility as inability of getting pregnant despite having frequent and sometime protected sex at least for a year. There are many factors on which fertility of a person male and female depends and male is contributing up to 50% to infertility factors. Spontaneous abortion (SAB) can result as a factor of various factors like advanced maternal age, genetic factors such as Y chromosome micro deletions and chromosomal anomalies. The research was designed to explore the relationship male infertility and the deletion in few base pairs of on Y chromosome and also a spontaneous abortion. A small population was examined for these few factors which were considered as a cause of infertility, approximately. All the women were 46, XX and men were 46, XY. The research postulated that no micro deletions of y chromosomes were detected in all individuals who were a part of this research sample. To get into the grassroots level to the disease the samples study was enhanced to detect alteration/deletion on Y chromosome or spontaneous abortion and also the numbers of patients were also increased.

Hildebrand *et al.,* (2010) investigated clinically significant proportion of couples experience difficulty in conceiving a child. In more than 50% of all such cases of male infertility and some other factor are involved at gene level. Despite advances in clinical diagnostics B50% of male infertility cases remain idiopathic. Based on this, further analysis of infertile males is required to identify new genetic factors involved in male infertility. This review focuses on cation channel of sperm (CATSPER)-related male infertility.

Beforehand, touching quality cancellations with the CATSPER2 quality embroiled the sperm specific CATSPER direct in syndromic male infertility (SMI). As of late, they discovered insertion changes of the CATSPER1 quality in families with recessively inherited non syndromic male infertility (NSMI). The CATSPER channels in this manner speak to a novel human male

richness component. In this survey they outline the hereditary and clinical information demonstrating the part of CATSPER changes in human types of NSMI and SMI. More ever, they examine clinical administration and helpful alternatives for these patients. At long last, they depict how the CATSPER channel could be utilized as an objective for improvement of a male preventative.

Totonchi *et al.,* (2012) detected micro deletion of Y-chromosome is useful to obtain reliable genetic information for assisted reproductive techniques, thus avoiding unnecessary treatment and vertical transmission of genetic defects. Purposes this research was conducted over a six-year period to analyze clinical data, somatic cytogenetic abnormalities, and types of micro deletions in men with fertility disorders in Iran. Semen samples were analyzed according to standard methods. Conventional chromosomal karyotyping was used to analyze chromosome abnormalities. Polymerase chain reaction (PCR) amplification using nine specific sequence-tagged sites (STS) was used to detect AZF micro deletions. Out of the 3654 patients who were analyzed, AZF region micro deletions were detected in 185 cases (5.06 %). Karyotype analyses was available for 157 men and among them abnormal karyotypes were found in 51 cases (32.48 %). One hundred and forty-seven cases with Yq micro deletions suffered from azoospermia and 38 from severe oligozoospermia. The research shows that the most frequent micro deletions were in the AZFc region, followed by the AZFb+c+d, AZFb+c, AZFb, AZFa, and AZF a+c regions. Conclusion The study has confirmed that the detection of micro deletions in the AZF region is significant from a diagnostic viewpoint. It is also useful to obtain reliable genetic information from infertile men to determine the etiology of the deletions, and to avoid unnecessary treatments and vertical transmission of genetic defects.

Qian *et al .,* (2013) was doing research on breast cancer and he noticed some other protein that are involved in the resistance mechanism of breast cancer and these protein are abbreviated as (Bcrp), actually these proteins are belonging to group of ABC that is an abbreviation of ATPs binding cassette that are helping in the transport of drug delivery. Many experiment were carried out to analyze these properly so Bcrp different polypeptides were dimerized to investigate its function as a drug transporter and the result shows that it function as ATP dependent drug efflux drug pump with a diversified spectrum of hydrophilic and hydrophobic substrates ranging from anticancer, antiviral and antihypertension drugs, organic anions, antibiotics, phyto estrogens (e.g., genistein, daidzein, coumestrol), xeno estrogens and

steroids (e.g., dehydroepiandrosterone sulfate, DHEAS). Bcrp belongs to membrane protein which have the role in both normal cells and in cancer too (e.g., testis, placenta, intestine and brain) that provides defense mechanism for cells to prevent the drug entry into cell or staying in cells for shorter interval of time. Brain also contains these cells which perform the function of creating barrier in blood and brain and these cell are located near tight junctions, however, Bcrp is absent at the Sertoli cell blood-testis barrier (BTB), instead, it is localized almost exclusively to the endothelial TJ in microvessels located in the interstitial space between somniferous tubules, and the peri tubular myoid cells in the tunica propriabehind the basement membrane. Recent studies have shown that Bcrp also expressed by Sertoli and germ cells stage-specifically, but its expression in the somniferous epithelium is restricted only to a testis-specific cell adhesion ultra structure known as apical ecto plasmic specialization (apical ES) at late stage VI to early VIII of the somniferous epithelial cycle of spermatogenesis, at the Sertoli-spermatid interface, namely step 17-19 spermatids. These research shows that Bcrp is being equipped by late spermatids and Sertoli cells to protect these delayed phase specialized spermatids in their development to complete spermatogenesis. Furthermore, Bcrp was found to associate with several actin regulatory proteins and actin at the apical ES, and it was involved in the reorganization of actin filaments at the apical ES in stage VII tubules.

Smith *et al.,* (2012) explored and identified spermatogenesis is a process with complex feature that involves biological interaction of two different types of cells that is somatic cells and germ cells. Re providing GCs nutrients and some defensive mechanism that is immunological support against certain immunogens. The transport of nutrients are helped by a group of microtubules which are placed parallel to one another and it facilitates movement of nutrients from cell to GCs as well as translocation of spermatids through the seminiferous epithelium during maturation. Their occur some abnormalities in the SC which results in GCs premature wastage of large number of cells. Some nonspecific mutagenesis mechanism some novel mouse line which are having similar function like male infertility which results due alteration in the ATPase region of the newly KATANIN p60 related microtubules severing protein Katanin p60 subunit A-like1 (KATNAL1). The results indicate Katanal1 is expressed in testicular Sertoli cells (SC) from 15.5 days post-coitum (dpc) and that, and their occur alteration in the chemical structure continuously which in turn results in loss of function of that particular gene which contribute to male-specific infertility by alteration of SC microtubule dynamics and premature

exfoliation of spermatids from the seminiferous epithelium. KATNAL1 was deeply studied and it was identified that it have primary role in the regulation of male fertility which provides a starter point for the understanding of SC microtubules and also evidences how it promote male fertility.

Xu *et al.,* (2013) investigate the conceivably utilization inherited variation of these loci on spermatogenic hindrance, particularly oligozoospermia. A sum of 784 guys having low sperm count (oligospermia) and 592 solid controls were enlisted to this study from March, 2004 and jan-2016. They led two phase studies to seek the relationship amongst oligozoospermia and new creators close NOA hazard loci. In the principal stage, they utilized cutting edge sequencing as part of 96 low sperm count cases and 96 sound control, to explore low sperm count powerless hereditary variations. In next stage, they approve these variations in an extensive partner containing 688 cases and 496 controls by SNP genotyping. They watched absolutely 7 oligozoospermia related variations. These variations in the conceivable utilization districts of qualities found by GWAS. This study serves as compelling methodology for finding hazard alleles later on.

Lopes *et al.,* (2013) was conducting research on failure of hormones associated with gonads with additional characteristics of early pregnancy loss and early death. This research investigated some important mutation that is harmful in human population. This research also explored with men with inability of spermatogenesis and also with unknown impairment. This research also investigated some other mutations that have deleterious alteration which are then compared with those individuals with normal spermatogenesis. Many genetic analysis were carried out like single nucleotides polymorphism and CNVs in 323 Caucasian men with idiopathic spermatogenic impairment and more than 1,100 controls, we estimate that each rare autosomal deletion detected in study multiplicatively changes a man's risk of disease by 10 % (OR 1.10 [1.04–1.16], p, 261023), rare X-linked CNVs by 29 %, (OR 1.29 [1.11–1.50], p,1he 61023), and rare Y-linked duplications by 88 % (OR 1.88 [1.13–3.13], p, 0.03). Different properties of CNVs were compared with case CNV with some other CNVs call set autism having additional schizophrenia, intellectual disability and also having bipolar disorders. This research also identified spermatogenesis as neurodevelopment disorders. Some patients were identified with two deletions of DMRT1 gene having chromosomal location 9p24.3 which is considered sex determinants locus of the avian ZW chromosome system. In an independent sample of Han

Chinese men, we identified 3 more DMRT1 deletions in 979 cases of idiopathic azoospermia and none in 1,734 controls, and found none in an additional 4,519 controls from public databases. The combined results indicate that DMRT1 loss-of-function mutations are a risk factor and potential genetic cause of human spermatogenic failure (frequency of 0.38% in 1306 cases and 0% in 7,754 controls, p=6.261025). This study also clarified that other recurrent CNVs as causes of idiopathic azoospermia and generates hypotheses for directing future studies on the genetic basis of male infertility and IVF.

Ghorbel *et al.,* (2014) led probe relationship between AZFc microdeletion that evacuate numerous qualities of the Y chromosome fluctuates among various nations and male sterility. The point of this research was to examine the recurrence and the attributes of various DAZ quality duplicate cancellations and their relationship spermetogenic disappointment and male childlessness in Tunisian men, 241 sterile males (30.7% no sperm count (n=74), 31.5% oligospermic (n=76) and 37.7% normozoospermia (n=91) and 115 sterile guys who fathered no less than one tyke were incorporated into the study. Three DAZ specific single nucleotide variation loci and six bi-allelic DAZ-SNVs (I-V1) were examined utilizing (Polymerase chain reaction) restriction fragment length polymorphism and PCR. These outcomes demonstrated high rate of infertile men (78.85%) and controls (78.26%) having just three DAZ gene copies (DAZ1, DAZ2, DAZ3, or DAZ1, DAZ3 and DAZ4 variations) so micro deletion of DAZ2 or DAZ4 were regular both in infertile (36.5% and 37.3%, individually) and fertile groups (33.9% and 44.3%, separately) and expelling DAZ4 copy was essentially more continuous in oligospermic than in normospermic men (p=0.04) in sterile group. This is additionally reported for the first time that prompt microdeletion of both DAZ2 and DAZ4 duplicates was significantly more basic in barren men (12.4%) than in ripe men (4.3%) (p=0.01). On the other hand, deletions of DAZ1/DAZ2 and DAZ3/DAZ4 cluster were exceptionally uncommon. Investigation of DAZ quality duplication in Tunisian individuals prescribed recommended that the synchronous erasure of DAZ2 and DAZ4 quality duplicates is connected with male barrenness, and that oligospermia is by all accounts advanced by expelling DAZ4.

Xiao Hua Jiang *et al.,* (2014) was doing research on blood flow and its indirect relationship with blood testis barrier analyzed effects of blood flow and its relationship with blood testis barrier (BTB) which was first observed in the cells that are known as sertoli cell which are placed adjacent to one another, these cells help out the best environment which help in

the maturation and nourishment of the germ cells at both meiotic and post meiotic cycles. There are many cells which are arranged with one another forming pretentious structure which forms a complex structure called tight junctions (TJs), and some other are called adhesion junctions and gap junctions (GJs). The abnormalities in these genes are because of deletion and functionally inhibition of gene through process of silencing which contributes to these genes which are producing these genes that disrupt BTB which in turn causes immunological or other noxious effects on cell cycles that on meiotic and post meiotic division lead to spermatogenic arrest and infertility.

Patrick Lorès *et al.*, (2014) proposed that MgcRacGAP is a (RACGAP1) is a GTPase activating protein (GAP), very created in the mouse embryonic cerebrum and in the human and mouse post-natal testis. MgcRacGAP adversely controls the action of Rac and Cdc42, which are key molecular switches following up on the microtubule and actin cytoskeleton and controlling different cell procedures, for example, expansion, grip and motility. Past studies showed that MgcRacGAP assume a basic part in the cytokinesis of substantial cells; thus homozygous inactivation of the quality in the mouse and transformation in caenorhabditiselegans prompted embryonic lethality because of the failure of MgcRacGAP-invalid developing lives to amass the focal axle and to finish cytokinesis. In the testis, the germ cells don't finish cytokinesis and stay associated as a syncytium everywhere throughout the entire methodology of spermatogenesis, fascinatingly, MgcRacGAP was appeared to situate to the intercellular extensions, connecting these germ cells. Keeping in mind the end goal to decide the function of MgcRacGAP in the male germ line, they produced a restrictive thump out mouse utilizing Stra8 promoter driven cre recombination to incite the specific erasure of MgcRacGAP in the pre meiotic germ cells. They found that the non appearance of MgcRacGAP impelled a germ line consumption and male sterility. Predictable with the part of MgcRacGAP in the foundation of the cytoplasm narrowing amid cytokinesis of the physical cells, they watched that MgcRacGAP erasure in the germ cells kept the arrangement of the intercellular scaffolds and instigated an expansion capture. While they expect that acquired homozygous loss of capacity changes in MgcRacGAP would be deadly in human, again transformation in the testis may represent a few instances of non-obstructive oligo and or azoo-spermia disorders, whose hereditary causes are out and out still ineffectively known oligospermia is one of the cruel types of idiopathic male infertility. Be that as it may, its pathology is generally obscure, and couples of hereditary variables have been characterized.

Khabour *et al.*, (2014) conducted research on AZF region of the human Y chromosome contains necessary genes for spermatogenesis. AZF region micro deletion has been shown to cause men sterility. The purpose of this research was to show the prevalence of AZF micro-deletions in Jordanian infertile males. A sample of 100 infertile males (36 with azoospermia and 64 with oligozoospermia) was screened for micro deletions using 16 AZF markers and polymerase chain reaction (PCR) technique. Two subjects were found to have micro deletions in AZFc region and one subject has micro deletion that includes AZFb and part of AZFc and AZFa. The three deletions were found in azoospermic subjects (8.3%). No micro deletions were found in oligozoospermic group. The prevalence of AZF region micro-deletion in Jordanian azoospermic infertile males is equal to that observed in other populations (1%-15%). The results suggest the importance of AZF micro deletion investigation for genetic counseling prior to providing assisted reproduction technique.

Noveski *et al.*, (2014) investigated that in approximately all individuals who were having CFTR gene abnormalities were suffering from missing of vas deference the most known CFTR disease. Some researchers reported data that is in contrast to other scientists that reveals that mutation in CFTR in infertile males were having non obstructing azoospermia and oligospermia. There results indicated a high percentage of CFTR and cystic fibrosis with double heterozygosity, but was not observed in those males who were fertile. These results are counted important in studying role of CFTR alteration in spermatogenesis process in human males.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Families description

In current study three families of male infertility were selected from different areas of Khyber Pakhtunkhwa province, Pakistan. Before any proceeding, informed consent of all members of families was obtained. Families were visited personally for the collection of blood samples and information that was used for pedigree construction. Different members of the families were interviewed to collect information. The history and information of all these families about infertility was carefully traced. The lab identification numbers were assigned to these families as: INFM1, INFM2 and INFM3.

3.2 Construction of pedigrees

After investigation of the families with a particular disease condition the information given by the elders of the families were used for constructing pedigree chart of the effected families according to the standard method of genetics.

It is necessary to show the relationship between different individuals in a family symbolically and there is a special representation called pedigree. Through this symbolic representation we can get knowledge about a specific family and can easily interpret their inheritance from their ancestors. To determine the mendalian inheritance of genetic traits, especially familial diseases, across several generations of a family this phylogenetic pedigree is prerequisite.

Pedigree chart contain certain specific symbols including circles, squares, filled squares and circles, double lines, single line and straight lines. Each and every symbol has its own genetic meaning.

Male and female are represented by squares and circles respectively and those who were abnormal were shown by filled circles and squares for both male and females respectively.

To show those individuals who or died is represented by fully black circle/square for male/female. Number in roman style indicates generation of the respective family. Family or cousin marriage was showed double line.

3.3 Clinical diagnosis

Clinical diagnosis of all the affected members of the families was performed. Semen analysis of affected individuals of the families was carried out with the help of clinical pathologists to determine the concentration of sperms that is necessary for evaluating male infertility. Semen was collected and liquefied within 15 minutes. The color of the semen was recorded and volume of semen was measured. The viscosity and pH of samples were measured.

3.4 Blood Sample Collection

After pedigree construction and all possible and relevant information that were necessary were taken and then the blood from all affected individuals and their other family members were taken up to by disposable syringes.

3.5 Storage of Blood

The collected blood was stored in BD vacutainer K2E (EDTA), Plymouth, PL6 78B. UK and was stored for isolation of DNA at freezing temperature to bring its maximum density so place at 4 degree Celsius.

3.6 Genomic DNA Extraction

Genomic DNA from human white blood cells was extracted utilizing using phenol-chloroform technique. For this reason, around 0.75 ml of human blood was blended with 0.75 ml of solution A (sucrose=0.32 M, Tris (pH 7.5) = 10mM, MgCl2 1 % (v/v) = 5mM, Triton X-100) in an eppendorf tube by reverting the eppendorf tube 4 to 6 times. Exposure to the room temperature for 15 minutes was given to the sample. The tube was then centrifuged (Eppendorf centrifuge 5415D) at 13,000 rpm fir one minute. And then the upper layer was removed and the pellet was re suspends and dissolved in 400 µl of solution A. and the mixture was centrifuged at the same rate. Again the supernatant was removed and pellet was dissolved in 400 µl of solution B [400mM NaCl, 2mM EDTA, 10 mM Tris] and 5-8 ul proteinase K (20mg/ml) were added to re suspend the pellet. Micro centrifuge tube was placed at 37 OC for overnight incubation. Next day, 500 µl mixtures of solution C (phenol) and solution D was added to the micro centrifuge tube and kept for centrifugation at 13,000 rpm for 13 minutes. Two layers were formed after centrifugation and the upper layer (aqueous) was separated in a new eppendorf and 500 µl of solution D (1 volume isoamyl alcohol, 24 volumes chloroform) was added to it. This new suspension was again spine for 10 minutes at 13,000rpm and upper layer was removed into

another micro centrifuge and blended after adding 55 µl of sodium acetate (3M, pH6) and 500 µl chilled isopropanol followed by centrifugation at the same rate. Tube was inverted many times for precipitation of DNA. Supernatant was carefully removed and 70 % ethyl alcohol was added to wash the DNA and kept the tube in incubator (Memmet, Germany) for drying. 100-200 µl TE or Tris EDTA (0.1 mM EDTA, 10mM Tris) was added to dissolve the DNA and kept at 37 ^{o}C for overnight incubation.

3.7 Molecular Analysis of Extracted DNA

One per cent Agarose gel was used for analyzing the extracted DNA. Gel solution was made by adding 0.5 g Agarose powder in fifty ml of 1XTBE solution in a flask and heated it for one minute in microwave oven. Gel rack was prepared by setting comb in it for the formation of wells. 5 µl Ethidium Bromide was added in solution to stain DNA and poured it into gel rack. Gel rack was allowed for some time to solidify. Preparation of loading of DNA was done by mixing 5 µl DNA and 3 µl stacking color on paraffin sheet and stacked in the wells of the gel. Electrophoresis was done at 100 volts for twenty minutes. DNA was envisioned under ultra violet transluminator (Wealtec Taiwan) and result was archived by using gel documentation system.

3.8 STS Markers

The STS markers used in the present study were selected from the previous reports (Krausz *et al.*, 2013 and Mehdi Totonchi *et al.*, 2012) and confirmed by UCSC genome browser (http://genome.ucsc.edu/). The list of STS markers used in the current study are shown in the table 1.

3.9 PCR Amplification of STS Markers

The STS markers in the DNA of infertile individuals and normal members were amplified for detection of deletions in the long arm of Y-chromosomes. Total 12 different STS markers were used in the current study. The total volume of reaction was 25 µl containing 1 µl of diluted genomic DNA (with concentration of 40-50nd/ ul), 0.5 ul of 10mM Deoxy-nucleotide Triphosphates (dNTPs), 0.3 ul of forward primers and 0.3 ul of reverse primer, 2.3 ul of 25mM MgCl2, 0.1 ul OF 0.3 unit Taq DNA polymerase, 17.8 ul PCR water and 2.5 ul of 10X PCR buffer composed of 750 mM Tris-hydrochloric acid, 200 mM ammonium sulphate and 0.1 % Tween 20. PCR was operated for 35 cycles with thermo cycling conditions initial denaturation at

95 °C for 4 minutes, remaining denaturation was carried out at 95 °C for 30 seconds, annealing temperature was 55 °C, extension was set at 72 °C for 30 seconds and final extension was programmed at 72 °C for six minutes. These reactions were performed in Biometra (Germany) thermal cycler.

3.10 Agarose Gel Electrophoresis

The amplified products were run on two per cent Agarose gel. Agarose gel was setup by blending one gram of Agarose in 50ml of one X solution, took after by warming in a microwave oven (PEL) for 50 seconds. After some time when the solution become cool then 5 ul Ethidium bromide was added in the solution for visualization of PCR product in ultra violet transluminator. The gel solution was poured in the gel tray and permitted to harden on normal condition. When the gel is completely solidified then the outright volume of the PCR item was blended with tracking or staking dye, and loaded into the wells. Electrophoresis was carried out at 110 volts (400mA) for half an hour. And then to visualize the amplified product the gel was put in gel documentation system. Pictures were caught for documentation and investigation reason.

3.11 Hormonal Analysis

Beside genotypic study hormonal analysis was also carried out such as LH, FSH, Prolactin TSH and Testosterone using "AIA-360 TOSOH" instrument employing chemiluminescent technology.

For hormonal analysis fresh blood was taken from the affected individuals and serum was separated to process it for further analysis.

Table 1: List of primers for Y chromosome microdeletion.

STS	Primer Sequence	Product Size (BP)	Region/ Gene
ZFY-F	ACCRCTGTACTGACTGTGATTACAC	495	SRY
ZFY-R	GCACYTCTTTGGTATCYGAGAAAGT		GENE
sY84-F	AGAAGGGTCTGAAAGCAGGT	326	AZFa
sY84-R	GCCTACTACCTGGAGGCTTC		
sY86-F	GTGACACAGACTATGCTTC	320	AZFa
sY86-R	ACACACAGAGGGACAACCCT		
sY117-F	GTTGGTTCCATGCTCCATAC	261	AZFb
sY117-R	CAGGGAGAGAGCCTTTTACC		
sY127-F	GGCTCACAAACGAAAAGAAA	274	AZFb
sY127-R	CTGCAGGCAGTAATAAGGGA		
sY134-F	GTCTGCCTCACCATAAAACG	301	AZFb
sY134-R	ACCACTGCCAAAACTTTCAA		
sY254-F	GGGTGTTACCAGAAGGCAAA	380	AZFc
sY254-R	GAACCGTATCTACCAAAGCAGC		
sY255-F	GTTACAGGATTCGGCGTGAT	123	AZFc
sY255-R	CTCGTCATGTGCAGCCAC		
sY 157-F	CTTAGGAAAAAGTGAAGCCG	285	AZFc
sY 157-R	CCTGCTGTCAGCAAGATACA		
sY 14-F	GAATATTCCCGCTCTCCGGA	380	SRY
sY 14-F	GCTGGTGCTCCATTCTTGAG		GENE
sY 83-F	CTTGAATCAAAGAAGGCCCT	275	AZFa
sY 83-R	CAATTTGGTTTGGCTGACAT		
sY 154-F	TTTGCACCAGGATTAAGTGA	451	AZFc
sY 154-R	TTTTTTCAGATAAACTTTCAGTGG		

(Simoni M *et al.*, 2004)

RESULTS

4. RESULTS

4.1 Description to collected families of male infertility

4.1.1 Family A (INFM-1)

Family A of male infertility was collected from village Mir Azam Machan khel, District lucky marwat, Province Khyber Pakhtunkhwa (KPK), Pakistan. Generation to generation pedigree was scripted upto four generations on the bases of information obtained from investigations and discussion with the notable of the family. This family consists of nine members including three affected males. The medical reports shows that all the affected individuals were only having azoospermic phenotype. And their previous history shows no other disease related to phenotype either syndromic or non syndromic was found. . Blood was collected from all individuals, including normal brothers and parents. Squares and circles show male and female respectively while filled squares and circles shows affected individuals. Two adjacent double lines show consanguineous marriage and cross diagonal line on circles or squares shows demised person. The roman numbers indicates the generation number of the individuals in the pedigree while that of an Arabic numbers indicates position of the individuals in the respective family.

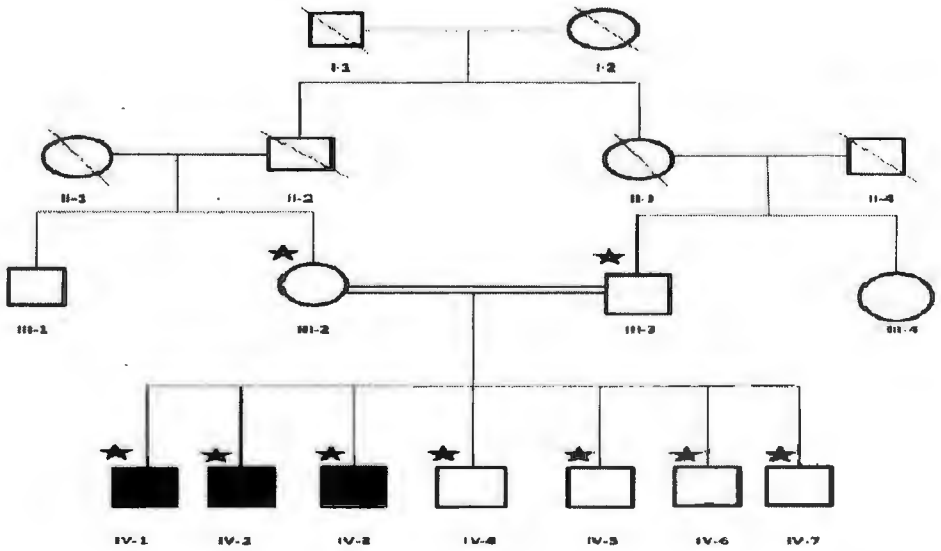


Figure 4.1 : Pedigree of Family A(INFM-1) having three azoospermic affected individuals.

4.1.2 Family B (INFM-2)

Family B (INFM-2) was collected from sokari, Tehsil and distracts Bannu, Province of Khyber Pakhtunkhwa, Pakistan. The pedigree of the family shows that it is consanguineous marriage and this disease was not present in the first three generation and it appeared in the fourth generation of the family. The medicals reports of the affected individual's shows that they are severe oligospermic which means very low sperm count and azoospermic which may be the cause of male infertility. The blood samples were collected from all individuals of the fourth generation which is indicated by star about the individuals symbols. Squares and circles show male and female respectively while filled squares and circles shows affected individuals. Two adjacent double lines show consanguineous marriage and cross diagonal line on circles or squares shows demised person. The roman numbers indicates the generation number of the individuals in the pedigree while that of an Arabic numbers indicates position of the individuals in the respective family

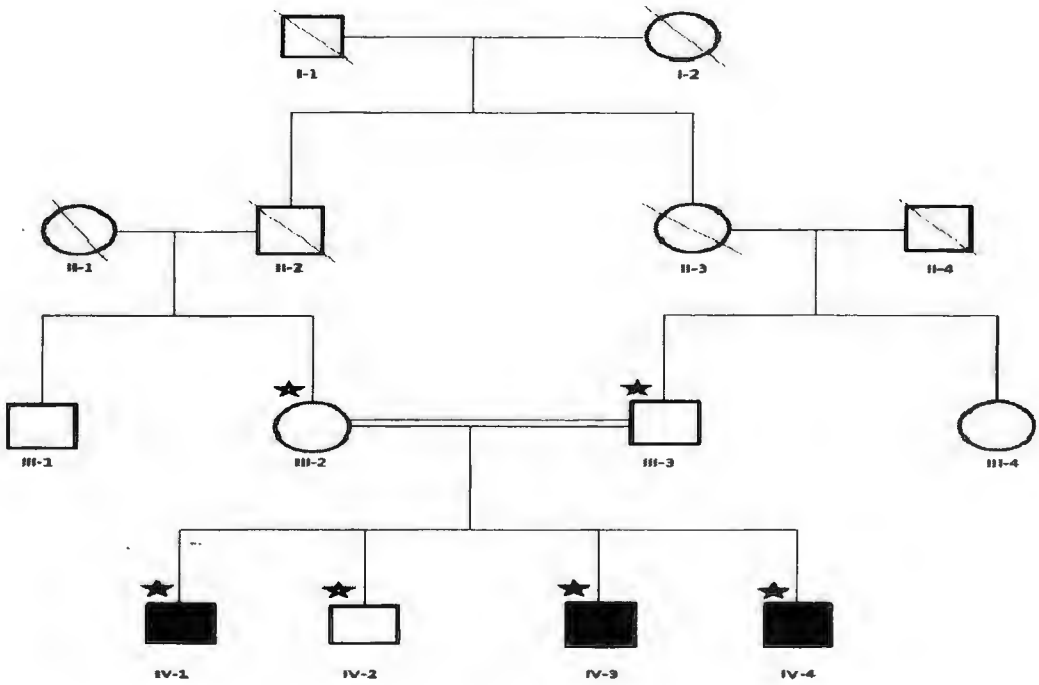


Figure 4.2: IV-1 (severe oligospermic), IV-3(Azoospermic), IV-4(Severe oligospermic).

Pedigree of Family A(INFM-1) having three affected individuals.

4.1.3 Family C (INFM-3)

Family C (INFM-3) was collected from Surrani, Tehsil and district Bannu, Province of Khyber Pakhtunkhwa, Pakistan. The pedigree of the family shows that it is consanguineous marriage and this disease was not found in the previous generations of the family. The medicals reports of the affected individual's shows that the two affected individuals are azoospermic which may be the cause of male infertility. The blood samples were collected from all individuals of the last generation which is indicated by star about the individuals symbols. Squares and circles show male and female respectively while filled squares and circles shows affected individuals. Two adjacent double lines show consanguineous marriage and cross diagonal line on circles or squares shows demised person. The roman numbers indicates the generation number of the individuals in the pedigree while that of an Arabic numbers indicates position of the individuals in the respective family.

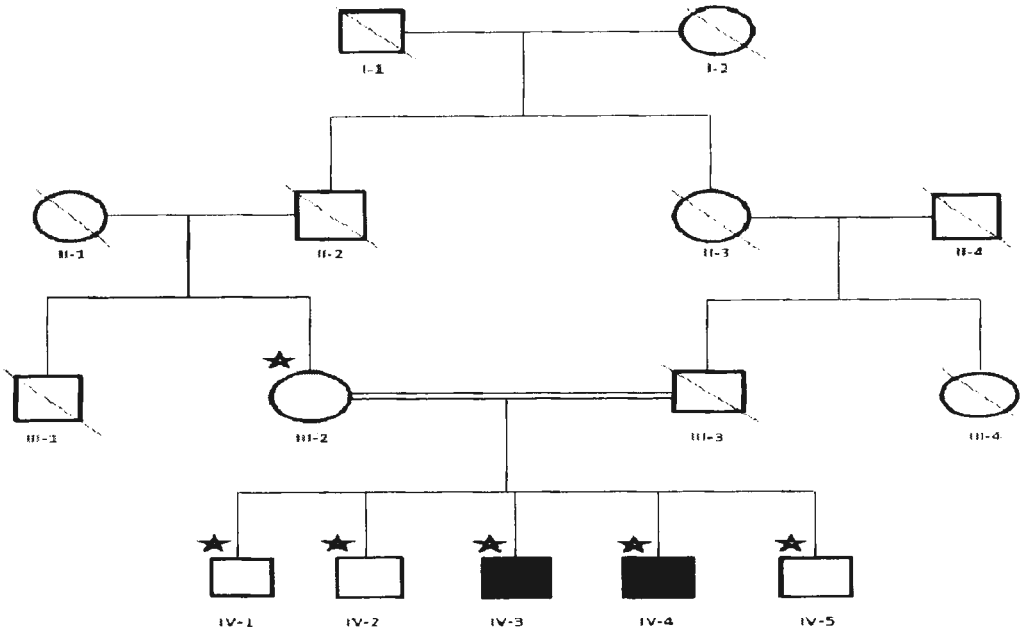


Fig 4.3 : IV-3 and IV-4 shows Azoospermic phenotype.

Pedigree of Family C (INFM-3) having two azoospermic affected individuals.

4.1.4 Mutational Analysis of Selected Genes Using STS Marker

To identify mutation in the selected genes which are suggested to be a cause of male infertility, different STS markers were used although microdeletion can directly be detected by sequencing but here we used indirect approach that is using of STS markers for mutational analysis. This study includes three families A, B and C which were examined for the microdeletion of Y chromosome that is considered as a cause of male infertility. The table 1 clearly shows the summery of all the STS markers which were used for the known locus of Y chromosome. Analysis of the STS marker was carried out by using PCR reaction according to standard scientific protocol. These PCR products were observed on 2% Agarose gel and were observed under UV documentation system and photos were taken through digital camera attached to UV gel documentation system. To investigate mutation the proposed chromosome was scanned through micro-deletion analysis and the banding pattern was studied to know whether any micro deletion of Y chromosome exists or not in the respective families. For normal individuals it is necessary to give bands on 2% agarose gel while in case of affected individuals there should be no band on gel due to micro deletion on Y chromosome region. All the individuals showed same banding pattern on both normal and affected individuals which suggest that there was no micro- deletion in affected individuals so the family was considered excluded for Y chromosome micro-deletions markers.

Family A (INFM-1):

Figure 4.5 Banding pattern of PCR product of Y-chromosome microdeletion on marker "SY14" designed for SRY of family A (INFM-1).

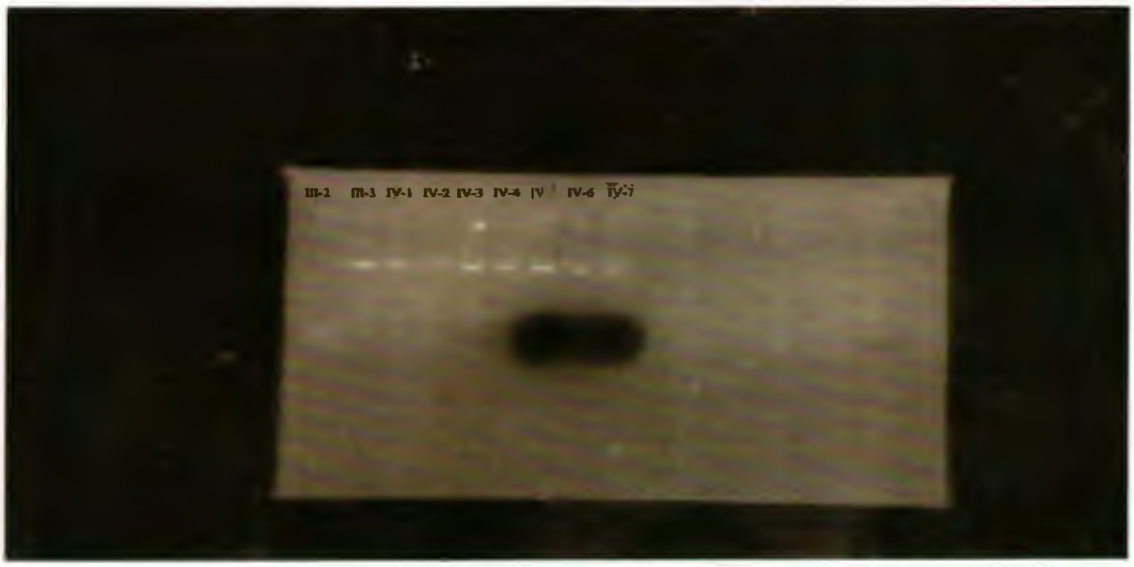


Figure 4.6 Banding pattern of PCR product of Ychromosome microdeletion on "ZFXY" marker designed for SRY of family A (INFM-1).

Figure 4.7 Banding pattern of PCR product of Ychromosome microdeletion on "SY83" marker designed for AZFa region of family A (INFM-1).

Figure 4.8 Banding pattern of PCR product of Y-chromosome microdeletion on "SY84" marker designed for AZFa region of family A (INFM-1).

Figure 4.9 Banding pattern of PCR product of Y-chromosome microdeletion on "SY86" marker designed for AZFa region of family A (INFM-1).

Figure 4.10 Banding pattern of PCR product of Y-chromosome micro deletion on "SY117" marker designed for AZFb family A (INFM-1).

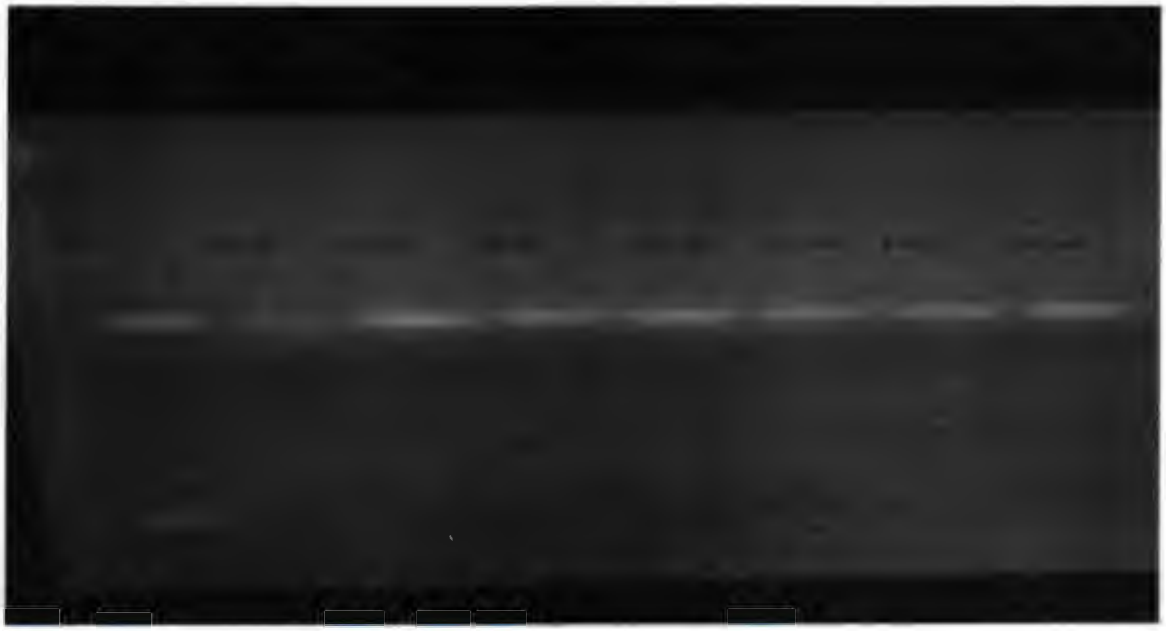


Figure 4.11 Banding pattern of PCR product of Ychromosome microdeletion on "SY127" marker designed for AZFb region of family A (INFM-1).

Figure 4.12 Banding pattern of PCR product of Y-chromosome micro deletion on "SY134" marker designed for AZFb region of family A (INFM-1).

Figure 4.13 Banding pattern of PCR product of Y-chromosome micro deletion on "SY255" marker designed for AZFc region of family A (INFM-1).

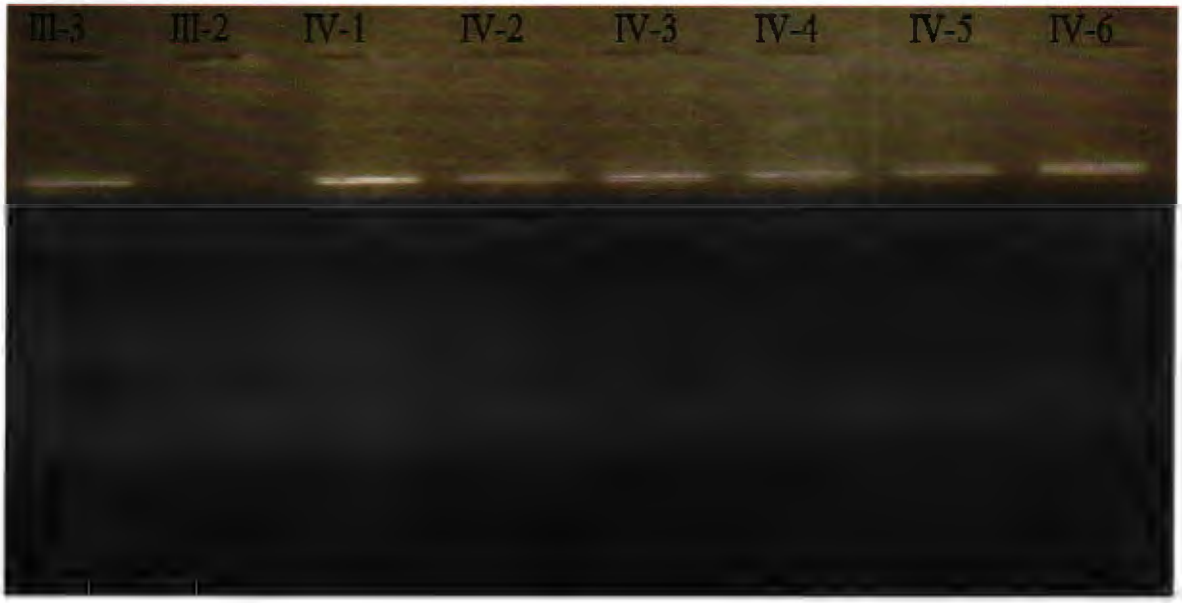


Figure 4.14 Banding pattern of PCR product of Y-chromosome micro deletion on "SY154" marker designed for region AZFc region of family A (INFM-1).

Family B (INFM-2):

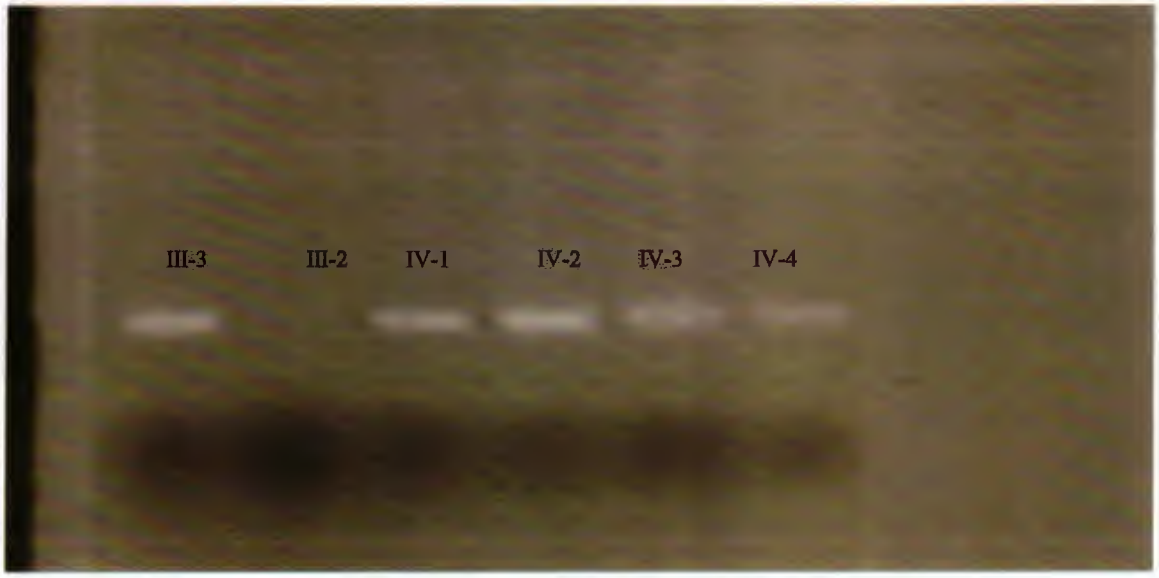


Figure 4.17 Banding pattern of PCR product of Y-chromosome micro deletion on "ZFXY" marker designed for SRY gene of family B (INFM-2).

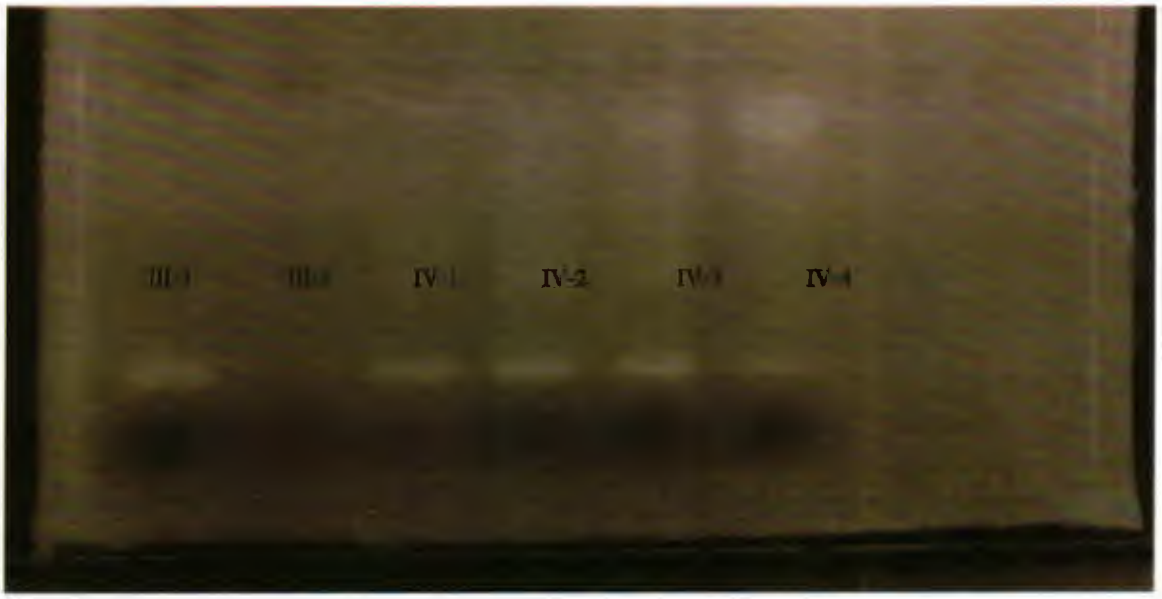


Figure 4.18 Banding pattern of PCR product of Y-chromosome micro deletion on "SY14" marker designed for SRY gene of family B (INFM-2).

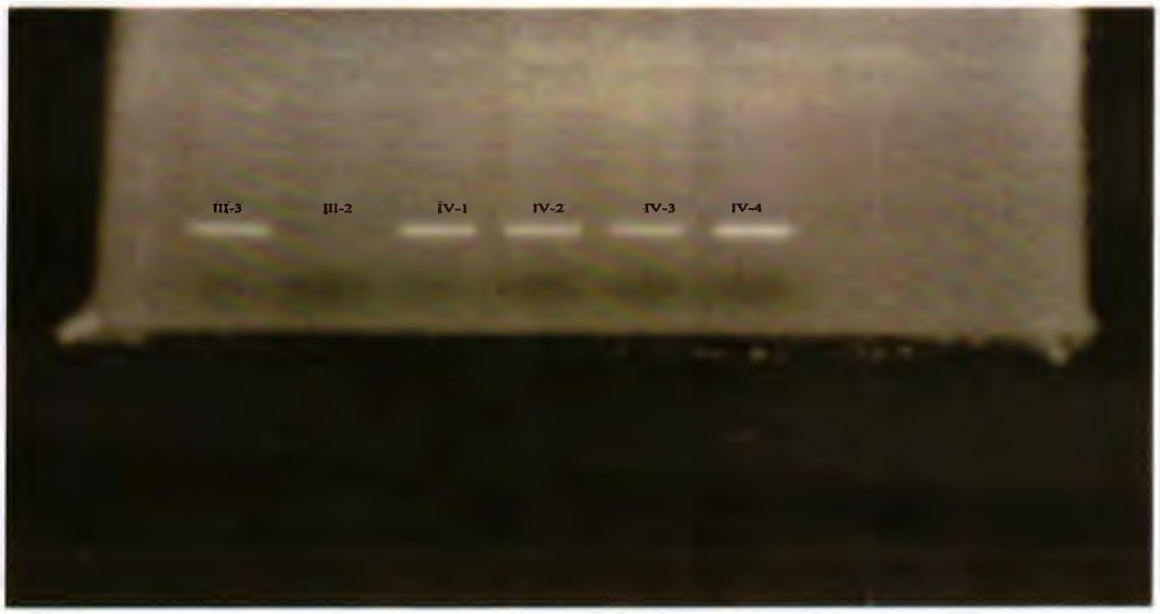


Figure 4.19 Banding pattern of PCR product of Y-chromosome micro deletion on "SY83" marker designed for AZFa region of family B (INFM-2).

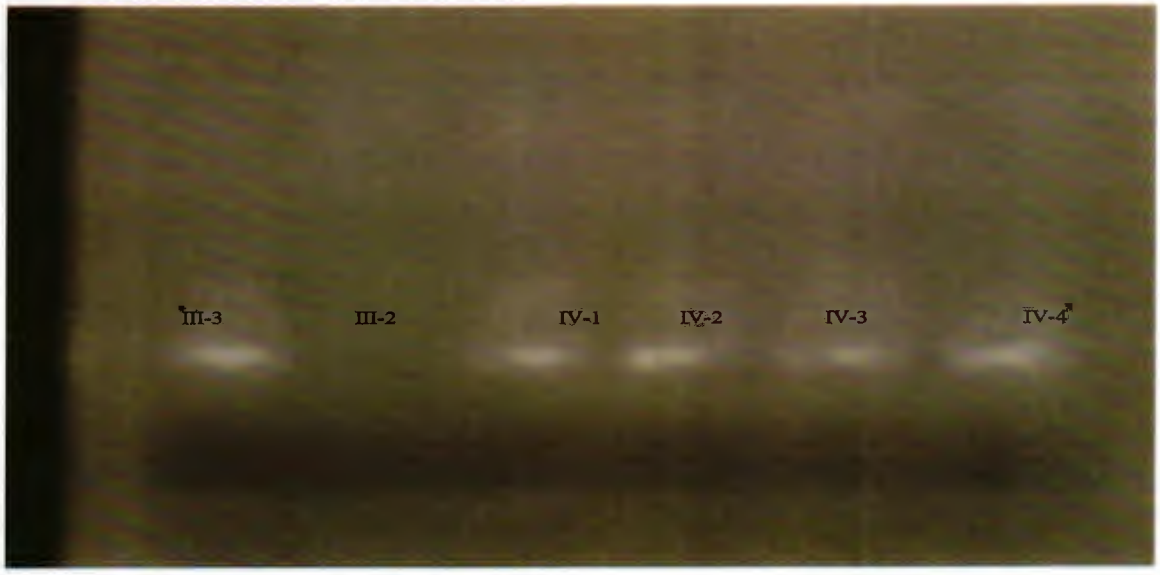


Figure 4.20 Banding pattern of PCR product of Y-chromosome micro deletion on SY84 marker designed for AZFa region of family B (INFM-2).

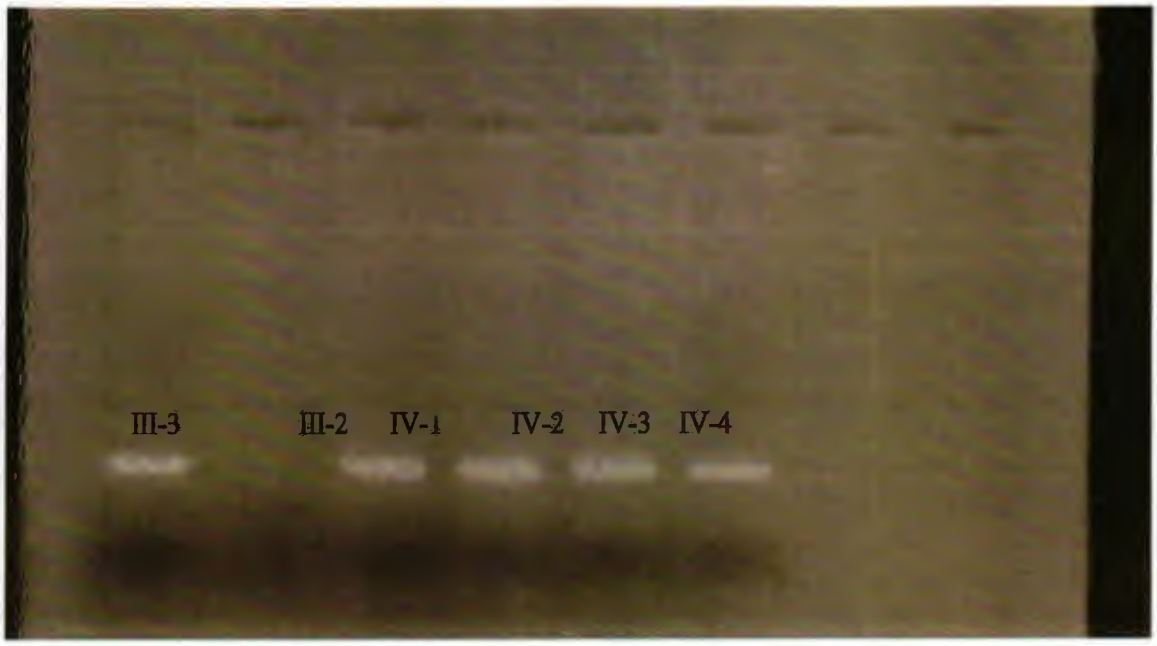


Figure 4.21 Banding pattern of PCR product of Y-chromosome micro deletion on "SY86" marker designed for AZFa region of family B (INFM-2).

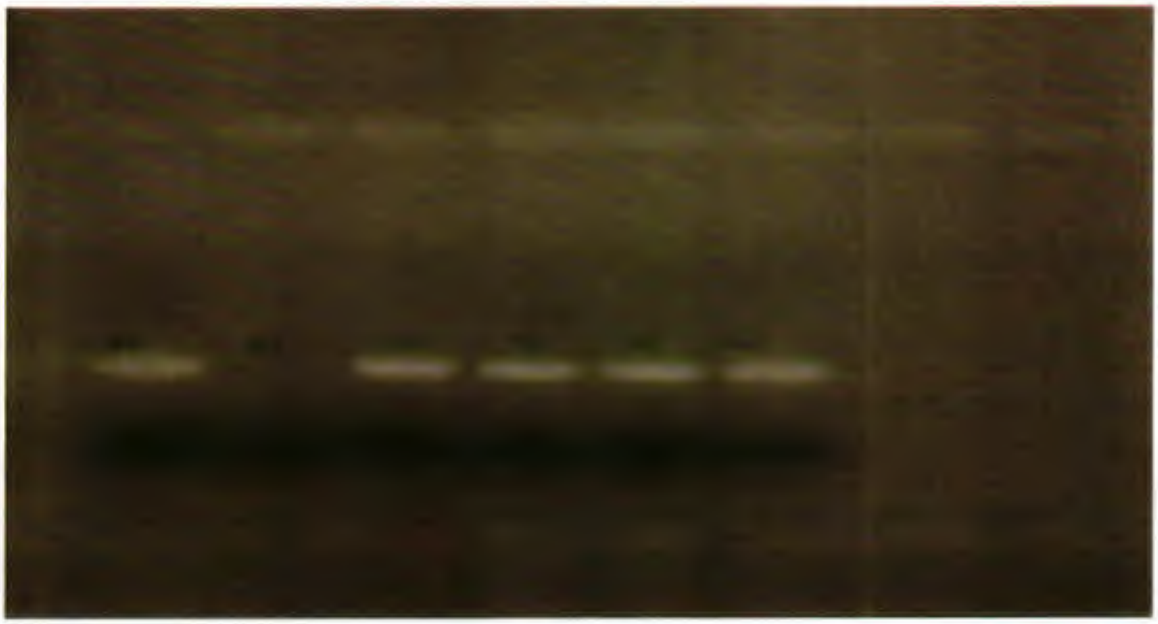


Figure 4.22 Banding pattern of PCR product of Y-chromosome micro deletion on "SY117" marker designed for AZFb region of family B (INFM-2).

Figure 4.23 Banding pattern of PCR product of Y-chromosome micro deletion on "SY127" marker designed for AZFb region of family B (INFM-2).

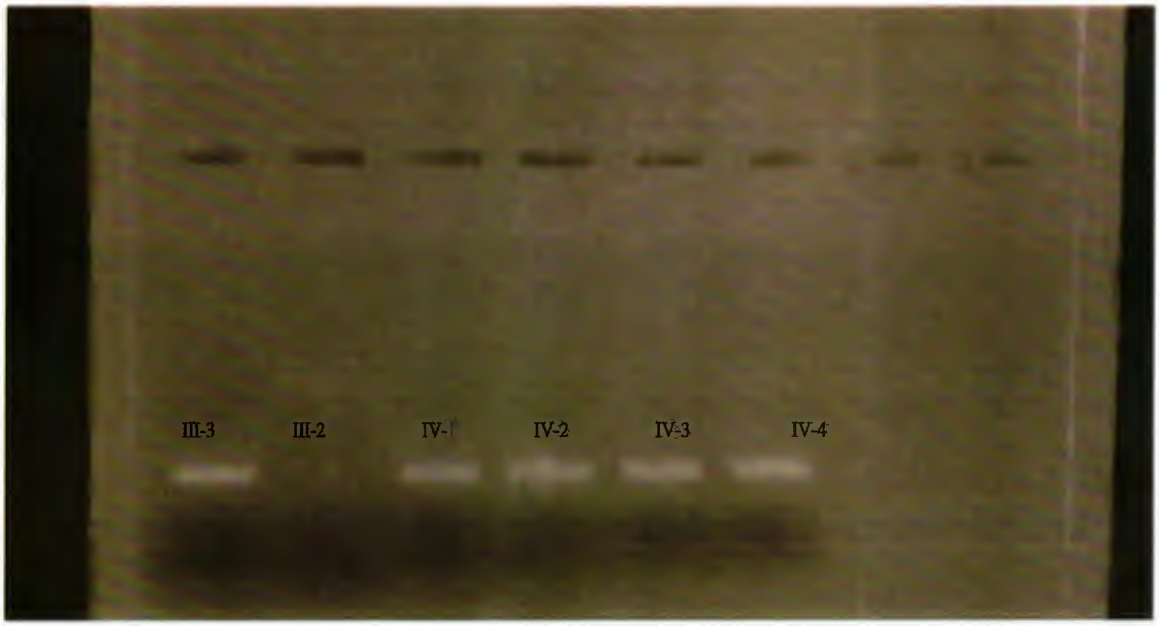


Figure 4.24 Banding pattern of PCR product of Y-chromosome micro deletion on "SY134" marker designed for AZFc region of family B (INFM-2).

Figure 4.25 Banding pattern of PCR product of Y-chromosome micro deletion on "SY157" marker designed for AZFc region of family B (INFM-2).

Figure 4.26 Banding pattern of PCR product of Y-chromosome micro deletion on SY154 marker designed for AZFc region of family B (INFM-2).

Figure 4.27 Banding pattern of PCR product of Y-chromosome micro deletion on SY254 marker designed for AZFc region of family B (INFM-2).

Figure 4.28 Banding pattern of PCR product of Y-chromosome micro deletion on SY255 marker designed for AZFc region of family B (INFM-2).

Family C (INFM-3):

Figure 4.29 Banding pattern of PCR product of Y-chromosome micro deletion on ZFXY marker for SRY gene and SY117 designed for AZFa region of family C (INFM-3).

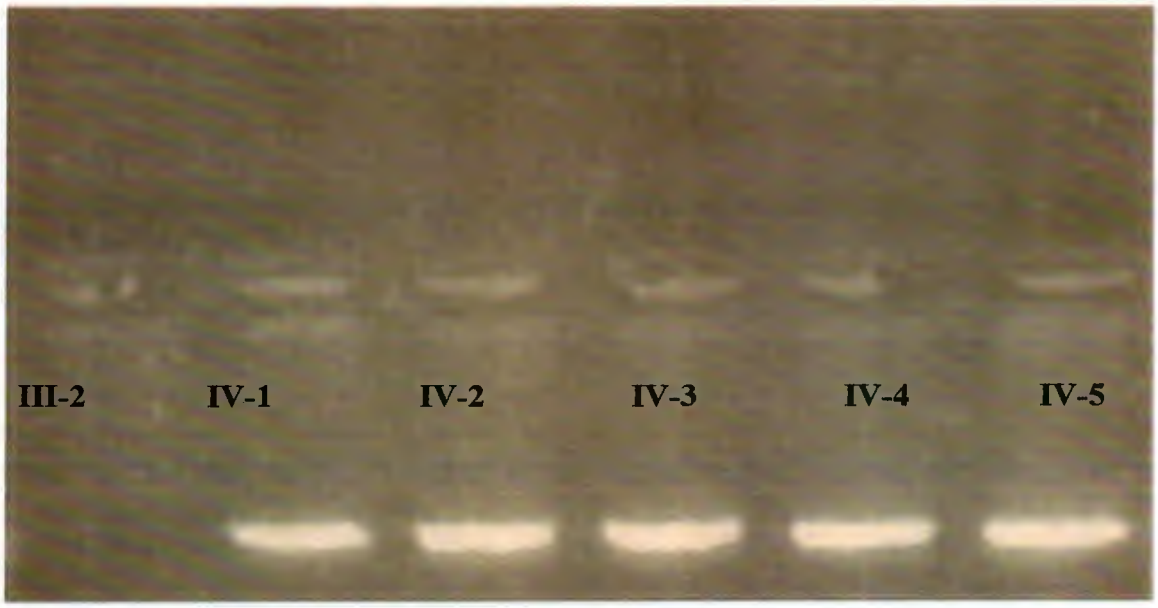


Figure 4.30 Banding pattern of PCR product of Y-chromosome micro deletion on SY14 marker designed for SRY region of family C (INFM-3).

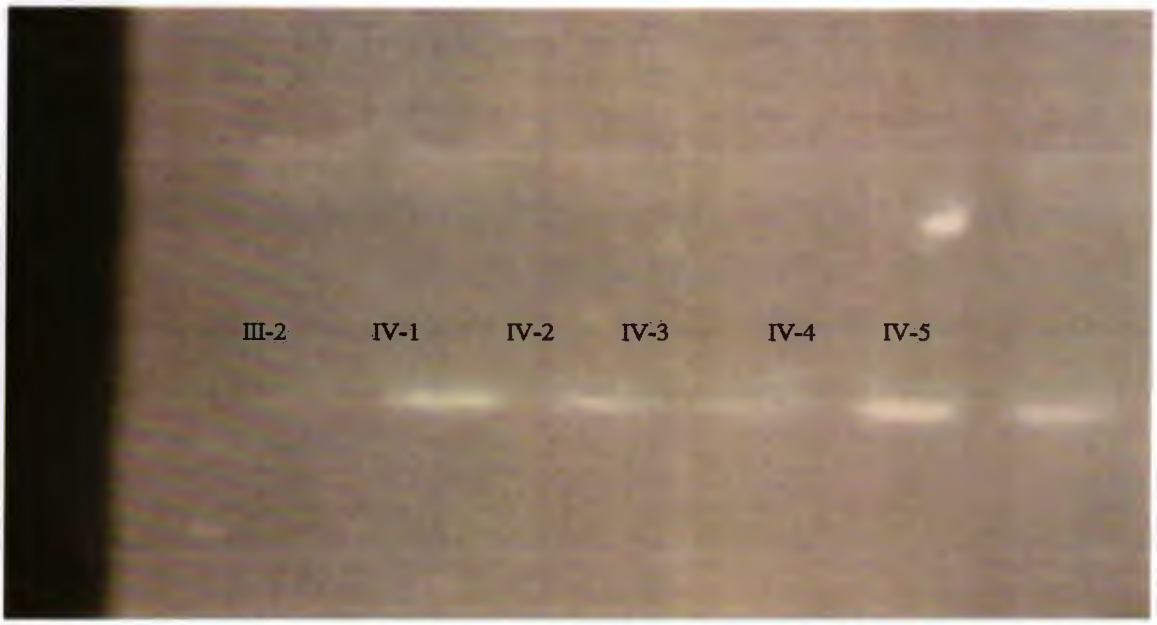


Figure 4.31 Banding pattern of PCR product of Y-chromosome micro deletion on "SY83" marker designed for AZFa region of family C (INFM-3).

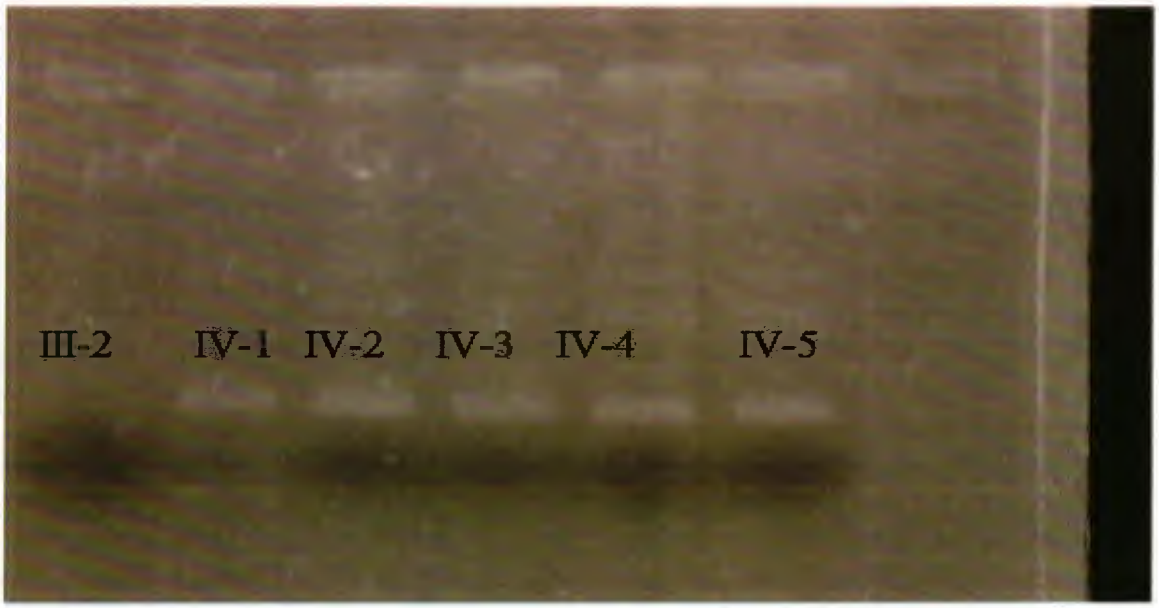


Figure 4.32 Banding pattern of PCR product of Y-chromosome micro deletion on "SY84" marker designed for AZFa region of family C (INFM-3).

Figure 4.33 Banding pattern of PCR product of Y-chromosome micro deletion on "SY86" marker designed for AZFa region of family C (INFM-3).

Figure 4.34 Banding pattern of PCR product of Y-chromosome micro deletion on SY127 marker designed for AZFb region of family C (INFM-3).

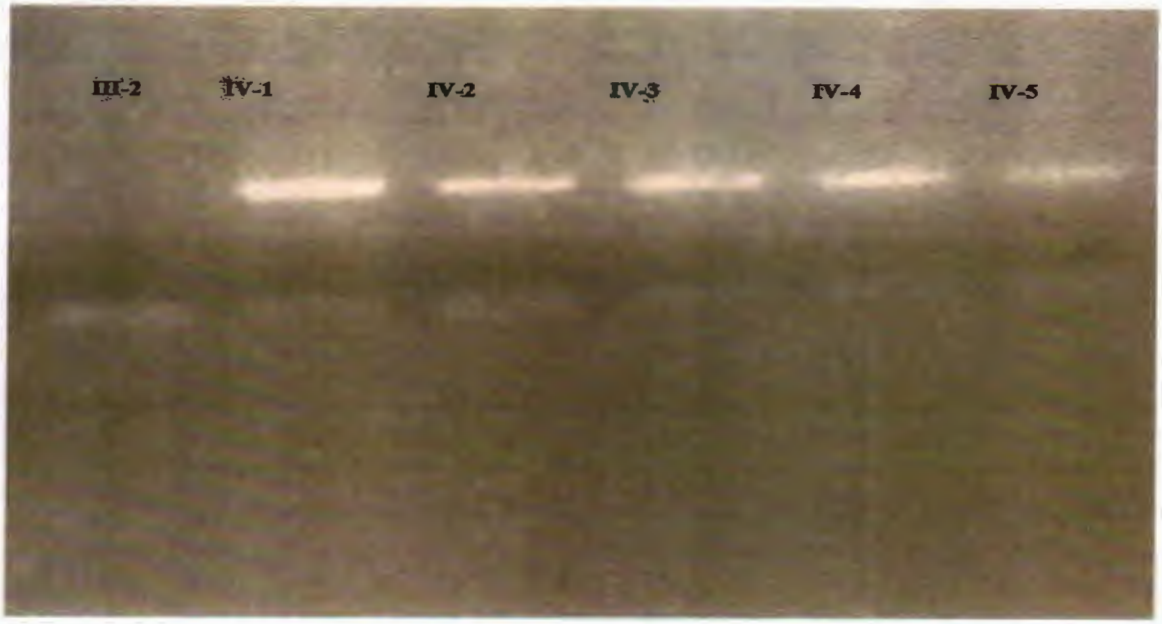


Figure 4.35 Banding pattern of PCR product of Y-chromosome micro deletion on "SY134" marker designed for AZFb region of family C (INFM-3).

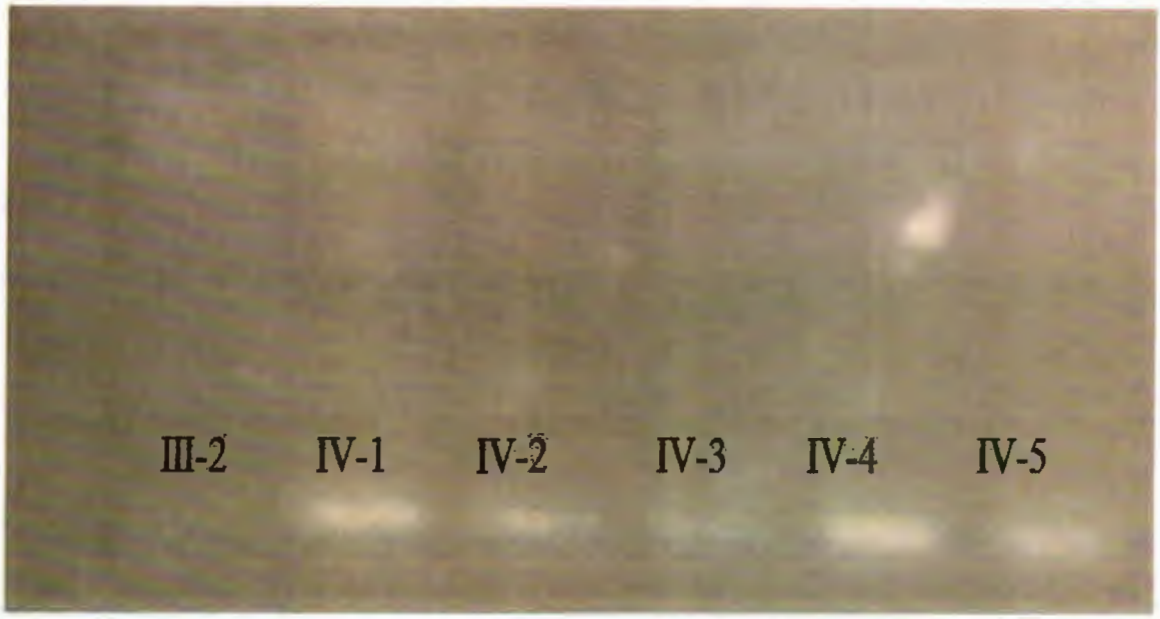


Figure 4.36 Banding pattern of PCR product of Y-chromosome micro deletion on "SY154" marker designed for AZFc region of family C (INFM-3).

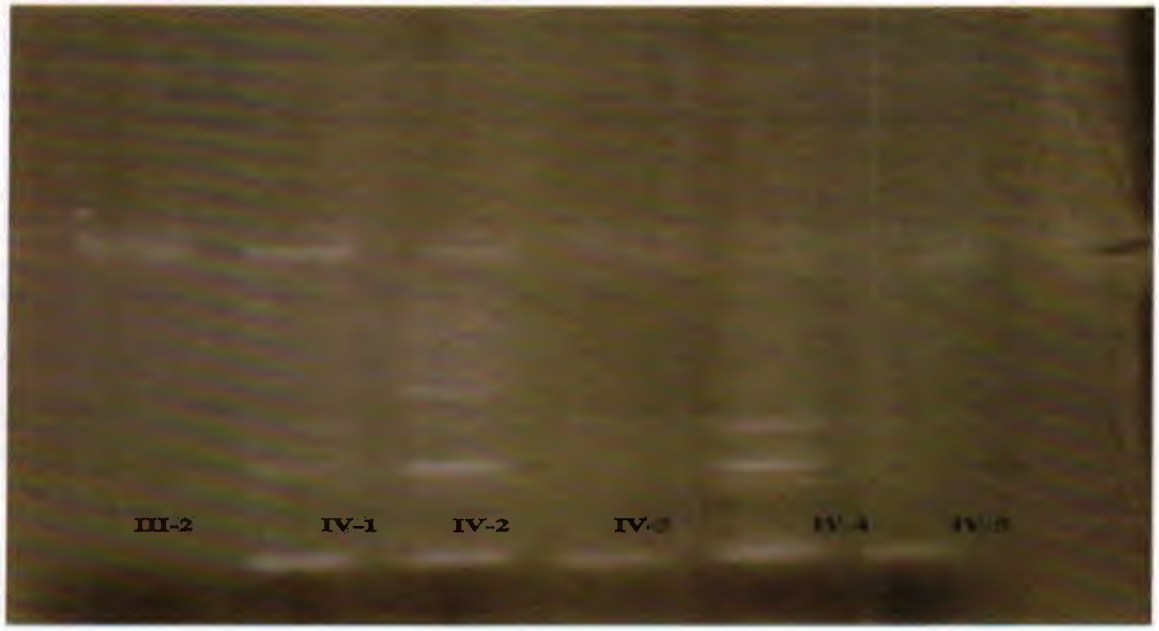


Figure 4.37 Banding pattern of PCR product of Y-chromosome micro deletion on "SY157" marker designed for AZFc region of family C (INFM-3).

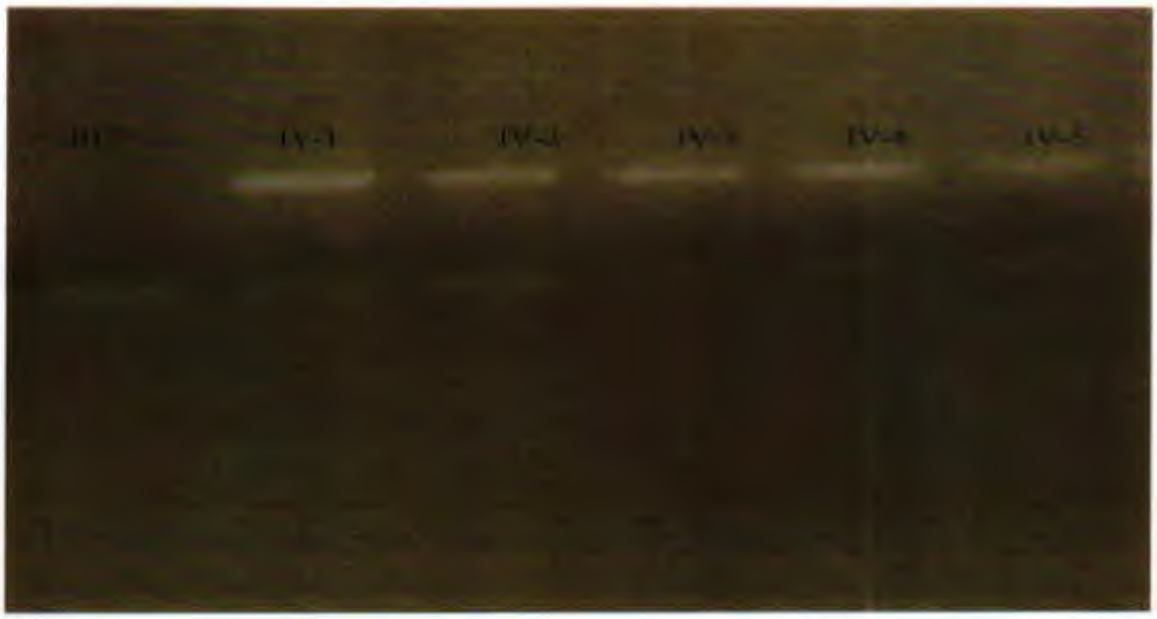


Figure 4.38 Banding pattern of PCR product of Y-chromosome micro deletion on "SY254" marker designed for AZFc region of family C (INFM-3).

Figure 4.39 Banding pattern of PCR product of Y-chromosome micro deletion on "SY255" marker designed for AZFc region of family C (INFM-3).

4.2 Hormonal analysis of individuals with male infertility.

Table 4.2 Effect of Hormonal Variation on Individuals Etiology in case of male infertility.

Testosterone	LH	FSH	Etiology
Low	High	High	Primary testicular disorder(Hypo gonadotrophic/Hypogonadotrophism)
Low	Low	Low	Hypogonadotrophic/hypogonadotrophism Secondary disorders includes pituitary or Hypothalamic disorders
Normal	Normal	High	Seminiferous tubular failure Steroli cells only syndromic Status after hamicastration
High	High	Normal	Androgen resistance Syndrome Hyperthyroidism
Normal	Normal	Normal	Disorder of sperm transport Ductal or epididymal obstruction and spermogenetic arrest

Table 4.3 Different hormones level of Family A infertile individuals in compare with standard range.

Sub Generation No Normal Range	LH 1.7-11.2	FSH 2.1-18.6	Prolactin 3.6-16.3	Testosterone 262-870	TSH 0.38-4.31
IV-1	16mIU/mL	52.8mIU/mL	15.5ng/ml	423.16ng/dL	3.787mIU/L
IV-2	10.8mIU/mL	45.2mIU/mL	14ng/ml	500ng/dL	4.01mIU/L
IV-3	9.6mIU/mL	43.23mIU/mL	11ng/ml	740ng/dL	2.87mIU/L

Table 4.4 Different hormones level of Family B infertile individuals in compare with standard range.

Sub Generation No Normal Range	LH 1.7-11.2	FSH 2.1-18.6	Prolactin 3.6-16.3	Testosterone 262-870	TSH 0.38-4.31
IV-1	4.6mIU/mL	1.6mIU/mL	13.1ng/ml	704.16ng/dL	1.622mIU/L
IV-3	3.2mIU/mL	4.5mIU/mL	8.3ng/ml	439.32ng/dL	1.559mIU/L
IV4	14.9mIU/mL	22.0mIU/mL	16.9ng/ml	937.36ng/dL	1.334mIU/L

Table 4.5 Different hormones level of Family C infertile individuals in compare with standard range.

Sub Generation No Normal Range	**LH** 1.7-11.2	**FSH** 2.1-18.6	**Prolactin** 3.6-16.3	**Testosterone** 262-870	**TSH** 0.38-4.31
IV-3	5.5mlU/mL	12.4mlU/mL	7.9ng/ml	858.94ng/dL	1.187mlU/L
IV-4	9.7U/mL	16.1mlU/mL	13.1ng/ml	535.09ng/dL	2.168mU/L

DISCUSSION

5. DISCUSSION

Large population of the world is suffering with infertility. Infertility is a disorder which may be by genetic disorder or may be caused due to certain reason. Infertility is a condition with disability to conceive even if a couple has unprotected intercourse for more than a year. As a huge population of the world is suffering with infertility that is both male and female. Approximately 15% of the world couples are facing this problem in which 50% is contributed by male and 50% is contributed by females (Poongothai *et al.*, 2009). According to (Zeger *et al.*, 2009) 30-40% of couples are sterile in nature is contributed by males while 10-30% is either because of both male and females or by other reasons. Infertility in males is caused due to sperm disability which is dived on the bases of their morphology like shapes, motility and sperm count etc in the semen. When sperm count is very less is known as oligospermic and when sperm count is absolutely nil is known as azoospermic. There are many causes of infertility like obesity, smoking and the main is the defect in genetic material like chromosomes or genes on these chromosomes. In male, infertility is caused due to Y chromosomes microdeletion which carries important genes which have a key role in fertility maintenance.

The recent study was designed to investigate male infertility in the population of district Lucky Marwat and district Bannu, KPK, Pakistan. Three different families of male infertility were collected from different regions of district Bannu. All the medical reports and brief history of the patients and normal individuals were collected along with their medical diagnostics test. All the collect families were having recessive mode of chromosomal disorders. The aim of the study was investigate mutation of different genes that are supposed to be involved in the fertility of these males. For this purpose Y chromosome microdeletion was investigated because genes for male fertility factor reside on Y chromosomes. Mostly deletion is observed in AZF region and this AZF is further divided into three sub portion that are AZFa, AZFb and AZFc regions. SRY is another key gene reported having function in male fertility. This study were composed of investigating three different families that were named as A (INFM-1), B (INFM-2) and C (INFM-3) in which approximately eight members were infertile. Families A and C affected individuals were pure azoospermic while in family B one was azoospermic and two were severe Oligospermic. All these families were screened for Y chromosome microdeletion. Sequence tagesites (STS) markers for SRY genes are ZFXY and SY14 while AZFa region STS markers

are SY83, SY84, SY86 and AZFb region markers are SY127, SY117 and SY 134 and last AZFc region markers are SY254, SY255 and SY154 were used to identify Y chromosome microdeletion.

Two types of control were used in the mutational analysis that is positive control and negative control, in case of positive control normal male of the family and in negative control mother of the respective individuals were used. Under normal analysis condition there should be no bands of PCR product of a negative control that is of female because it lack Y chromosomes and band should be observed of positive control means in normal males while in case of any microdeletion in infertile males there should be no bands on gel of PCR products.

In our study in the three families which were investigated for AZF region microdeletion in infertile males were not observed with microdeletion of this AZF region when it were screened through STS markers specific for AZF region while in contrast (Carvalho *et al.*, 2004) reported Y chromosomes microdeletion on q arm of the chromosome in about 7-15 % males. Similar results are also reported by some other scientist who also reported microdeletion of AZF sub region base pair deletions (Kim *et al.*, 1999).

Kuroda-Kawaguchi *et al.,* (2001) also explained some new microdeletion and he also elaborated the possible mechanism through which these micro deletions might have happened on the long arm of the Y chromosome but our study do not matches the reports of these scientists because no microdeletion been noted. So all the families on the bases of microdeletion analysis were found no deletions so were considered excluded for Y chromosome microdeletion analysis.

Proposed study was designed to know the root cause behind this genetic disorder in different population and also to investigate its association with the phenotype. In this research indirect research approach was used in spite of direct sequencing method.

Beyond this genetic analysis some other parameter were taken to check because they might have some role in controlling or contributing to the disease. Hormonal levels of these individuals were examined. These hormones are involved in different biological activities that contribute to sperm formation and its proper regulation. These groups of hormones include LH, FSH, Prolactin and testosterone which is male sex hormone. Different individual were tested for these hormones and they were labeled as their on the bases of their generation number. Member IV-1 was having TSH LH and prolactin normal while low testosterone and FSH. While IV-II two was having normal was having low testosterone and high FSH. IV-3 was found with low LH,

low FSH and also with low testosterone.IV-3 was found with low TSH and while IV-4 was detected with relatively low testosterone with low This hormonal study was carried out to analysis whether there exists any other gene defect other than Y chromosome microdeletion because all the three families were not found with deletion when they were observed for SRY and AZF microdeletion which are important portion of Y chromosome.

CONCLUSION

Current research study was designed to explore the major root causes of male infertility at genome level and to scrutinize all the possible causes of this genetic disorder. A significant number of world populations are suffering from this disease affecting their social lives and values of their families as well. Different genes were investigated which were reported for possible causes of male infertility. The main focus of this study was to target Y chromosome genetic analysis specially the alteration due to deletion of certain genes on Y chromosome. The important genes which have a key role in fertility located on Y chromosome are SRY gene and genes located on AZF region which have further sub regions AZFa, AZFb and AZFc. The results of current study show variations and were different from previously reported results because majority of previous studies reported micro-deletions while no microdeletion was observed in this study. For this purpose some other hormonal assay were also carried out to know whether some other genes or hormones might have the lethal effects and role in male infertility. Those hormonal assays concluded that some other genes might be involved in male infertility as well.

REFERENCES

6. REFERENCES

Bamford S., Dawson, E., Forbes S., Clements, J., Pettett, R., Dogan A. and Wooster R., (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website, British journal of cancer, 91(2), p. 355-358.

Carrell D. T., (2008) Contributions of spermatozoa to embryogenesis, assays to evaluate their genetic and epigenetic fitness, Reproductive biomedicine online, 16(4), p. 474-484.

Carvalho C. M. B., Rocha J. L., Santos F. R., Kleiman S. E., Paz G., Yavetz H. and Pena S. D. J., (2004) Y-chromosome Haplotypes in azoospermic Israeli men, Human biology, 76(3), p. 469-478.

Casals T., Bassas L., Egozcue S., Ramos M. D., Giménez J., Segura A. and Estivill X., (2000) Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens, Human Reproduction, 15(7), p. 1476-1483.

Claustres M., (2005) Molecular pathology of the CFTR locus in male infertility, Reproductive biomedicine online, 10(1), p. 14-41.

De Braekeleer M. and Dao T. N., (1991) Review Cytogenetic studies in male infertifity, Human Reproduction, 6(2), p. 245-250.

Dörk T., Dworniczak B., Aulehla-Scholz C., Wieczorek D., Böhm I., Mayerova A. and Pander H. J., (1997) Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens, Human genetics, 100(3-4), p. 365-377.

Edwards A. L., Hammond H. A., Jin L., Caskey C. T. and Chakraborty R., (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups, Genomics, 12(2), p. 241-253.

Elliott D. J. and Cooke H. J., (1997) The molecular genetics of male infertility, Bioassays, 19(9), p. 801-809.

Emery B. R. and Carrell D. T., (2006) The effect of epigenetic sperm abnormalities on early embryo-genesis, Asian journal of andrology, 8(2), p. 131-142.

Evens E. M., (2004) A global perspective on infertility, an under recognized public health issue, The University of North Carolina at Chapel Hill, p. 18.

Ferlin A., Arredi B., Speltra E., Cazzadore C., Selice R., Garolla A. and Foresta C., (2007) Molecular and clinical characterization of Y chromosome microdeletions in infertile men, a 10-year experience in Italy, The Journal of Clinical Endocrinology and Metabolism, 92(3), p. 762-770.

Ferlin A., Raicu F., Gatta V., Zuccarello D., Palka G. and Foresta C., (2007) Male infertility, role of genetic background, Reproductive biomedicine online, 14(6), p. 734-745.

Foresta C., Garolla A., Bartoloni L., Bettella A. and Ferlin A., (2005) Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection, The Journal of Clinical Endocrinology and Metabolism, 90(1), p. 152-156.

Foresta C., Moro E. and Ferlin A., (2001) Y chromosome microdeletions and alterations of spermatogenesis 1, Endocrine reviews, 22(2), p. 226-239.

Galan J. J., Buch B., Cruz N., Segura A., Moron F. J., Bassas L. and Ruiz A., (2005) Multilocus analysis of estrogen-related genes reveal involvement of the ESR1 gene in male infertility and the polygenic nature of the pathology, Fertility and sterility, 84(4), p. 910-918.

Galan J. J., Guarducci E., Nuti F., Gonzalez A., Ruiz M., Ruiz A. and Krausz C., (2007) Molecular analysis of estrogen receptor alpha gene AGATA haplotype and SNP12 in European populations, potential protective effect for cryptorchidism and lack of association with male infertility, Human reproduction, 22(2), p. 444-449.

Georgiou I., Syrrou M., Pardalidis N., Karakitsios K., Mantzavinos T., Giotitsas N. and Tzoumis P., (2006) Genetic and epigenetic risks of intracytoplasmic sperm injection method, Asian journal of andrology, 8(6), p. 643-673.

Ghorbel M., Baklouti-Gargouri S., Keskes R., Chakroun N., Sellami A., Fakhfakh F. and Ammar-Keskes L., (2014) Combined deletion of DAZ2 and DAZ4 copies of Y chromosome DAZ gene is associated with male infertility in Tunisian men, Gene, 547(2), p. 191-194.

Gianaroli L., Magli M. C., Ferraretti A. P., Munne S., Balicchia B., Escudero T. and Crippa A., (2002) Possible interchromosomal effect in embryos generated by gametes from translocation carriers, Human Reproduction, 17(12), p. 3201-3207.

Giannouli C., Goulis D. G., Lambropoulos A., Lissens W., Tarlatzis B. C., Bontis J. N. and Papadimas J., (2004) Idiopathic non-obstructive azoospermia or severe oligozoospermia, a cross-sectional study in 61 Greek men, International journal of andrology, 27(2), p. 101-107.

Gianotten J., Lombardi M. P., Zwinderman A. H., Lilford R. J. and Van Der Veen F., (2004) Idiopathic impaired spermatogenesis, genetic epidemiology is unlikely to provide a short-cut to better understanding, Human reproduction update, 10(6), p. 533-539.

Griffin D K. and Finch K A., (2005) The genetic and cytogenetic basis of male infertility, Human Fertility, 8(1), p. 19-26.

Gülşah KOÇ., Korkut ULUCAN ., Deniz KIRAÇ ., Deniz ERGEÇ., Tufan TARCAN. and İlter GÜNEY., (2010) Molecular and cytogenetic evaluation of Y chromosome in spontaneous abortion cases, Journal of Cell and Molecular Biology, 7(2) , p. 45-52.

Hardee K. and Leahy E., (2008) Population, fertility and family planning in Pakistan, a program in stagnation, Population Action International, 3(3).

Hildebrand M. S., Avenarius, M. R., Fellous M., Zhang Y., Meyer N. C., Auer J. and Smith R. J., (2010) Genetic male infertility and mutation of CATSPER ion channels, European Journal of Human Genetics, 18(11), p. 1178-1184.

Johnson M. D., (1998) Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility, recommendations for genetic counselling and screening, Fertility and sterility, 70(3), p. 397-411.

Jungwirth A., Diemer T., Dohle G R., Giwercman A., Kopa Z., Tournaye H. and Krausz C., (2004) Guidelines for the investigation and treatment of male infertility, EurUrol, 46(5), p. 555-558.

Kaku M. and Freire-Maia N., (1992) Inbreeding effect on morbidity, IV, Further data in Brazilian populations, American journal of medical genetics, 42(4), p. 420-423.

Kee K., Angeles V. T., Flores M., Nguyen H. N. and Pera R. A. R., (2009) Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation, Nature, 462(7270), p. 222-225.

Khabour O. F., Fararjeh A. S. and Alfaouri A. A., (2014) Genetic screening for AZF Y chromosome microdeletions in Jordanian azoospermic infertile men, International journal of molecular epidemiology and genetics, 5(1), p. 47.

Kim S W., Kim K D and Paick J. S. (1999) Microdeletions within the azoospermia factor subregions of the Y chromosome in patients with idiopathic Azoospermia, Fertility and sterility, 72(2), p. 349-353.

Krausz C., Degl'Innocenti S., Nuti F., Morelli A., Felici F., Sansone M. and Forti G., (2006) Natural transmission of USP9Y gene mutations, a new perspective on the role of AZFa genes in male fertility, Human Molecular Genetics, 15(18), p. 2673-2681.

Krausz C., Hoefsloot L., Simoni M. and Tüttelmann F., (2014) EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions, state-of-the-art 2013, Andrology, 2(1), p. 5-19.

Krausz, C. And Giachini C., (2007) Genetic risk factors in male infertility, Archives of andrology, 53(3), p. 125-133.

Kuroda-Kawaguchi T., Skaletsky H., Brown L. G., Minx P. J., Cordum H. S., Waterston R. H. and Page, D. C., (2001) The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men, Nature genetics, 29(3), p. 279-286.

Lardone M. C., Parodi D. A., Valdevenito R., Ebensperger M., Piottante A., Madariaga M. and Castro A., (2007) Quantification of DDX3Y, RBMY1, DAZ and TSPY mRNAs in testes of patients with severe impairment of spermatogenesis, Molecular human reproduction, 13(10), p. 705-712.

Li Q., Qiao D., Song N. H., Ding Y., Wang Z. J., Yang J. and Yin C. J., (2013) Association of DAZ1/DAZ2 deletion with spermatogenic impairment and male infertility in the South Chinese population, World journal of urology, 31(6), p. 1403-1409.

Lichtenstein P., Yip B. H., Björk C., Pawitan Y., Cannon T. D., Sullivan P. F. and Hultman C. M., (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families, a population-based study, The Lancet, 373(9659), p. 234-239.

Lin Y. W., Chia-Ling Hsu L., Kuo P. L., Huang W. J., Chiang H. S., Yeh S. D. and Yen P. H., (2007) Partial duplication at AZFc on the Y chromosome is a risk factor for impaired spermatogenesis in Han Chinese in Taiwan, Human mutation, 28(5), p. 486-494.

Liu J., Larsen U. and Wyshak G., (2005) Prevalence of primary infertility in China, in-depth analysis of infertility differentials in three minority province/autonomous regions, Journal of biosocial science, 37(01), p. 55-74.

Lopes A. M., Aston K. I., Thompson E., Carvalho F., Gonçalves J., Huang N. and Seabra C., (2013) Human spermatogenic failure purges deleterious mutation load from the autosomes and both sex chromosomes including the gene DMRT1, PLoS Genet, 9(3), e1003349.

Lourenço D., Brauner R., Lin L., De Perdigo A., Weryha G., Muresan M. and McElreavey K., (2009) Mutations in NR5A1 associated with ovarian insufficiency, New England Journal of Medicine, 360(12), p. 1200-1210.

Lunenfeld B. and Van Steirteghem A., (2004) Infertility in the third millennium, implications for the individual, family and society, condensed meeting report from the Bertarelli

Foundation's second global conference, Human Reproduction Update, 10(4), p. 317-326.

Mak V., Zielenski J., Tsui L. C., Durie P., Zini A., Martin S. and Jarvi K. A., (2000) Cystic fibrosis gene mutations and infertile men with primary testicular failure, Human Reproduction, 15(2), p. 436-439.

Mateizel I., Verheyen G., Van Assche E., Tournaye H., Liebaers I. and Van Steirteghem A., (2002) FISH analysis of chromosome X, Y and 18 abnormalities in testicular sperm from azoospermic patients, Human Reproduction, 17(9), p. 2249-2257.

Mitra A., Dada R., Kumar R., Gupta N. P., Kucheria K. and Gupta S. K., (2008) Screening for Y-chromosome microdeletions in infertile Indian males, utility of simplified multiplex PCR, Indian Journal of Medical Research, 127(2), p. 124.

Mueller B. A. and Daling J. R., (1989) Epidemiology of infertility, Extent of the problem risk factors and associated social changes, Controversies in reproductive endocrinology and infertility, New York, Elsevier, 3, p. 1-13.

Mumtaz Z., Shahid U. and Levay A., (2013) Understanding the impact of gendered roles on the experiences of infertility amongst men and women in Punjab, Reproductive health, 10(1), p. 1.

Nathanson K. L., Kanetsky P. A., Hawes R., Vaughn D. J., Letrero R., Tucker K. and Lohynska R., (2005) The Y deletion gr/gr and susceptibility to testicular germ cell tumor, The American Journal of Human Genetics, 77(6), p. 1034-1043.

Neale M. C. and Kendler K. S., (1995) Models of comorbidity for multifactorial disorders, American journal of human genetics, 57(4), p. 935.

Noveski P., Madjunkova S., Mircevska M., Plaseski T., Filipovski V. and Plaseska Karanfilska D., (2014) SNaPshot Assay for the Detection of the Most Common CFTR Mutations in Infertile Men, PloS one, 9(11), e112498.

Nuti F. and Krausz C., (2008) Gene polymorphisms/mutations relevant to abnormal spermatogenesis, Reproductive biomedicine online, 16(4), p. 504-513.

Palermo G. D., Colombero L. T., Hariprashad J. J., Schlegel P. N. and Rosenwaks Z., (2002) Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI, Human Reproduction, 17(3), p. 570-575.

Park I. H., Arora N., Huo H., Maherali N., Ahfeldt T., Shimamura A. and Daley G. Q., (2008) Disease-specific induced pluripotent stem cells, 134(5), p. 877-886.

Patrick Lorès., Nadège Vernetd., Tomohiro Kurosakig., Tom Van de Puttef., Danny Huylebroeckf., Masaki Hikidae., Gérard Gacon., Aminata Tour (2014) Deletion of MgcRacGAP in the male germ cells impairs spermatogenesis and causes male sterility in the mouse , Developmental Biology, 386, p. 419-427.

Poongothai J. E. N. S., Gopenath T. S. and Manonayaki S. (2009) Genetics of human male infertility, Singapore Medical Journal, 50(4), p. 336-347.

Purcell S. M., Wray N. R., Stone J. L., Visscher P. M., O'Donovan M. C., Sullivan P. F. and O'Dushlaine C. T., (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder, Nature, 460(7256), p. 748-752.

Qian X., Cheng Y. H., Mruk D. D. and Cheng C. Y., (2013) Breast cancer resistance protein (Bcrp) and the testis, an unexpected turn of events, Asian Journal Andrology, 15(4), p. 455-460.

Reijo R., Lee T. Y., Salo P., Alagappan R., Brown L. G., Rosenberg M. and Chapelle A. D. L., (1995), Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene, Nature genetics, 10(4), p. 383-393.

Reynolds N. and Cooke H. J., (2005) Role of the DAZ genes in male fertility, Reproductive Biomedicine online, 10(1), p. 72-80.

Ron-El R., Strassburger D., Gelman-Kohan S., Friedler S., Raziel A. and Appelman Z., (2000) A 47, XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome, case report, Human Reproduction, 15(8), p. 1804-1806.

Ruan Y. C., Shum W. W., Belleannée C., Da Silva N. and Breton S., (2012) ATP secretion in the male reproductive tract, essential role of CFTR, The Journal of physiology, 590(17), p. 4209-4222.

Schaefer A. M., Taylor R. W., Turnbull D. M. and Chinnery P. F., (2004) The epidemiology of mitochondrial disorders past, present and future, Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1659(2), p. 115-120.

Scriver C. R., Neal J. L., Saginur R. and Clow A., (1973) The frequency of genetic disease and congenital malformation among patients in a pediatric hospital, Canadian Medical Association Journal, 108(9), p. 1111.

Sertié A. L., Sossi V., Camargo A. A., Zatz M., Brahe C. and Passos-Bueno M. R., (2000) Collagen XVIII, containing an endogenous inhibitor of angiogenesis and tumor

growth, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome), Human Molecular Genetics, 9(13), p. 2051-2058.

Simoni M., Bakker E. and Krausz C., (2004) EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions, State of the art 2004, International journal of andrology, 27(4), p. 240-249.

Simoni M., Tüttelmann F., Gromoll J. and Nieschlag E., (2008) Clinical consequences of microdeletions of the Y chromosome, the extended Münster experience, Reproductive biomedicine online, 16(2), p. 289-303.

Skaletsky H., Kuroda-Kawaguchi T., Minx P. J., Cordum H. S., Hillier L., Brown L. G. and Chinwalla A., (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes, Nature, 423(6942), p. 825-837.

Smith L. B., Milne L., Nelson N., Eddie S., Brown P., Atanassova N. and Napper D., (2012) KATNAL1 regulation of sertoli cell microtubule dynamics is essential for spermiogenesis and male fertility, PLoS Genet, 8(5), e1002697.

Sriram G., Martinez J. A., McCabe E. R., Liao J. C. and Dipple K. M., (2005) Single-gene disorders, what role could moonlighting enzymes play, The American Journal of Human Genetics, 76(6), p. 911-924.

Tahir F., Shahab M., Afzal N. M., Subhan F., Sultan S., Kazi B. M. and Dil A. S., (2004) Male reproductive health, An important segment towards improving reproductive health of a couple, In a Chapter in Population Research and Policy Development in Pakistan in Fourth Population Association of Pakistan Conference Held at Faisalabad.

Tiepolo L. and Zuffardi O., (1976) Localization of factors controlling spermatogenesis in the non-fluorescent portion of the human Y chromosome long arm, Human genetics, 34(2), p. 119-124.

Tomlinson I. P. and Bodmer W. F., (1995) The HLA system and the analysis of multifactorial genetic disease, Trends in genetics, 11(12), p. 493-498.

Totonchi M., Meybodi A. M., Boroujeni P. B., Gilani M. S., Almadani N. and Gourabi H., (2012) Clinical data for 185 infertile Iranian men with Y-chromosome microdeletion, Journal of assisted reproduction and genetics, 29(8), p. 847-853.

Travaglini P., Pizzocaro A., Beck-Peccoz P. and Colpi G., (2006) Malattie endocrine del testicolo, Malattie del sistema endocrino e del metabolismo. Milano: McGraw-Hill, p. 257-286.

Tüttelmann F. and Simoni M., (2008) Current recommendations for genetic testing in male infertility, European Urological Review, p. 88-92.

Tyler-Smith C., (2008) An evolutionary perspective on Y-chromosomal variation and male infertility, International journal of andrology, 31(4), p. 376-382.

Visser L. and Repping S., (2010) Unravelling the genetics of spermatogenic failure, Reproduction, 139(2), p. 303-307.

Vogt P. H. (2005) Azoospermia factor (AZF) in Yq11 towards a molecular understanding of its function for human male fertility and spermatogenesis, Reproductive Biomedicine online, 10(1), p. 81-93.

Vogt P. H., (2004) Genomic heterogeneity and instability of the AZF locus on the human Y chromosome, Molecular and cellular endocrinology, 224(1), p. 1-9.

Xiao Hua Jiang., Ihtisham Bukhari., WeiZheng., Shi Yin., Zheng Wang., Howard J. C., Qing Hua Shi., (2014) Blood testis barrier and spermatogenesis, lessons from genetically modified mice, Asissan Journal of Andrology, 16, p. 1-9.

Xu M., Qin Y., Qu J., Lu C., Wang Y., Wu W. and Sha, J., (2013) Evaluation of five candidate genes from GWAS for association with oligozoospermia in a Han Chinese population, PloS one, 8(11), e80374.

Yamamoto Y., Sofikitis N., Kaponis A., Georgiou J., Giannakis D., Mamoulakis C. H. and Chatzikyziakidou A., (2002) Use of a highly sensitive quantitative telomerase assay in intracytoplasmic sperm injection programmes for the treatment of 47, XXY non-mosaic Klinefelter men, Andrologia, 34(4), p. 218-226.

Yu J., Chen Z., Ni Y. and Li Z., (2012) CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD), a systemic review and meta-analysis, Human reproduction, 27(1), p. 25-35.

Yu J., Chen Z., Zhang T., Li Z., Ni Y. and Li Z., (2011) Association of genetic variants in CFTR gene, IVS8 c. 1210-12T [5_9] and c. 1210-35_1210-12GT [8_12], with spermatogenetic failure, case–control study and meta-analysis, Molecular human reproduction, 17(9), p. 594-603.

Zegers-Hochschild F., (2010) Algunas reflexiones éticas en el uso de la tecnología reproductiva moderna para el tratamiento de la infertilidad, Revista Medica Clinica Las Condes, 21(3), p. 469-478.

Zenzes M. T., (2000) Smoking and reproduction, gene damage to human gametes and embryos, Human Reproduction Update, 6(2), p. 122-131.

Zhang F., Lu C., Li Z., Xie P., Xia Y., Zhu X. and Wang X. (2007) Partial deletions are associated with an increased risk of complete deletion in AZFc, a new insight into the role of partial AZFc deletions in male infertility, Journal of medical genetics, 44(7), p. 437-444.

Zuccarello D., Ferlin A., Cazzadore C., Pepe A., Garolla A., Moretti A. and Foresta C., (2008) Mutations in dynein genes in patients affected by isolated non-syndromic asthenozoospermia, Human reproduction, 23(8), p. 1957-1962.