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Cytogenetics and Y chromosome microdeletions analysis for a cohort of Palestinian azoospermic and oligospermic men

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Abstract:

Genetic causes account for 10-15% of male infertility in Western countries and include single gene disorders and chromosomal aberrations. Klinefelter Syndrome and submicroscopic deletions on the Y chromosome involving AZF region seem to be the most genetic causes of male infertility associated with azoospermia and oligospermia. The aim of this study was to do genetic tests in a group of azoospermic and oligospermic infertile men in the Southern part of the West Bank (Palestine) to assess the frequency of these defects. **Method**: 27 samples referred from doctor's clinics and medical laboratories were analyzed by standard G-banding techniques for numerical and structural chromosomal rearrangements. 26 samples were analyzed for Y chromosome microdeletions by multiplex PCR using primers for AZFa, AZFb and AZFc regions. **Results:** We reported that 7 of the 27 patients (26 %) had Klinefelter Syndrome. We reported the first case of rare variant of Klinefelter Syndrome 48,XXXY in Palestine. No subject was positive for deletions of the classical AZF regions. **Conclusion:** our study revealed a higher incidence of chromosomal aberrations than is reported in Western populations, and absence of Y chromosome microdeletions. Follow-up studies are now justified to determine the exact causes of such higher incidence of chromosomal aberrations among infertile Palestinian men. On a more practical level, our study indicates the need for cytogenetic analysis for diagnosis and genetic counseling for infertile men. Such data would also help design the most appropriate therapeutic interventions for example by testicular biopsies and/or intracytoplasmic sperm injection (ICSI).

Key words: Cytogenetics, Y chromosome microdeletion, ICSI, azoospermia and Oligospermia.

دراسة الخلل الكروموسومي و الحذف في كروموسوم Y لمجموعة من الرجال الفلسطينيين الذين يعانون من ضعف أو عدم قدرة على إنتاج الحيوانات المنوية

ملخص الدراسة:

تمثل الأسباب الجينية لعقم الرجال في البلاد الغربية ما نسبته 15% من الأسباب الكلية للعقم، و تتضمن اضطرابات الجين الواحد و الإعتلالات الكروموسومية. متلازمة كلاينفلتر و الحذف في كروموسوم Y في منطقة AZF يبدوان أكثر الأسباب الجينية المرتبطة بعقم الرجال الذين ليس لديهم حيوانات منوية أو عدد قليل من الحيوانات المنوية . الهدف من هذه الدراسة إجراء اختبارا ت جينية لدى مجمو عة من الرجال الذين لديهم ضعف أو عدم قد رة على إنتاج الحيوانات المنوية في الجزء الجنوبي من الضفة الغربية (فلسطين) لمعرفة نسبة وجودها . **الطريقة** : تم تحليل الخلل الكروموسومي ل 27 مريض محولين من أطباء و مختبرات طبية بواسطة الطريقة المعيارية لدراسة الكروموسومات. و تم دراسة الحذف في كروموسوم Y لدى 26 مريض بواسطة التحليل الجزيئي. **النتائج** : أظهرت الدراسات أن 7 مرضى لديهم متلازمة كلاينفلتر منهم مريض واحد لديه حالة نادرة جدا 48,XXXY تسجل لأول مرة في فلسطين ، كما و أظهرت النتائج عدم وجود الحذف في كروموسوم Y لدى أي من المرضى . **الخلاصة**: يبدو أن لدينا نسبة مرتفعة من الخلل الكروموسومي لدى الرجال الذين يعانون من العقم ، و يبدو أن الحذف في كروموسوم Y لا يمثل سببا جوهريا للعقم في فلسطين . أظهرت الدراسة الحالية أهمية وجود دراسات تبين الأسباب الدقيقة وراء إرتفاع الخلل الكروموسومي لدى الرجال الذين يعانون من ضعف أو عدم قدرة على إنتاج الحيوانات المنوية، كما و بينت أه مية الفحص الجيني للرجال الذين يرغبون في الإنجاب بطريقة الحقن المجهري للمساعدة في التشخيص و إختيار الطرق المناسبة للعلاج و إعطاء المشورة الوراثية المناسبة لمنع إنتقال الإعتلالات الكروموسومية للأبناء.

الكلمات المفتاحية: الإعتلالات الكروموسومية، الحذف في كروموسوم Y ، متلازمة كلاينفلتر، ضعف إنتاج الحيوانات المنوية و عدم القدرة على إنتاج الحيوانات المنوية.

DECLARATION

I declare that the Master Thesis entitled " Cytogenetics and Y chromosome microdeletions analysis for a cohort of Palestinian azoospermic and oligospermic men" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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Date: 2011

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Dedication

I would to dedicate this thesis to my parents, who gave me all they could to enter the university and complete the education. They gave me the support needed throughout this difficult phase from my life.

I would to dedicate this thesis to my husband, who encouraged me to complete the master degree, and gave me his time and efforts to reach this stage.

I would to dedicate this thesis to my sisters and brothers, and I mention my sister Hind, who gave her time to complete this thesis.

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Abbreviations

KS	Klinefelter syndrome
TESA	Testicular sperm extraction
ICSI	Intracytoplasmic sperm injection
PGD	Preimplentation genetic diagnosis
AZF	Azoospermia factor
P 1-8	Palindrome 1 to 8
Yp	Short arm of chromosome Y
Yq	Long arm of chromosome Y
CFTR	Cystic fibrosis transmembrane conductance regulator gene
CBAVD	Congenital bilateral aplasia of vas deference
AR	Androgen Receptor
IVF	In vitro fertilization
AIS	Androgen insensitivity syndrome
EAA	European Academy of Andrology
EMQN	European Molecular Genetics Quality Network
PCR	Polymerase chain reaction
STS	Sequence tagged sites
PAR1 and 2	Pseudoautosomal region 1 and 2 on Y chromosome
SCO	Sertoli cell only
MSY	Male specific of chromosome Y
DAZ	Deleted in azoospermia
SRY	Sex-determining region on Y
FSH	Follicular stimulating hormone
IQ	Intelligence Quotient
LH	Luteinizing hormone

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Chapter one

The study

1.1 Introduction

Infertility is a serious problem around the world accounting for 15% of married couples (Irvine, 1998). In Palestine, one study on fecundability (the capacity to reproduce) conducted on two agricultural Palestinian villages of Hebron district showed that 13.4% of 205 newly married couples could not achieve pregnancy during one year of follow-up (Issa et al., 2010), which indicates similar rate of infertility. However, additional studies from other parts of Palestine are warranted.

Infertility is caused by male factor alone in 20% of cases, and as a contributory factor with other factors in about 30% (Thonneau et al., 1991). There are many etiologies of male infertility including genetic causes and other causes like varicocele, cryptorchidism, endocrinopathies, infections, obstructions and idiopathic (no reason to explain the cause of infertility). In Palestine, there is a shortage in studies on etiology of male infertility, there was one study in Gaza strip on the genetic causes of infertility (Shaqalaih, 2007), in which they studied the chromosomal aberrations and Y chromosome microdeletions in a sample of azoospermic (no sperms in ejaculate) and oligospermic (less than 5 million/ml of sperms in ejaculate) infertile men.

Genetic causes of male infertility include the chromosomal aberrations and monogenetic disorders. The most common chromosomal abnormality which causes male infertility is Klinefelter syndrome. Y chromosome microdeletions are considered as the second most common genetic cause of male infertility (Katherine et al., 2010). The most common monogenetic disorders are mutations of cystic fibrosis and androgen receptor genes.

Klinefelter syndrome is caused by at least one extra X chromosome in the male genome. About 80% of patients have the karyotype of 47,XXY, the other have variants including 48,XXXY,48,XXYY, 49,XXXXY, 46,XY/47,XXY mosaicism and structurally abnormal X

chromosomes (Lanfranco et al., 2004). Its prevalence among the general population is 1 every 600 newborns (Nielsen and Wohlert, 1991), and its prevalence among oligospermic men is ≈ 1.5% and in azoospermic men is ≈ 13% (Van Assche et al., 1996; Vincent et al., 2002). Newborns of KS appear normal, and generally, adult men don't have obvious facial dysmorphology. So, the syndrome is underdiagnosed, only one-fourth of adult male are diagnosed, and less than 10% of the expected number are diagnosed before puberty (Bojesen et al., 2003). Most patients are diagnosed in infertility clinics as they are passing the routine cytogenetic analysis. Most KS patients are azoospermic (have not sperms in ejaculates), and few patients have few motile sperms in their ejaculates (Foss and Lewis, 1971). There are two reported cases with approved paternity for a spontaneous pregnancy in KS patients, but such cases are exceptional and very rare (Laron et al., 1982; Terzoli et al., 1992). In Azoospermic KS patients, the possibility to find sperms in testicular tissues by surgical methods is around 50% (Vernaeve et al., 2004), and can reach up to 70% by the method of microdissection TESE (testicular sperm extraction) (Schiff et al., 2005). So patients of KS can reproduce and have biological paternity with the aid of assisted reproductive techniques, specifically intracytoplasmic sperm injection (ICSI). But, as this technique can bypass the natural selection, the abnormal sperm can fertilize the egg resulting in abnormal embryo. Many studies reported a modest significant increase in sex chromosomal hyperploidy in testicular sperms of KS patients (Blanco et al., 2001; Foresta et al., 1998). Some studies showed an increase in autosomal chromosomal hyperploidy in chromosomes; 13, 18 and 21 in sperms of KS (Hennebicq et al., 2001; Morel et al., 2003). Preimplentation genetic diagnosis (PGD) which is performed by taking an embryo biopsy for diagnosis, was applied on ICSI-embryos for KS patients by Staessen et al , they found an increase in sex and autosomal chromosomal abnormalities compared with controls who did PGD for sex selection (Staessen et al., 2003).

Although most infants born after ICSI with sperms from KS patients were normal, genetic counseling is mandatory and PGD should be done in order to prevent transfer of abnormal embryos.

The second most common genetic cause of male infertility is Y chromosome microdeletions. Their incidence among azoospermic and severely oligospermic men is about 10% (Stahl et al., 2010). Y chromosome has on its long arm a region contains genes responsible for spermatogenesis called AZF region (Tiepolo and Zuffardi, 1976), it is subdivided into 3 regions; AZFa, AZFb and AZFc (Vogt et al., 1996). Within AZF region, there are ampliconic

sequences (highly repetitive sequences) composed of eight large palindromes P 1-8 (inverted repeats with very little intervening sequence). A homologous recombination between these identical repeats will cause a deletion and loss of genetic materials between repeats. And this is the mechanism by which microdeletions occur. After sequencing of Y chromosome, Repping et al defined a new model for deletion pattern; AZFa, AZFb (P5/proximal P1), AZFbc (P5/distal P1 or P4/distal P1) and AZFc (b2/b4) (Repping et al., 2002).

A complete deletion of AZFa is characterized by a testes histology of Sertoli cell only (SCO) and azoospermia, and implies the impossibility of finding sperms in testes (Kamp et al., 2001). Complete deletion of AZFb or AZFbc is characterized by a histological picture of SCO or spermatogenic arrest, and also implies the virtual impossibility of finding sperms in testes (Hopps et al., 2003). Partial deletion of AZFb is associated with sperm retrieval of 50% (Krausz et al., 2000). Complete deletion of AZFc (b2/b4) is the most common deletion, and can cause different degrees of spermatogenic failure, which range from absence of germ cells in testes to presence of sperms in ejaculate (oligospermia). Partial deletions within AZFc can occur, and different partial deletions were reported include; gr/gr, b2/b3 and b1/b3 deletions. These partial deletions are associated with extremely variable phenotypes, ranging from normospermia to azoospermia, and there is a major controversy on their real impact on male fertility (Ferlin et al., 2005; Hucklenbroich et al., 2005). Some of these partial deletions were reported to be a neutral in certain Y chromosome haplogroup (genealogical tree of Y chromosome constructed on the basis of Y-chromosomal single nucleotide polymorphisms SNPs) (Sin et al., 2010). In rare cases, complete AZFc may present in fertile male and transmitted to infertile sons (Kuhnert et al., 2004; Saut et al., 2000). In azoospermic AZFc-deleted men, the possibility to find sperms in testes is about 67% (Oates et al., 2002). So they can undergo ICSI and have a biological offspring, but sons will have the same deletions as their fathers, or even expanded to a larger area (Komori et al., 2002; Lee et al., 2006). Siffroi et al showed that some individuals with AZFc microdeletions harbored a significant population of 45,XO cells in both peripheral blood lymphocytes and in germ cells, which may lead to formation of monosomic embryos with ICSI (Siffroi et al., 2000). Also Ferlin et at assessed the sperm aneuploidy rate in men with the b2/b4 deletions and compared it with non-deleted severely oligozoospermic men and normozoospermic control men, they found a significant reduction in the percentage of normal Y-bearing spermatozoa with respect to controls and found a significant increase in XY-disomic sperms than the two groups (Ferlin

et al., 2007). These two studies highlighted the risk of formation of abnormally karyotyped embryos for AZFc-deleted men with ICSI.

On the other hand, many studies reported normal karyotyped males born after ICSI for AZFc-microdeleted men (Gambera et al., 2010; Oates et al., 2002).

For these reasons, many studies emphasized on the importance of screening for Y chromosome microdeletions, to aid in prognosis (AZFc-deleted men have the opportunity to find sperms in the testes and not AZFb or AZFa) and to discuss the transmission of deletion to sons with infertile couple to get a suitable genetic counseling before ICSI and doing PGD (Stahl et al., 2010).

Other numerical chromosomal abnormalities other than Klinefelter syndrome that can be found in azoospermic and oligospermic men are: chromosomal abnormalities with extra Y chromosomes (47,XYY, 48,XYYY, 49,XYYYY and 49,XXYYY), Mosaic 45,X /46,XY and 46,XX male.

Structural chromosomal abnormalities of autosomes that have high incidence among azoospermic and oligospermic men are: Robertsonian translocations (result from fusion of 2 acrocentric chromosomes) and reciprocal translocations (mutual exchange of chromosomal segments between two chromosomes). Robertsonian translocations have a higher incidence among infertile men nine times than the general population, and Reciprocal translocations have a higher incidence of seven times (Ferlin et al., 2006). Men with these abnormally karyotypes have normal phenotypes, but show infertility because of spermatogenic defects due to disturbances in meiotic process (Anton et al., 2004), and show increase in pregnancy loss due to rearrangements in embryos. Studies on segregation process in Robertsonian carriers showed an increase in unbalanced sperms, Chen et al showed a frequency for adjacent segregation varied between 11.70 and 19.53% and a higher frequencies of aneuploidy for sex chromosomes and interchromosomal effect (with sperm aneuploidy not only for the chromosomes involved in the translocation, but also for other chromosomes) (Chen et al., 2007). Regarding reciprocal translocations, as a general rule, carriers produce more unbalanced sperms than normal or balanced sperms (Mardesic et al., 2011).

So, karyotyping in azoospermic and oligospermic men is very important before ICSI to detect these arrangements and to do PGD to prevent abnormal embryos from implantation and to decrease pregnancy loss due to rearranged embryos.

Structural chromosomal abnormalities of sex chromosomes also increase in azoospermic and oligospermic men and include: X-autosome translocations, Xp deletions, Xq deletions, isochromosome Y, ring Y chromosome.

Y Isochromosome is a Y chromosome that has lost one of its arms and replaced it with an exact copy of the other arm. It is divided into monocentric and dicentric isochromosome Y. Dicentric Isochromosomes Y are the most common Y structural abnormalities (Abdelmoula and Amouri, 2005). Azoospermia is common in 46, X,dic(Yq or p), may be due to disturbance of spermatogenesis because the presence of two centromeres which disturb the normal segregation of chromosomes in meiosis (Yoshida et al., 1997). Ring Y chromosome which result from breaks in chromosome and fusion of proximal broken ends, can be inherited with ICSI. Spinner et al reported transmission of ring y from father to newborn infant with ICSI, this newborn had 47,XXr(Y)[10]/46,XX[40 (Spinner et al., 2008). Another study reported transmission of ring Y from father to an embryo, which was found to have 45,X/46,X,r(Y) karyotype at 18 weeks' gestation on genetic amniocentesis (Bofinger et al., 1999). These studies confirm on the importance of cytogenetic analysis before ICSI and doing PGD before transfer of embryos.

The most common monogenic disorders that are found in azoospermic and oligospermic men are: mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene and androgen receptor gene (AR). Mutations of CFTR gene cause congenital bilateral Aplasia of vas deference (CBAVD) which is a failure of normal development of vas deference. CBAVD accounts for at least 6% of cases of obstructive azoospermia and is responsible for 1 to 2% of cases of infertility in men (Jequier et al., 1985). Mutations in AR gene can result in impaired embryonic sex differentiation in 46, XY genetic individuals in a disorder called androgen insensitivity syndrome (AIS). It is characterized by phenotypic variations from female external genetalia through ambiguous genetalia and normal male with azoospermia or oligospermia. Expansion of CAG repeats in AR gene was associated with decreased spermatogenesis in male infertility in some ethnic groups (Eldar-Geva et al., 2004).

So it is very important to do a cytogenetic analysis to exclude the abnormal karyotypes in such disorders, which aids in diagnosis and proper genetic counseling in the case of ICSI.

From the previous introduction, it is clear that cytogenetic analysis and screening for Y chromosome microdeletions are mandatory before ICSI for diagnostic and prognostic values and for proper genetic counseling.

1.2 Problem statements and objectives

In Palestine, there are many assisted reproductive centers, they don't request from azoospermic and oligospermic infertile men to do cytogenetic and Y chromosomal microdeletion analysis, although there is one cytogenetic and molecular lab in the West Bank (in AL-Maqased Hospital). May be the causes for that are: doctors don't realize the necessity of genetic counseling before ICSI. Or because the shortage in cytogenetic and molecular laboratories in the West Bank. Or because the shortage in studies of genetic causes of male infertility that clarify their incidence in Palestine and emphasize on the importance of analysis before ICSI. Also, these are no follow up studies on ICSI-children from IVF centers in West Bank.

Considering the above, we decided to do cytogenetic and molecular analysis for azoospermic and oligospermic infertile men in the south part of West Bank, in order to assess the frequency of chromosomal abnormalities and Y chromosomal microdeletions in this population.

1.2.1 Specific objectives:

- Compare frequency of chromosomal abnormalities and Y chromosome microdeletions in our population with those reported elsewhere.
- Relate cytogenetic and molecular analysis to assisted reproductive technologies.
- Develop best practices for genetic counseling in infertile couples.
- Get hands on the experience in karyotyping and molecular analysis.

1.3 Materials and methods

1.3.1 Subjects and samples

Gynecological and urological doctors and lab technicians were informed of the possibility of referring azoospermic and oligospermic men for cytogenetic and molecular analysis for this research study (in Hebron and Bethlehem, between June 2010 to February 2011). 9 men were refereed from doctors. 10 men were referred by lab technicians. 8 men of the sample were

taken retrospectively from Al-Hanan IVF center. Patients from Hebron were seen in Al-Hanan IVF center, while, patients from Bethlehem were seen in cytogenetic lab of Bethlehem university.

In total, 31 subjects were studied, 27 Azoospermic and Oligospermic infertile men and 4 control men. 18 men were azoospermic (66.6%). 11 men were oligospermic (33.4%). 4 control men included 2 negative control (fertile men with many children for each), and 2 positive control (cytogenetically abnormal, Down syndrome men). Geographic distribution included 13 men (48%) from Hebron city, 7 (26%) from Hebron rural Villages and 7 (26%) from Bethlehem.

Azoospermic and oligospermic men had 2 semen analysis testes. Oligospermic men had less than 5 million sperms\ ml in semen tests along their ages. Azoospermic men were tested by a single urologist for physical examination to exclude obstructive azoospermia. Every man was interviewed to give information about history of infertility, past cryptorchidism, past accidents, previous surgical operations and previous orchidism. All men in the sample gave a consent form for participating in the study.

1.3.2 Cytogenetic analysis

The blood samples were drawn into a 10 ml of sodium heparin tubes, and were karyotyped using standard G-banding technique.

1.3.2.1 Cell culture and harvest

1. 0.5 ml of blood sample was cultured into 5 ml PHA (phytoheamagglutinin) media for three days in incubator at 37C. PHA media is a mitogen which activates the cell divisions by triggering mitosis.
2. 50 µl colcemed (10µg/ml) was added to the cultured-sample for 1 hour. Colcemed acts by binding with microtubules to suppress its action, hence, stops the cells in metaphase stage.
3. The sample was centrifuged for 10 min at 1000 rpm.
4. The supernatant was removed, the pellet resususpended in 8-10 ml of hypotonic solution (0.56% KCl) and incubated for 20 min at 37 C.

5. 2 ml of fresh fixative solution (1:3 glacial acetic acid: absolute methanol) was added and tubes were inverted to mix. Hypotonic solution makes the cells fragile, and when adding the fixative solution, RBCs will be hemolyzed.
6. The sample was centrifuged for 10 min at 1000 rpm.
7. The supernatant was discarded, the pellet was gently suspended in the last few drops, and 8 ml of fixative solution was added (slowly in the beginning) and centrifuged for 10 min at 1000 rpm.
8. The last step was repeated for at least 2 times until pellet is clear white.
9. The pellet was suspended in a small volume of fixative solution.

1.3.2.2 Slides preparation and staining

1. Slides were cleaned by alcohol and rinsed with distilled water.
2. 2 to 3 drops of cell suspension are dropped on wet slides held at 45 degree angle, and placed at a humid chamber at 37- 45 C until dried.
3. The slides were placed at the hot plate at 90 C for 1 hour.
4. The slides were stained by placing them:

- First, in pH 7 buffered solution which prepared by dissolving 1Gurr buffer tablet in 1 litter 0.9% NaCl.
- Then, in Trypsin solution for 30 seconds, which prepared by 3 ml of stock Trypsin (1.25g of 1:250 pancreatic Trypsin in 200 ml of DW, Sigma, Cat. No. 93615) in 40 ml pH 7 buffer. Trypsin was used in order to digest the proteins in chromosomes to absorb the stain.
- Rinse in two jars of pH 7 buffer.
- Giemsa solution for 2 min, which prepared by placing 1.5 ml of stain with pH 6.8 buffered solution. PH 6.8 buffered solution was prepared by dissolving 1 Gurr tab in 1 litter of DW, and adjusted by pH meter to 6.8 by NaOH or HCl.
- Finally, in DW for rinsing the stain.

The slides were seen using the light microscope and 20 metaphases were analyzed for every sample, in case of mosaicism, 50 metaphases were seen.

1.3.3 Molecular analysis

Y chromosome microdeletions were analyzed by polymerase chain reaction (PCR), using the protocol approved by European Academy of Andrology (EAA) and the European Molecular

Genetics Quality Network (EMQN) (Simoni et al., 2004). They stated in the best practice guidelines that the use of the recommended primers will enable the detection of clinically relevant deletions (over 95% of the deletions reported in the literature in the three AZF regions).

The protocol includes using of 2 multiplex PCR; each multiplex contains 3 primers for the three AZF regions (AZFa, AZFb, and AZFc) and 2 internal controls (ZFY and SRY) (See table 1 for the used primers).

The STS (sequence-tagged sites) primers for AZF regions are:

For AZFa: sY84, sY86.

For AZFb: sY127, sY134.

For AZFc: sY254, sY255 (both in the DAZ gene) (Fig.1.1).

These STS primers are derived from non polymorphic regions of Y chromosome and are well known to be deleted specifically in azoospermic and oligospermic men according to the known, clinically relevant microdeletion pattern (Simoni et al., 2004).

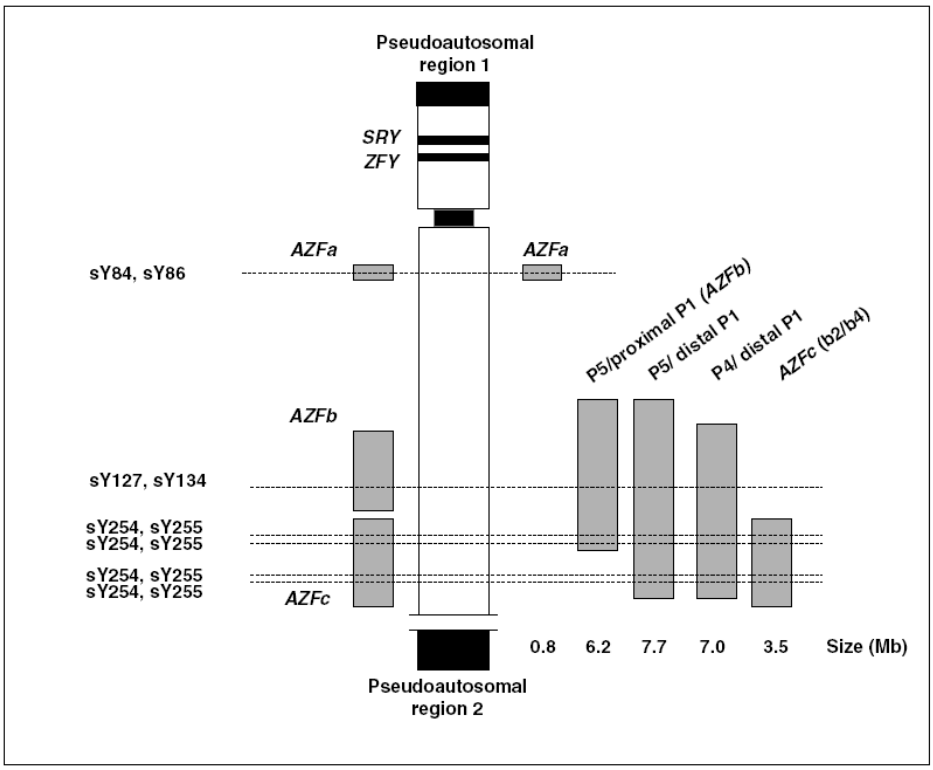

Figure 1.1: The location of STS primers within AZF regions. STS primers are indicated by dotted lines. The STS primers sY254, sY255 will amplify the four loci of DAZ gene in AZFc. The picture Adapted from (Simoni et al., 2004).

In each run of multiplex PCR, positive and negative controls were used. The positive control was female DNA, and used to control for specificity and contamination. The negative control was fertile male, and used to control for sensitivity and specificity of the test. In addition to these controls, a water sample, which contained all reaction components except DNA was used to control for reagent contamination.

1.3.3.1 DNA extraction

DNA was extracted using salt based method, and this is the procedure:

- 10 ml of whole blood was collected into a heparenized tube.
- The blood was put into 50ml-tube and RBCs-lysis buffer was added until filled the tube and kept on ice for 20 min.
- The sample was centrifuged at 2000 rpm for 10 min at 4 C.
- The supernatant was removed and the pellet was washed by adding 20 ml of RBCs-lysis buffer, and centrifuged again at 2000rpm for 10 minutes at 4 C.
- The pellet was broken by careful shaking, and then 3ml of DNA-lysis buffer, 50µl of 5 mg/ ml proteinase K and 100 µl of 20 % SDS was added and incubated at 37 C for 2 days.
- After incubation, the tube was clear with no pellet.
- 1.2 ml of 6M NaCl was added and shaken vigorously for 2 minutes.
- The tube was centrifuged at 3000rpm for 20 minutes at 25 C.
- The supernatant was taken and centrifuged again for 7min at 3000 rpm.
- The supernatant was taken, and 4 ml of cold absolute ethanol was added and shaken gently 4 – 5 times until DNA was observed in a shape of spider threads.
- DNA was picked by clean pasteur pipette and washed with 70% Ethanol 8-10 times carefully and dried for about 3-5 minutes.
- DNA was put into ependorf tube containing 400 µl (0.02 % Sodium Azide + D.W) and left for 1-3 days at room temperature to dissolve the DNA completely.
- DNA was stored at -20 C.

1.3.3.2 PCR protocol

- Primers of each multiplex were mixed and contained the forward and reverse primers of ZFY, SRY and 3 loci of AZF region (10 primers for each multiplex). The mix was prepared by taking 0.5 µl of each primer (10 pmol/µl) for 1X reaction (Primers were designed by Metabion international AG).
- 1X PCR reaction contained: 12.5µl of master mix (ABgene, thermo-scientific UK, which consists Taq polymerase, buffer and dNTPs mix), 5µl of primer mix, 6.5 µL of distilled water and 1µl of DNA.
- The tubes of patient's samples, in addition to negative and positive controls and DW sample (DW instead of DNA) were shaken and put in the thermocycler (gene Amp 9700, Applied Biosystem).
- The thermocycler was programmed as follow:
  15 minutes at 95 C --- > Initial Denaturation Step
  30 seconds at 95 C --- > Denaturation Step
  90 seconds at 57 C --- > Annealing Step
  60 seconds at 72 C --- > Extension Step
  10 minutes at 72 C --- > Final Elongation
  Cycle Number: 35
- The PCR product was mixed with loading dye, and loaded on 4% agarose gel (Seakem LE agarose, Lonza, USA) which prepared by adding 4 g of agarose in 100 ml 1x Tris Acetate EDTA (TAE) buffer with 0.5 µg/ml ethidium bromide.
- Electrophoresis was run at 64 V for 1 hour for multiplex B and 3 hours for multiplex A.
- The results were visualized and documented using gel documentation system (gel doc. Molecular imager, BioRad).
- Amplification with multiplex A and B for each sample was repeated two times, in the case of unclear results, simplex PCR was done (amplification for each primer set).

Multiplex Primers	
A and B	ZFY-F: 5′ – ACC RCT GTA CTG ACT GTG ATT ACA C – 3′
	ZFY-R: 5′ – GCA CYT CTT TGG TAT CYG AGA AAG T – 3′
A and B	*SRY* -F: 5′ – GAA TAT TCC CGC TCT CCG GA – 3′
	SRY -R: 5′ – GCT GGT GCT CCA TTC TTG AG – 3′
A.	sY86-F: 5′ – GTG ACA CAC AGA CTA TGC TTC – 3′
	sY86-R: 5′ – ACA CAC AGA GGG ACA ACC CT – 3′
A.	sY127-F: 5′ – GGC TCA CAA ACG AAA AGA AA – 3′
	sY127-R: 5′ – CTG CAG GCA GTA ATA AGG GA – 3′
A.	sY254-F: 5′ – GGG TGT TAC CAG AAG GCA AA – 3′
	sY254-R: 5′ – GAA CCG TAT CTA CCA AAG CAG C – 3′
B.	sY84-F: 5′ – AGA AGG GTC TGA AAG CAG GT – 3′
	sY84-R: 5′ – GCC TAC TAC CTG GAG GCT TC – 3′
B.	sY134-F: 5′ – GTC TGC CTC ACC ATA AAA CG – 3′
	sY134-R: 5′ – ACC ACT GCC AAA ACT TTC AA – 3′
B.	sY255-F: 5′ – GTT ACA GGA TTC GGC GTG AT – 3′
	sY255-R: 5′ – CTC GTC ATG TGC AGC CAC– 3′

Table 1.1: The used primers for 2 multiplex PCR. adapted from EAA/EMQN best practice guidelines (Simoni et al., 2004) .

1.4 Results

From 27 azoospermic and oligospermic infertile men analyzed for chromosomal abnormalities, 7 men (≈ 26%) had abnormalities; 6 men had 47,XXY and 1 man had 48,XXXY. All were azoospermic (See figures 1.2, 1.3).

From 26 azoospermic and oligospermic infertile men analyzed for y chromosomal microdeletions using 8 STSs approved by EAA/EMQN best practice guidelines, no subject had deletion for any of these STSs (sY14 (SRY), ZFX/ZFY, sY84, sY86, sY127, sY134, sY254, sY255) (See figures 1.4,1.5).

1.5 The case of karyotype 48,XXXY

The karyotype showed an extra 2 Xs chromosomes. This is the first case in Palestine (as our knowledge) is reported with this karyotype, which is a variant of Klinefelter syndrome. Its incidence is from 17,000 to 50,000 (Venkateshwari, Srilekha et al. 2010).
He was the first child for his parent. His mother age at his birth was 31 years, and his father was 33 years. His weight at the birth was 1.700 kg , he was placed in the incubator of hospital for 28 days, and he became 2.25 km. He suffered from lack in immunity against bacterial and viral respiratory tract infections. He was diseased by measles at age of 7 month. He walked at 2.5 years. He had a speech delay. At school, he had learning disabilities, he was less than peers. He stayed at the same class for many times, he stayed at school until seventh class, at this point he was ashamed from the students because he was the oldest, so the teachers told his father that he cant keep up with his peers and he should leave the school. After that, he learned the job of his father and he worked with his father until now; he works in maintenance of air compressor. He entered the puberty at late age. Now, he is 25 years old, he is tall, he has a mild facial dysmorphology; Hypertelorism (increased distance between eyes), flat nasal bridge, protruding lips, prominent mandible (lower jaw bone) and he has radioulnar synostosis (abnormal connection between the two bones of the forearm: the radius and ulna). He has hypergonadotropic hypogonadism, hypoplastic penis, gynecomastia and little beard hair. He talks very well and can express about his feeling, but he has a volatile mood. He is emotionally linked to his father very much. He is imitator like a child to his father. He is passive, cooperative and not particularly aggressive.

As kwon, the origin of extra Xs is a successive non-disjunction at the first and second paternal or maternal meiotic divisions. In most cases a single parent contributed all of the additional sex chromosomes, with the other parent contributing a single X or Y chromosome (Pfeiffer and Sanger, 1973).

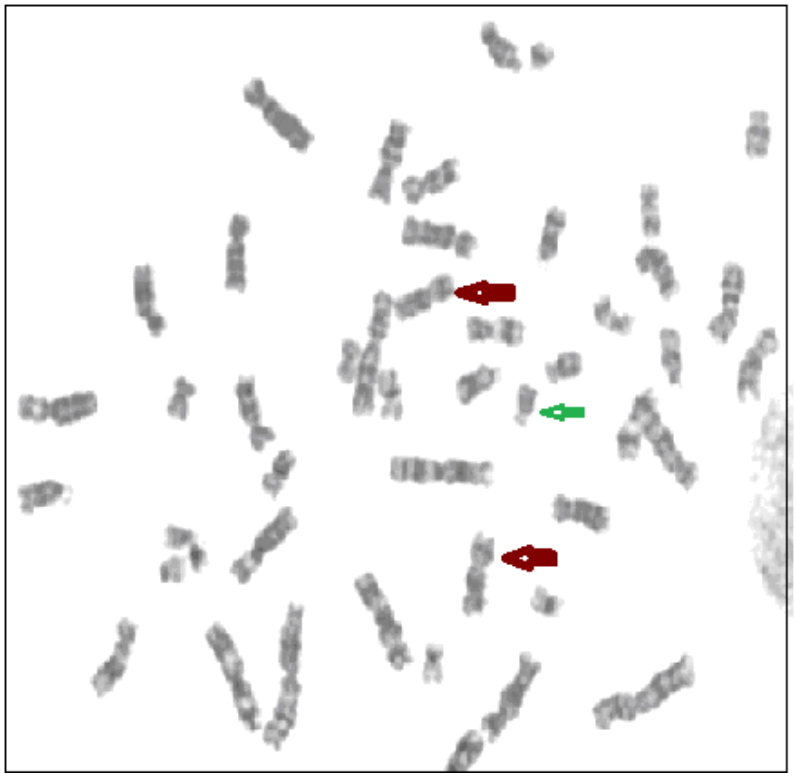

Fig.1.2: 47, XXY, Karyotype of patient # SB10- 0092. The karyotype shows a presence of extra X chromosome. The two X are showed by red arrows, Y chromosome is showed by green arrow (The picture was taken by camera of infinity analyze 0.5, Lumenara corporation).

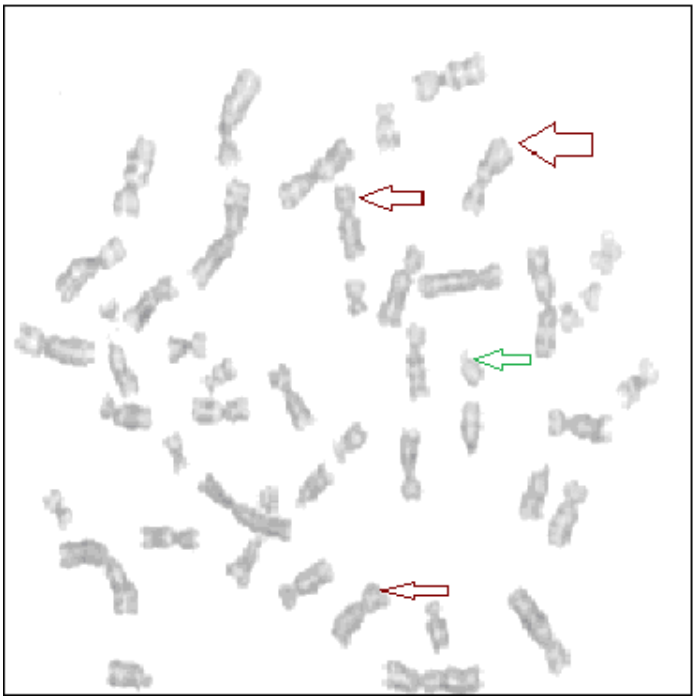

Fig. 1.3: 48, XXXY, Karyotype of patient # SB11-0028. It shows the presence of extra 2X chromosomes (3 Xs are showed by red arrows, Y chromosome is showed by green arrow).

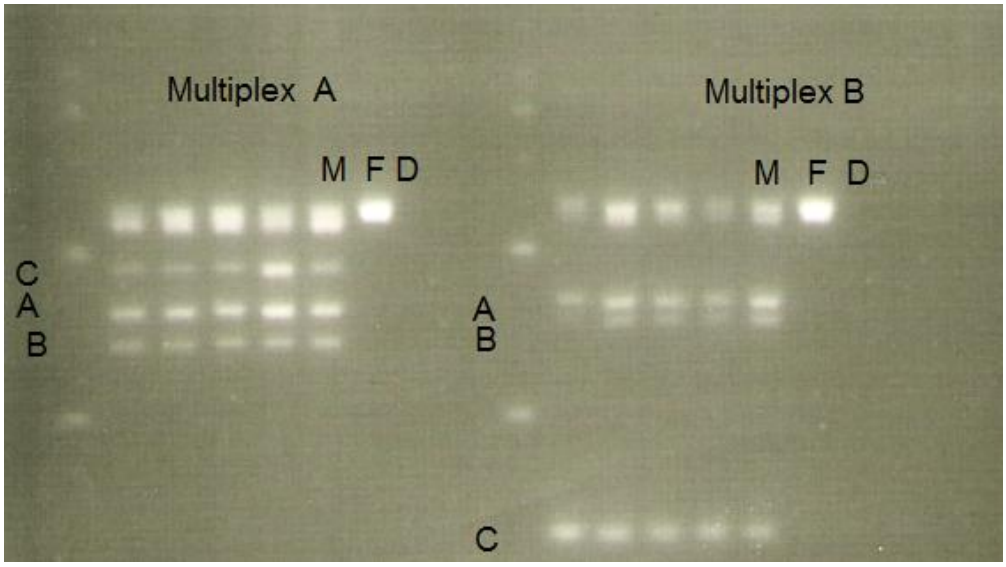

Fig.1.4: Multiplex PCR results. The picture shows the gel image of PCR result of 4 patients (SB10-0024, SB10-0025, SB10-26, and SB10-0027). In each multiplex, M lane represents a DNA of fertile male as a negative control, F lane represents a female sample as a positive control and D lane represents a distilled water sample as a control for reagent contamination. In each multiplex, A, B, and C, show the locations of AZF deletion for the three regions. The first band in real composed of adjacent two bands (ZFY (above) and SRY), they were used as internal controls of multiplex.

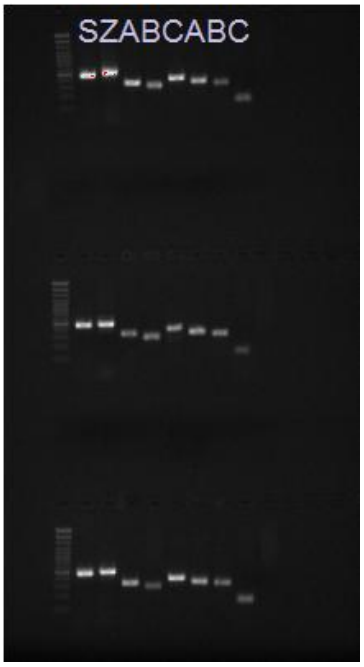

Fig.1.5: Simplex PCR results. The gel shows the results of 2 patients (SB10-0092, SB11-0028). The first gel for normal fertile male, the second was for SB10-0092 and the third for SB-11-0028. The bands show amplification of SRY, ZFY, sY86, sY127, sY254, sY84, sY134, and sY255 respectively.

1.6 Discussion

Chromosomal aberrations and Y chromosome microdeletions are the most two genetic factors of male infertility. The prevalence of chromosomal aberrations ranges from 4.3% to 17.5% in azoospermic and oligospermic infertile men attending reproductive centers (Akin et al., 2011; Huleyuk et al., 2010; Koşar et al., 2010; Kumtepe et al., 2009) and may reach up to 40% in azoospermic and oligospermic men attending laboratories of medical genetics (Zhang et al., 2006). The wide range of frequencies of chromosomal aberrations reflects different patient's selection criteria, e.g. chromosomal abnormalities are more prevalent in azoospermic than oligospermic infertile men. Also, selection of patients after excluding other causes of infertility such as cryptorchidism, varicocele, and endocrinopathies will increase the prevalence. Doing a chromosomal analysis in unselected patients, as a screening test before ICSI, may give prevalence as low as 4% or may be less. So selection criteria play a critical role in determining the prevalence of chromosomal abnormalities, in addition to the most important factor, which is the geographical region and environment. This factor plays a very critical role in decreasing or increasing the prevalence of chromosomal abnormalities.

The prevalence of Y chromosome microdeletions also differs among studies from 4% in oligozoospermic men, 14% in idiopathic severely oligozoospermic men, 11% in azoospermic men, to 18% in idiopathic azoospermic patients (Akin et al., 2011; Foresta et al., 2001). Idiopathic azoospermia or oligospermia includes stricter patient's selection criteria, and excludes kwon infertility factors.
From the chromosomal aberrations, the most common cause of male infertility is Klinefelter syndrome, it accounts for 60 to 90% of chromosomal aberrations, with an incidence in oligospermia is ≈1.5% and in azoospermia is ≈ 13% (Vincent et al., 2002).

In our study we reported a frequency of chromosomal abnormalities about 26% (7/27), with all patients had Klinefelter syndrome (6 had 47,XXY and 1 had 48,XXXY). This frequency is considered a high frequency. We explain this by many factors; the first factor is the geographic factor. Our environment may increase the chromosomal abnormalities in newborns. Dudin et al showed a high frequency of chromosomal abnormalities in malformed newborns, they reported a frequency of 7.9% for chromosomal abnormalities in malformed newborns (Dudin et al., 2001), while as a comparison, the Spanish Collaborative Study of Congenital Malformations reported a frequency of 5.4% of chromosomal abnormalities in

malformed newborns (Centeno Malfaz et al., 2001). The study of Dudin et al is the only study on chromosomal abnormalities in newborns in West Bank (done in AL-Maqased Hospital), and did not determine the incidence of Klinefelter syndrome, because they karyotyped the newborns that showed malformations, and as known, child with KS looks normal and does not show any malformations. So, we need other studies to calculate specifically the incidence of KS in the West Bank, these studies should karyotype all newborns in a certain period, and by this, the incidence of KS can be calculated, and can confirm or infirm our results.

Klinefelter syndrome arises mostly from non-disjunction in the meiosis of germ cells of the mother or father, and rarely from non-disjunction in the first mitotic events in embryo. Paternal non-disjunction accounts for 50% of cases and maternal non-disjunction accounts for the same percent (Lanfranco et al., 2004). The effect of chemical pollutants in the environment on the aneuploidy rate increase was well reported (Eastmond and Pinkel, 1990; Zhang et al., 1996). In Palestine, as we haven't strict regulation rules on using or discarding chemicals (leather tanning factory in Hebron discards the chemical wastes into sewage, overflowing of sewage in Zif region at south Hebron) we do not rule out the effect of these pollutants on aneuploidy rate and chromosomal aberrations.

The second factor that explains the increased frequency of chromosomal abnormalities is the patient's selection criteria. We depended for selection patients on referencing from doctors and lab technicians, and they might suspect a genetic defect and so referenced patients. 6 doctors and 7 lab technicians participated in referencing, and they were told to refer azoospermic and oligospermic men for genetic study, in order to agree for participating in the study.

After that, all patients were examined by a single urologist doctor to exclude obstruction, which added another selection criterion in the study.

In spite of that, and as our results imply, we strongly recommend doing a cytogenetic analysis before ICSI, in order to give appropriate genetic counseling for infertile couple, and to aid in diagnosis and prognosis of their infertility.

Diagnosis of KS patients will save unnecessary surgical or medicinal treatments, and give a prognosis of finding sperms in testicular tissues in azoospermic KS up to 50% by surgical methods (TESE) (Vernaeve et al., 2004), and up to 70% by microdissection TESE (testicular

sperm extraction) (Schiff et al., 2005). But the couple should know that KS patients have a moderate significant increase in sex chromosomal hyperploidy and autosomal chromosomal aneuploidies in their sperms (Foresta et al., 1998; Hennebicq et al., 2001). So they have a higher risk for conceiving abnormal embryos, and they can benefit from PGD to prevent implantation of abnormal embryos. PGD also is useful in increasing the pregnancy rate, because of implanting only normal embryos.

Also, the same in the cases of structural chromosomal aberrations, ICSI will transmit these abnormalities to offspring, so cytogenetic analysis before ICSI is mandatory, and PGD should be done to increase the pregnancy rate and to transfer only normal embryos. Presence of special type of Robertsonian translocation (translocation between the same two chromosomes, for example; two of 13 chromosomes) will nullify the usefulness of ICSI, because only abnormal embryos will result (monosomy of 13 or trisomy of 13) (Veld et al., 1997).

Diagnosis of male with mosaic 45,X/46,Xy will give a prognosis of higher incidence of testicular neoplasia (Gonadoplastoma).

In the case of disorders of sex development, cytogenetic analysis is very important to exclude the normal karyotype and search for single gene disorders to give appropriate genetic counseling.

We reported 26% of chromosomal aberrations with all patients have numerical aberrations; Klinefelter syndrome, and we did not report any structural chromosomal abnormalities, may be because our sample contained mainly azoospermic patients 66.6% , and as kwon structural abnormalities increase in oligospermic patients.

About Y chromosome microdeletions, we did not detect any of AZF deletions using these STS markers (sY14 (SRY), ZFX/ZFY, sY84, sY86, sY127, sY134, sY254, sY255). Our results are consistent with the study of Y chromosome microdeletions in Gaza strip (Shaqalaih et al., 2009); from 125 patients with primary idiopathic male infertility they did not detect any of AZFa, AZFb or AZFc deletions using the same STS markers as us and other 7 STS markers, they detected gr/gr partial AZFc deletions in fertile and infertile populations, and they hypothesized that the gr/gr deletion is not associated with male infertility .

Y chromosome microdeletions are affected by Y-haplogroups (a group of similar haplotypes that share a common ancestor with a single nucleotide polymorphism (SNP) mutation).

Haplogroup E was found to predispose for complete AZFc (b2/b4) deletion (Arredi et al., 2006), while haplogroup Db2 (in Japan) protect against the effect of gr/gr deletion, the same as b2/b3 deletion which seems to have no effect on fertility in Y haplogroup N (in northern Eurasian populations) (Repping et al., 2004).
So, it seems that our genetic background and haplogroup does not predispose for Y chromosome microdeletions, but we need another larger study to confirm our results.

We interested in determining the frequency of Y chromosome microdeletions in our patients to know whether the frequency is high or low. If it is high, it will be mandatory to analyze for y chromosome microdeletions in our country, to give appropriate genetic counseling, because these deletions will be transmitted by ICSI to sons or may be expanded to larger area of deletion (Lee et al., 2006).
We observed through the study of clinical history of our patients that 6 (23%) patients had a history of cryptorchidism, which is a high frequency. So we need methods and ways to increase the awareness of this problem and its solutions in our country.

Finally, we recommend that, another larger study should follow our study to confirm or infirm the high frequency of Klinefelter syndrome and the absence of classical AZF deletions in south part of West Bank. We also recommend for that study to collect the sample retrospectively from one large IVF center, and should be large enough to draw the conclusions.

1.7 Statistical analysis:

We can consider our study as binomial experiment. Binomial distribution is the probability of a given number of successes (x) from a fixed number of independent trials (n). Given that the probability of success in an individual trial is P, the binomial probability is:

$$\Pr[X] = \binom{n}{X} p^X (1-p)^{n-X}$$

$$\binom{n}{X} = \frac{n!}{X!(n-X)!}$$

In our study, the number of independent trials were 27, the number of successes were 7. The incidence of cytogenetic abnormalities in the population of azoospermic and oligospermic men in most studies is around 10% (Romero Tovar et al., 2009; Shaqalaih, 2007), hence probability (P) is equal 0.1. Given these informations, we calculated the binomial probability of our study which was P (7) = 0.01

So, in other word, the probability of getting 7 positive patients from 27 samples by random is 1%.

If we need to test a hypothesis that: does our sample proportion differ from most studies proportion (P=0.1)?

We stated:

Ho: the proportion of cytogenetic abnormalities in our sample does not differ from most studies proportion.

H1: the proportion of cytogenetic abnormalities in our sample differs from most studies proportion.

The Test Statistic (TS) for a binomial distribution for the differences between our proportion of 7/27 ($\hat{p}$) versus the reported proportion in most studies (P_0) of 0.1 is calculated by:

$$TS = \frac{\hat{p} - p_0}{\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}}$$

In our study, TS= 0.259-0.1 / 0.08= 1.99, with associated P value of 0.0233 which is lower than $\alpha = 0.05$, so we reject the null hypothesis that our proportion of cytogenetic abnormalities is not differ from most studies proportion.

1.8 Concluding remarks

- Infertility is a big problem any where, and many studies should be done in our country to decrease the infertility and its causes.
- We have a high frequency (26%) of chromosomal aberrations in our sample of azoospermic and oligospermic infertile men.

- We did not detect any classical AZF deletions (AZFa, AZFb, AZFc) in our sample, which is consistent with Gaza study.
- We strongly recommend doing cytogenetic analysis before ICSI to give appropriate genetic counseling for infertile couple.
- Doing Y chromosome microdeletion analysis before ICSI as a routine test is questionable under the present results.
- There is a strong need for searching for the environmental pollutants that increase the rate of aneuploidy and chromosomal aberrations in our environment.
- We reported a rare case with 48,XXXY karyotype for the first time in Palestine.

Chapter two

Review of literature

2.1 Preface

I expanded in writing this chapter to make it as a reference for doctors of IVF, to be familiar with the genetic causes of male infertility.

Genetic causes of male infertility are divided into chromosomal abnormalities and single gene disorders. I talked about endocrinopathies of male infertility to relate the genetic disorders to clinical manifestation, specifically because endocrine tests are considered a very important diagnostic tool in the hands of urologist in IVF centers. Also, I expanded on the talk of chromosomal abnormalities in order to realize the importance of doing cytogenetic analysis before assisted reproductive techniques.

2.2 Infertility

INFERTILITY is the inability of a couple to conceive in 12 months of regular unprotected Intercourse (ASRM, 2008). The World Health Organization defines the lower reference limit for Time to Pregnancy (TTP) by 12 months. Infertility is divided into primary or secondary infertility. A couple with primary infertility has never been able to conceive while secondary infertility is defined as the inability to conceive or carry a pregnancy to term after successfully and naturally conceiving one or more children.

The global incidence of infertility is about 15% (Irvine, 1998). Infertility may be due to male factor, female factor or both. Yet, infertility is not individual, it is a couple based outcome, slight reductions in fertility potential in both partners can be magnified by their union as a couple. According to a large scale study done in France (Thonneau et al., 1991), infertility due a male factor alone accounted for 20 % of infertile couples , female factor accounted for 30%, both female and male factors accounted for 40% and unexplained infertility accounted for 10% .

2.3 Etiology of male infertility

There are many causes for male infertility like varicocele, obstruction of genitourinary tract, infection, cryptorchidism, ejaculatory dysfunction and genetic disorders.

Varicoceles are the most common cause of male infertility, its incidence among infertile men is about 40% (Birmingham 2008). They are defined as abnormally dilated scrotal veins, and are believed to negatively affect spermatogenesis by impaired drainage or pooling of blood around the testis leading to increased scrotal temperature (Mariotti et al., 2011).

Cryptorchidism and Endocrinopathies are two major causes of male infertility, and they are related in some aspects to genetic causes of infertility, so they are discussed bellow.

2.4 Cryptorchidism

Cryptorchidism refers to absence of one or both testes from the scrotum. Cryptorchidism is a very common anomaly of the male genitalia, affecting 2-4% of male infants (Cortes et al., 2008). But after 3 months the incidence decreases to 1-2% because of normal descent of the testes (Berkowitz et al., 1993).

Testicular descent in fetal life is believed to occur in two distinct phases; the trans-abdominal phase and the inguino-scrotal phase. The first occurs in 8-15 weeks of the fetus, and involves enlargement of the gubernaculum which is a cylindrical, gelatinous structure attached cranially from one end to the testis and epididymis and at its other end to the region of inguinal canal, and involves also regression of the mullerian ducts. Enlarged gubernaculum will anchor the testes in the inguinal region. This stage is controlled by insulin-like hormone 3 (Insl3), which is produced by Leydig cells in the testis, and Anti-Mullerian hormone (AMH) from Sertoli cells. Insl3 hormone acts through its receptor (LGR8) on gubernaculum to stimulate the caudal gubernaculum to grow and become thicker, in a process known the swelling reaction. Insl3 was discovered about 12 years ago when transgenic mice with a mutant Insl3 gene were found to have intra-abdominal cryptorchidism (Nef and Parada, 1999). The receptor LGR8 is called a GREAT receptor. Mutations in LGR8 in the mouse and humans caused high intra-abdominal cryptorchidism (Gorlov et al., 2002). Anti-Mullerian hormone (AMH) also stimulates the swelling reaction in the gubernaculum and regression of mullerian ducts. Children with AMH mutations are born with persisting Mullerian ducts and

intra-abdominal undescended testes but normal masculinization of the external genitalia (Imbeaud et al., 1994).

The second phase occurs between 25 and 35 weeks of gestation, and involves the migration of the gubernaculum and testis from the inguinal region to the scrotum. The process is regulated by Androgens. Calcitonin gene-related peptide (CGRP), a neurotransmitter released from the genitofemoral nerve, increases the cell proliferation at the gubernacular tip during migration into the scrotum, androgens are necessary to preprogramme the proliferative response of the gubernaculum to CGRP (Shenker et al., 2006). In animals and humans with complete androgen insensitivity syndrome (CAIS), the gubernaculum ends in the inguinal abdominal wall and no migration occurs (Hutson et al., 2010).

Any defect in hypothalamic pituitary testicular (H-P-T) axis can cause cryptorchidism, for example, disrupting of gonadotropin synthesis in Kallmann's syndrome has been associated with cryptorchidism. Also, cryptorchidism may be caused by mechanical defects. For example, presence of an inguinal hernia is frequently associated with undescended testes, and any abnormality that can cause the inguinal canal to be closed prior to testicular descent or fail to close after descent has the potential to cause cryptorchidism.

The descent of the testes is thus a complex process, involving the coordination of both hormonal and mechanical mechanisms; disturbances in any of these mechanisms have the potential to cause cryptorchidism.

The temperature of the abdomen is higher than the scrotum, so the germ cells in undescended testis will rapidly deteriorate if descent is not corrected by surgical operation (orchidopexy) in the first year. Deterioration of germ cells causes infertility in the future. In the case of unilateral cryptorchidism, the thermal injury in undescended testis will cause autoimmune reactions, and these reactions have the potential to affect the descended partner of undescended testes (Rapaport et al., 1969).

Cryptorchidism is a risk factor for testicular cancer, because the abnormal location exposes the cryptorchid testes to malignant transformation. 5% of testicular cancers are caused by cryptorchidism (Thorup et al., 2010).

2.5 Endocrinopathies of genetic origin

The hypothalamic–pituitary–testicular (HPT) axis is the descriptive term for an integrated mechanism by which the brain signals the male reproductive system to carry out its two main functions; androgen secretion and spermatogenesis (Harris et al., 2011).

At puberty, the receptor called G-protein-coupled receptor-54 (GPR54) in hypothalamus is stimulated by kisspeptin for reactivation of the secretion of gonadotropin-releasing hormone (GnRH) following the pre-pubertal quiescent phase (Jayasena et al., 2009). GnRH reaches the anterior pituitary, where it stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) into the systemic circulation. FSH stimulates spermatogenesis through testicular Sertoli cells, which also secrete a hormone down-regulating the release of FSH called inhibin B that acts at the level of the pituitary gland. LH stimulates testicular Leydig cells to release testosterone which is necessary for spermatogenesis.

Any problem in HPT axis will lead to infertility, and these are the most common endocrinopathies in infertile men:

2.5.1 Hypogonadotropic Hypogonadism (HH)

HH is a state of low androgen secretion as a result of absent or low gonadotropic stimulation, which may be either congenital or acquired. There are many causes for HH:

2.5.1.1 Failure of Hypothalamus

- Kallmann's syndrome, which results from a mutation in Kal1 gene on chromosome Xp22.3, which encodes anosmia protein (the protein responsible for migration of nerve cells that produce GnRH in hypothalamus) (Hardelin, 2001).
  Other autosomal genes can be mutated in this syndrome are: FGFR1 (MIM 136350) , PROK2 (MIM 607002) , PROKR2 (MIM 607123), CHD7 (MIM 608892) and FGF8 (MIM 600483). Patients are often present with anosmia (absence of smell) or hyposmia.
- Mutations of GnRH: One mutation was described in a Romanian brother and sister who had normosomic hypogonadotropic hypogonadism (Bouligand et al., 2009).
- Mutations of GnRH receptor: GnRH-R is a member of the G protein-coupled receptor, Located on the cell surface of pituitary gonadotropes, which modulates the

synthesis and secretion of LH and FSH. 14 different mutations were described in OMIM. A homozygous mutation (Gln106Arg) in the GnRH-R causes a *fertile eunuch syndrome* (Idiopathic hypogonadotropic hypogonadism, IHH with normal testicular size and some degree of spermatogenesis). This mutation decreases but not eliminates GnRH binding, so after treatment with coriogonadotropin CG, a reversible IHH can occur and normal pregnancy will result (Pitteloud et al., 2001).

2.5.1.2 Hyperprolactinemia

Presence of abnormally high levels of prolactin, which results in depression of GnRH and consequently depression in LH and FSH secretions leads to reduced levels of serum testosterone and impairment of spermatogenesis causing oligospermia and infertility. There are no reported mutations in prolactin, prolactin releasing hormone or prolactin releasing hormone receptor. Hyperprolactinemia may be a result of pituitary tumor, decreased dopamine secretion from hypothalamus (because of lesions and others), hypothyroidism, systemic disease or medications (anti-depression drugs). The most common phenotypes are: decreased libido, erectile dysfunction, gynecomastia and infertility (Harris et al., 2011).

2.5.1.3 Pituitary Insufficiency

Hypopituitarism in the form of partial or complete failure of the anterior pituitary can result in Hypogonadotropic hypogonadism. Hypopituitarism may be caused by brain surgery, brain tumor, head trauma, infections, stroke, radiations and subarachnoid hemorrhage. Occasionally, Hypopituitarism is due to: hemochromatosis, histiocytosis X and Lymphocytic hypophysitis (Bellastella et al., 2003).

Abnormalities of transcription factors required for ontogenesis of the pituitary gland can cause HH with or without deficiencies of other pituitary hormones. Pituitary transcription factors associated with combined pituitary hormone deficiency have been described and include the PROP1 (MIM 601538), HESX1 (MIM 601802), and LHX3 (MIM 600577) genes.

2.5.1.4 Isolated LH or FSH deficiencies

Isolated LH deficiencies are due to mutation in the gene of B chain of the hormone. If the mutation changes the protein and prevents it from binding with the receptor, the patient fails

to go through puberty. But, if the mutation lower the receptor-binding activity, a milder phenotype is result with a delayed puberty and oligoasthenozoospermia (Shiraishi and Naito, 2003). The drug of choice in these patients is HCG (human chorionic gonadotropin), which may correct infertility.

Isolated FSH deficiencies are due to mutation in the gene of B chain of the hormone or in regulatory genes of FSH activity (Murao et al., 2008). Patients have normal levels of LH but low levels of FSH. FSH levels do not respond to GnRH stimulation. Due to normal levels of LH, patients are normally virilized and have normal testicular size and testosterone. Because of FSH deficiency, patients have azoospermia to oligospermia. The drug of choice in these patients is human menopausal gonadotropin (hMG).

2.5.1.5 Congenital adrenal hyperplasia (CAH)

Is a group of autosomal recessive disorders resulting from defects in enzymes involved in cortisol synthesis (21-Hydroxylase deficiency accounts for 90% of cases, then, 11-b (beta) hydroxylase deficiency). This will lead to virilized male, with suppressed gonadotropins (FSH,LH) secondary to high levels of adrenal androgen, so spermatogenesis will not stimulated due to low level of FSH (Torresani and Biason-Lauber, 2007). Defects in enzymes that involved in both cortisol and androgen biosynthesis will cause a combination of male pseudohermaphroditism and hypoadrenalism.

2.5.1.6 Syndromes

These syndromes are associated with hypogonadotropic hypogonadism:

- Prader–Willi syndrome is caused by deletion in paternal chromosome 15q11-13. Necdin is a gene deleted in PWS, is highly expressed in mature hypothalamic neurons, it activates expression of gonadotropin-releasing hormone (GnRH) by binding to repressor Msx, it is also necessary for generation of the full complement of GnRH neurons during mouse development and extension of GnRH axons to the median eminence. So PWS is associated with hypogonadotropic hypogonadism (Miller et al., 2009).
- Familial cerebral ataxia (co-occurrence of cerebellar ataxia ;lack of coordination of muscle movements, and hypogonadism, unknown genetic causes (Seminara et al., 2002))

- Laurence–Moon–Biedl syndrome (mutations in BBS genes (14 different genes) lead to problems with the structure and function of cilia).

2.5.2 Hypergonadotropic Hypogonadism

Increase in FSH and LH levels due to primary testicular failure. The causes may be congenital or acquired; congenital causes include chromosomal abnormalities or genetic mutations in enzymes or receptors. Others are congenital multi-organ diseases with associated hypogonadism. The acquired causes include environmental or biological factors that disturb gonadal function at the level of the testes.

2.5.2.1 Chromosomal abnormalities that preset hypergonadotropic hypogonadism

- Klinefelter syndrome, 47,XXY or its variants; 48,XXXY, 48,XXYY, 49,XXXXY or mosaic 46,XY/ 47,XXY.
- 46,XX male.
- Mosaic 45,X/46,XY, mixed gonadal dysgenesis (chromosomal abnormalities will be discussed in details later).

2.5.2.2 Single gene disorders that present hypergonadotropic hypogonadism

2.5.2.2.1 LH and FSH resistance

LH resistance is caused by LH receptor mutations. LHR is G-protein coupled receptor with seven transmembrane domains (MIM152790). There are two ligands for binding the receptor: LH or CG (coriogonadotropin). Mutations in the receptor prevent ligand bindings, therefore, preventing activation cascade mediated by c-AMP. This will lead to **Leydig cell hypoplasia** (complete or relative absence of Leydig cells) in testes, hence, no androgen biosynthesis.

Complete loss-of-function mutations are characterized by male pseudohermaphroditism (female external genetalia with internal undescended testes, vas deference and epididymis), low testosterone, high LH, normal FSH in most cases, and abnormally elevated in a minority

of patients (Kremer et al., 1995). Mutations with a residual activity are characterized by micropenis, severe hypospadias, hypogonadism without sexual ambiguity, and isolated infertility.

FSH resistance is caused by FSH receptor mutations, FSHR is also G-protein coupled receptor with seven transmembrane domains (MIM 136435). Only one complete Loss-of-function mutation in the FSH receptor was described (Tapanainen et al., 1997), which may indicate that the resulting phenotype is subtle and escapes identification. In the study of Tapanainen et al, five men had a homozygous FSH mutation. They had normal testosterone levels, normal or slightly elevated LH levels, moderately elevated FSH levels, and slightly to markedly reduced testicular volume. They have variable degrees of spermatogenic failure, but, surprisingly, do not show azoospermia or absolute infertility; two of the men had successfully fathered two children each. So, FSH hormone is not essential to the onset of spermatogenesis at puberty, as believed, it may contribute to testicular size and quality of the ejaculate.

2.5.2.2.2 Androgen receptor defects (Androgen Insensitivity Syndrome)

Is a condition that results in the partial or complete inability of the cell to respond to androgens (will be discussed in details later in the most common monogenetic disorders).

2.5.2.2.3 Steroidogenesis defects

Cholesterol is the common precursor of all steroid hormones, including androgens (testosterone and 5α-dihydrotestosterone). Defects in the enzyme 7-dehydrocholesterol reductase (7-DHCR), the last enzyme in cholesterol biosynthesis steps, will lead to hypergonadotropic hypogonadism in a Smith-Limli-Opitz syndrome.

Testosterone is formed from cholesterol by five well-characterized enzymatic steps, with the assistance of StAR (steroidogenic acute regulatory protein), a transport protein which transfer cholesterol from the outer membrane of mitochondria to the inner membrane. Any defects in StAR and any enzyme in testosterone biosynthesis will lead to different degrees of male pseudohermaphroditism (presence of female external sex organs and male internal organs like testes). These enzymes are: cytochrome P450, 3β-hydroxysteroid dehydrogenase (3β-

HSD), 17α -hydroxylase (the same as 17,20-lyase) and 17β-hydroxysteroid dehydrogenase (17β-HSD).

2.5.2.2.4 Multi-organ diseases associated with hypergonadotropic hypogonadism

- Alstrom syndrome, a rare autosomal recessive disease caused by mutations in the gene ALMS1,characterized by childhood obesity, blindness due to congenital retinal dystrophy, and sensoneural hearing loss, hyperinsulinemia, early-onset type 2 diabetes, and hypertriglyceridemia.

- Myotonic dystrophy is an autosomal dominant disease characterized by a progressive wasting of muscles. MD1 is caused by an expanded CTG repeat in the 3′untranslated region of the dystrophia myotonica-protein kinase (DMPK) gene. MD2 is caused by an expanded CCTG repeats in intron 1 of the zinc finger protein 9 (ZNF9) gene (Schara and Schoser, 2006). Although these expansions are in untranslated regions, they alter RNA processing and splicing of other genes. For example, in both DM1 and DM2, altered splicing of chloride channel and insulin receptor transcripts leads to myotonia and insulin resistance, respectively (Day and Ranum, 2005). Hypergonadotropic hypogonadism occurs as a result of Testicular atrophy in 75% of affected males (Sobel and McGinley, 2004).

- Testicular regression syndrome (MIM 273250) is an XY gonadal dysgenesis syndrome. It is characterized primarily by the absence of gonads in an XY person. The male embryo should develop his testes within the first 8 weeks of gestation, if he fails to develop the testes, he will have female genetalia. If the testes are lost between 8 to 10 weeks, the child will have ambiguous genetalia (parts of both male and female genitals). However, if the testes are lost after the time when the male genitals differentiate (between 12 and 14 weeks), the baby will have normal male genitals (penis and scrotum), but no testes. The cause of disappearance of testes is unknown, but presence of multiple affected individuals in the same pedigree for relatives parents suggests a possible autosomal-recessive transmission (Linden and Overzier, 1956). Anorchid patients have high gonadotropins, low testosterone and undetectable level of anti-Mullerian hormone AMH.

Acquired causes of hypergonadotropic hypogonadism may include gonadal toxicity, in which the testes are damaged from chemotherapy or radiation, and viral orchitis

2.6 Chromosomal abnormalities that cause male infertility

Chromosomal abnormalities are divided into numeric disorders or structural aberrations.

Numeric disorders (aneuploidy): is the gain or loss of one or more chromosomes. These defects can occur throughout an individual's genome or only in specific tissues, a condition termed mosaicism.
Structural chromosomal disorders: can occur in single or multiple chromosomes.
Examples of defects that occur in a single chromosome are deletions, duplications, and inversions. When more than one chromosome is involved, a translocation can occur, in which genetic information is exchanged in either a balanced or unbalanced way between involved chromosomes.

2.6.1 Numeric disorders of male infertility

2.6.1.1 Klinefelter syndrome:

Klinefelter syndrome was first described in 1942 as an endocrine disorder characterized by small firm testes, gynecomastia, hypogonadism, and higher than normal concentrations of follicle-stimulating hormone (FSH). It is the most common chromosomal disorder associated with male infertility found in 1 every 600 live-born males (Nielsen and Wohlert, 1991). It is caused by at least one extra X chromosome in the male genome. About 80% of patients have the karyotype of 47,XXY, the other have the variants of: 48,XXXY; 48,XXYY; 49,XXXXY, 46,XY/47,XXY mosaicism, or structurally abnormal X chromosomes (Lanfranco et al., 2004). The prevalence of KS among infertile men ranges from 1.5% in oligospermic men to 13% in azoospermic men (Vincent et al., 2002).
The extra X in 47,XXY males is caused by non-disjunction either during meiotic divisions in germ cell development or mitotic cell divisions in early embryo (Fig 2.1). The supernumerary X in KS cases originates equally from paternal and from maternal non-

disjunction (Thomas and Hassold, 2003). Maternal non-disjunction can be caused during the first and second meiotic divisions or during early mitotic divisions in the developing zygote. Paternal non-disjunction arises in meiosis I, because paternal nondisjunction during meiosis II leads to XXX or XYY zygotes (Lanfranco et al., 2004). An association between increased maternal age and increased prevalence of KS was reported only in the cases of MI non-disjunction (Harvey et al., 1990; Lorda-Sanchez et al., 1992). An association between increased paternal age and increased prevalence of KS was suggested by some studies (Lorda-Sanchez et al., 1992) and rejected by others (Jacobs et al., 1988). Some studies attributed the increase in paternal non-disjunction in advanced age due to increase in sperm aneuploidies (Eskenazi et al., 2002).

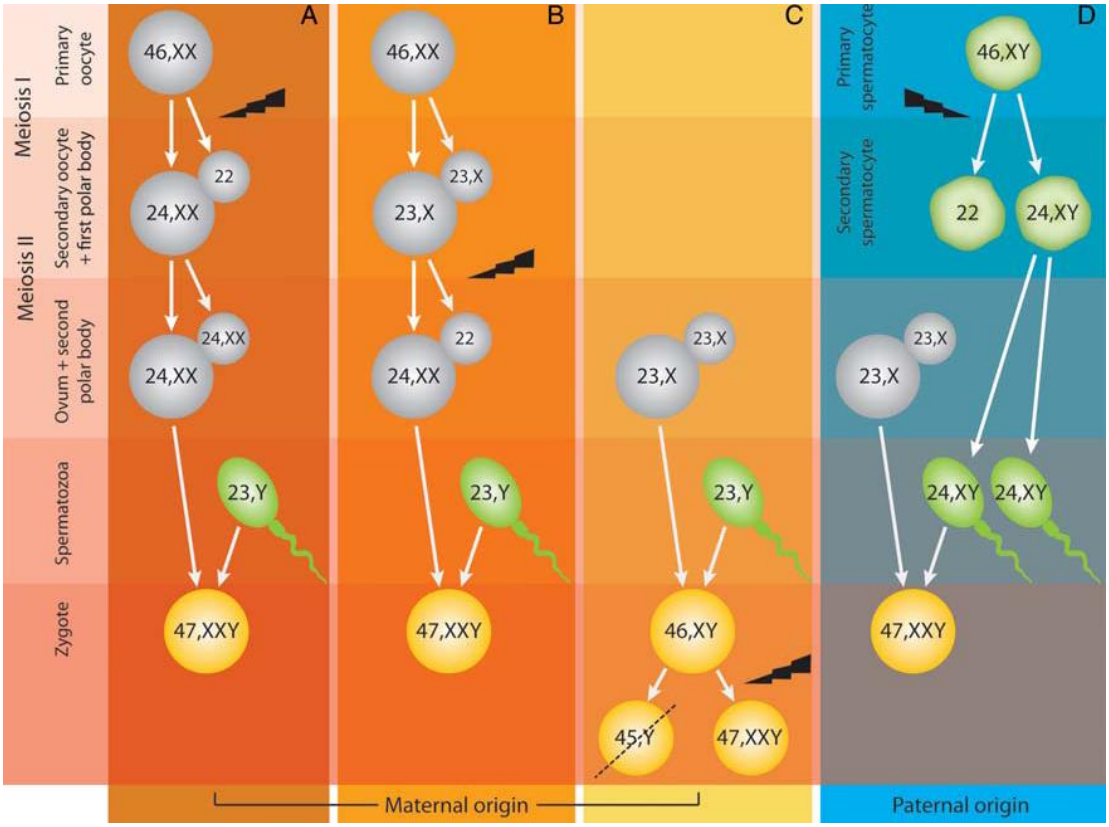

Fig 2.1: Parental origin of non-disjunction in KS. A: maternal MI, B: maternal MII, C: post-zygotic divisions, D: paternal MI. Adapted from (Tuttelmann and Gromoll, 2010).

The phenotype of KS varies considerably between patients of the same ages, and they do not have obvious facial dysmorphology. So the disorder is underdiagnosed; only approximately one-fourth of adult males with KS receive diagnoses, and fewer than 10% of the expected

number are diagnosed before puberty (Bojesen et al., 2003). The prenatal diagnosis of KS is very rare, because of weak /no association of maternal age with KS (Lanfranco et al., 2004). Most of 47,XXY neonates appear normal. During school-age childhood, the KS boy often presents with speech development delay, learning disabilities or behavioral problems. Child neurologists or child psychiatrists, with the aid of chromosomal study, often make the diagnosis. Many KS boys appear to enter the puberty normally, with a tendency for testosterone to decrease at late adolescence and early adulthood, which causes less muscle development, reduced facial and body hair, and in some cases, gynecomastia. Adult men have hypergonadotropic hypogonadism, azoospermia (very few are oligospermia).
Mosaic KS patients show very few clinical symptoms, they may have normal size testes. Endocrine abnormalities are also less severe, and gynecomastia and azoospermia are less common, they may have sperms in their ejaculates .

The wide variability in phenotypes of KS may be due to gene-dosage effects or the parental origin of the supernumerary X chromosome, or skewed X-chromosome inactivation. Genes in the two pseudoautosomal regions on X and Y (PARs, which recombine in meiosis) and an additional 15% of other genes escape inactivation. So, the KS phenotype may reflect increased gene dosage originating either from two active copies of these strictly X-linked genes or from three active copies of the X–Y homologous genes of the PAR. For example, the triplicate of SHOX gene (a growth-related gene) which is located on PAR1 is responsible for tall stature and long extremities in KS (Ottesen et al., 2010).
The supernumerary X chromosome is maternal in 50% of cases and paternal in 50%. Many studies analyzed parental origin with respect to KS phenotypes which may affect the phenotype by differential expression of paternal versus maternal alleles, i.e. imprinting. Some studies did not find any difference in phenotypes between maternal or paternal origin of supernumerary X (Zeger et al., 2008). Other studies such as (Stemkens et al., 2006) demonstrated a higher incidence of developmental problems in speech/language (88% versus 59%) and motor impairment (77% versus 46%) when the supernumerary X chromosome was paternally inherited. Another study found that KS boys with additional paternal X chromosome seem to have later onset and slower progression of puberty (Wikstrom et al., 2006). More studies with larger samples are needed to clarify the effect.
The androgen receptor gene on the X chromosome may play a particular role in differences in the KS phenotype. The AR contains a highly polymorphic trinucleotide repeat (CAG)n in

exon1 with the normal length varying between 9 and 36/37 repeats. The length is inversely associated with androgen action. In Klinefelter syndrome, a positive correlation exists between (CAG)n length and body height and presence of gynecomastia, but an inverse association exists with bone density, social status, testicular volume, and response to androgen substitution (Zitzmann et al., 2004). Other study described an inverse relationship of (CAG)n and penile length (Zinn et al., 2005). X-inactivation is assumed to occur randomly, but with analyzing x-inactivation in KS, skewed inactivation was detected with a preferential to inactivate the shorter allele which would magnify the impact of the (CAG)n length (Zitzmann et al., 2004).

2.6.1.1.1 Fertility in KS

Histomorphometric and immunohistochemical analysis reveal that in early adolescence the majority of KS boys have germ cells in their testes. But, depletion of these cells is accelerated with the activity of pituitary-gonadal axis at the onset of puberty (Wikstrom et al., 2007). These germ cells at least partially are arrested at the spermatogonium or early primary spermatocyte stage. It seems that spermatogonia have difficulty entering meiosis; instead they proceed at onset of puberty to apoptosis (Wikstrom et al., 2007). This is one explanation for germ cell degeneration, the other explanation is the disturbed testicular environment involving Sertoli and Leydig cells don't support normal germ-cell development (Aksglaede et al., 2006). Hunt et al showed that prenatal germ-cell proliferation is impaired in vivo, but not in vitro, which suggests a communication defect between Sertoli cells and germ cells in the differentiating XXY testis (Hunt et al., 1998).

Histology of the testes in the adult KS patient is characterized by extensive fibrosis and hyalinization of the seminiferous tubules, absence of spermatogenesis, and hyperplasia of Leydig cells and interstitium (Gordon et al., 1972). However, the presence of tubules with residual foci of spermatogenesis has also been reported (Warburg, 1963). So, ejaculates from most KS patients have no sperms, few patients may have few motile sperms (Foss and Lewis, 1971). The study done by Lanfranco et al. showed that from189 patients with KS who were asked to provide a semen sample at the time of the first visit, 131 (69·3 %) were able to or agreed to provide an ejaculate. Spermatozoa were observed in only 11 (8·4 %) of these men (Lanfranco et al., 2004). In very exceptional cases, 2 KS patients

were able to carry spontaneous pregnancies and had 1 child for each (with proved paternity) (Laron et al., 1982; Terzoli et al., 1992).

With the presence of foci of spermatogenesis, a common question comes in consideration: whether 47,XXY spermatogonia are able to complete meiosis or, in contrast, some spermatogonia lose the supernumerary X chromosome becoming normal 46,XY cells and then proceed through meiosis?

Foresta postulated that 47,XXY spermatogonia are able to undergo and complete the spermatogenic process leading to mature spermatozoa (Foresta, 1999). He analyzed sex chromosome aneuploidy in sperms from oligospermic KS patients, he reported 20% of sex chromosome aneuploidy and a 2:1 bias toward X-bearing sperm (Foresta et al., 1998).

Gonsalves et al reported that XXY germ cells can proceed at least partway through meiosis, because 47 out of 100 pachytene cells had an XXY sex chromosome constitution (Gonsalves et al., 2005). On the other hand, Sciurano et al showed by FISH analysis that meiotic spermatocytes were euploid 46,XY (Sciurano et al., 2009). Also Bergere et al arrived for the same result (Bergere et al., 2002).

Hall et. al hypothesized that If 47,XXY germ cells are able to complete meiosis, a high incidence (50%) of sperms aneuploidy will result. However, several FISH studies of sperm aneuploidy in KS indicated only modest — albeit significant — increases in both sex chromosomal and autosomal disomy. And, ICSI-children from KS fathers are almost always chromosomally normal, with as many 46,XY as 46,XX children. While, if 47,XXY germ cells are meiotically competent, this will yield a high percentage of 47,XXY and 47,XXX offspring with female to male percent of 2 to 1 (Hall et al., 2006).

Studies on XXY mice showed that presence of XXY germ cells is incompatible with life, and these cells are eliminated within several days after birth (Mroz et al., 1999a). The testes of adult mice may show a foci of spermatogenesis, but these foci composed of XY germ cells as showed by studies on these germ cells (Mroz et al., 1999a). Aneuploidy studies on the mice sperms indicate a 1% rate of disomy, which is significantly increased in comparison with controls (Mroz et al., 1999b). Because of non existence of XXY germ cells, they hypothesized that the increase in disomy rate is attributed to meiotic nondisjunction in XY cells due to compromised testicular environment.

2.6.1.1.2 Assisted Reproduction in KS

Intracytoplasmic sperm injection (ICSI) offers the hope for KS patients for reproduction, because the technique needs only few sperms from ejaculate or from testes by testicular sperm extraction (TESE). Many live-births were reported using ICSI and TESA from non-mosaic KS (Aboulfotouh et al., 2011; Nodar et al., 1999; Palermo et al., 1998; Poulakis et al., 2001; Reubinoff et al., 1998; Ron-el et al., 1999; Ron-El et al., 2000a; Rosenlund et al., 2002; Yarali and Bozdag, 2006). A Birth of a healthy infant after ICSI and cryopreserved testicular spermatozoa from non-mosaic KS also was reported (Greco et al., 2008).
Few live-births were reported using ejaculated sperms from non-mosaic KS and ICSI (Bourne et al., 1997; Hinney et al., 1997). Although the fertilization rate from ejaculated sperms is high, the live-birth rate is low, which suggest an increased of chromosomal abnormalities in ejaculated sperms in comparison with testicular sperms (Kitamura et al., 2000).
The recovery rate of sperms using testicular sperm extraction was reported around 50%, and there are currently no clinical parameters predicting successful sperm retrieval in non-mosaic KS patients (Vernaeve et al., 2004). Younger KS men have higher opportunity for sperm retrieval than older men (Bakircioglu et al., 2006). A new technique, microdissection TESE , has shown significantly better sperm recovery rates compared to conventional TESE, sperm recovery rates may reach as high as 70% (Schiff et al., 2005). The live-birth rate by ICSI was reported 20% by (Staessen et al., 2003).

FISH studies of chromosomal aneuploidy on sperms from KS patients showed a modest but significant increase in sex chromosomal hyperploidy (Blanco et al., 2001; Foresta et al., 1998). The incidence of sex-chromosomal hyperploidy varied from 0.9% to 2.5% in the mosaic KS and from 2.5% to 21.6% in the non-mosaic KS (Lanfranco et al., 2004).
Also, some studies showed an increase in autosomal chromosomal hyperploidy; Hennebicq et al found a much higher frequency of disomy 21 in the spermatozoa of non mosaic KS patients than in controls (6.2 vs. 0.4%) (Hennebicq et al., 2001). Morel et al found an increase incidence of autosomal aneuploidies for chromosomes 13, 18, and 21 in the sperms from non-mosaic KS patients than in the general population, the same as increase found in sperms from 46,XY males with oligozoospermia or oligoasthenoteratozoospermia (Morel et

al., 2003). PGD which is applied by taking an embryo biopsy for diagnosis, also confirmed the increase in sex and autosomal chromosomal abnormalities (Staessen et al., 2003).

There are more than 100 children born after ICSI with sperms from KS patients (Fullerton et al., 2010). Most of the reported infants have normal karyotype, there was 1 report of conceiving 47,XXY fetus, but it was reduced on the 14th gestational week (Ron-El et al., 2000b). Birth of healthy children, although the increase of aneuploidy rate in sperms of KS may be explained by doing PGD and implantation of normal embryos only with ICSI for KS by most laboratories because they know the high risk of chromosomal abnormalities. Or it seems that laboratories prefer to report the birth cases, not the miscarriaged cases, because it will be embarrassing for them to do ICSI for KS without doing PGD.

So, because of increase of sperm hyperploidy, there is a higher risk of fathering 47,XXY or 47,XXX child after successful ICSI. PGD or prenatal diagnosis should be considered before doing ICSI in KS patients. Because of our culture rejects the termination of pregnancy, PGD is the method of choice to prevent implantation of abnormal embryos.

Another issue related to KS patients is cryopreservation of semen sample from prepubertal KS boys, since degeneration of seminiferous tubules in KS seems to accelerate with puberty, but one should take into account the limited ability of prepubertal boys to give semen sample. So, testicular biopsy can be obtained and cryopresereved to derive gametes for fertilization in the future by in vitro maturation (including meiosis), which at present, however, remains entirely experimental. Early results suggest that meiosis and spermatogenesis may resume under culture conditions, yielding normal spermatids with some fertilization potential (Sousa et al., 2002).

Since the testosterone level decreases in mid-puberty, lifelong substitution therapy of testosterone is indicated to prevent symptoms and consequences of androgen deficiency, and subsequently to improve quality of life. But the sperm retrieval rate appeared to be lower in KS men who previously received exogenous testosterone, so it is beneficial to take semen sample or testicular biopsy before start of testosterone substitution (Wikström and Dunkel, 2011).

2.6.1.1.3 Variants of KS

As a general rule, the more X chromosomes, the more severe the somatic manifestations of Klinefelter syndrome. Each X reduces the overall IQ (Intelligence Quotient test) by 15–16 points, with language most affected (Linden et al., 1995).

- **48,XXXY**

48,xxxy was described in 1959 (Barr et al., 1959) with a reported incidence of 1 per 17,000 to 1 per 50,000 male births (Venkateshwari et al., 2010).

Patients have mild facial dysmorphisms: epicanthal folds (a skin fold of the upper eyelid, covering the inner corner of the eye), Hypertelorism (increased distance between eyes), flat nasal bridge, protruding lips, prominent mandible (lower jaw bone). 48,XXXY males are average or tall stature and have radioulnar synostosis (abnormal connection between the two bones of the forearm: the radius and ulna) and clinodactyly (curvature of the fifth finger). They have hypergonadotropic hypogonadism, azoospermia and infertility. 25% of patients have hypoplastic penis and gynecomastia is common (Cynthia 2005). 48,XXXY males have mild to moderate mental retardation, their IQ occurs mostly between 40–60, however, patient with an IQ of 79 has been reported. Most have speech delay, slow motor development, and poor coordination (Cynthia, 2005). Their behavior is often immature and consistent with IQ level, they are typically described as passive, cooperative and not particularly aggressive (Visootsak et al., 2007).

The mild mental retardation could be due to the gene dosage effect of non contiguous genes present on the X chromosome and their over-expression, which may lead to disruption of genes involved in specific aspects of brain development (Venkateshwari et al., 2010).

The origin of extra Xs is a successive non-disjunction at the first and second paternal or maternal meiotic divisions. In most cases a single parent contributed all of the additional sex chromosomes, with the other parent contributing a single X or Y chromosome (Pfeiffer and Sanger, 1973).

- **48, XXYY**

Its incidence is estimated at 1 in 50,000 male births. Men are tall with long thin legs, they have eunuchoid body habitus (resemble female) and sparse body hair. Men have hypergonadotropic hypogonadism, small testes, and frequently Gynecomastia (Cynthia 2005).Their IQ ranges from 60 to 80, with delayed speech. They tend to be shy but can be

aggressive and impulsive (Visootsak and Graham 2003). The cause of 48,XXYY is successive non-disjunction in both the first and second male meiotic divisions leading to an XYY sperm. It seems that the incidence of this aneuploidy increases with the age of male (Zhang and Li, 2009).

- **49,XXXXY**

It was first described by (Fraccaro et al., 1960). The incidence is estimated to be approximately 1 in 85,000 newborn males (Kleczkowska et al., 1988). The affected males have craniofacial features consisting of round face in infancy, coarsening of features in older age, ocular hypertelorism, epicanthal folds, and prognathism (an individual's top teeth do not align with the lower teeth), short broad neck and short stature in some patients. 15–20% of patients have congenital heart defects. Skeletal anomalies include radioulnar synostosis, genu valgus (the knees angle in and touch one another when the legs are straightened), pes cavus (the sole of the foot is distinctly hollow), and fifth finger clinodactyly. In addition, they may have Muscular hypotonia and hyperextensible joints. Patients have hypergonadotropic hypogonadism, hypoplastic genetalia, and cryptorchidism (Cynthia 2005) .
Their IQ ranges from 20 to 60, with severely Language impairment. They tend to be shy and friendly, with occasional irritability and temper tantrums (an emotional outburst), low frustration tolerance and difficulty changing routine (Visootsak and Graham, 2003).
The origin of extra Xs is successive non-disjunction of M1 and M2 in oogenesis (Deng et al., 1991).

- **49,XXXYY**

Few cases were reported (Bray and Sister, 1963; Cowie et al., 1986; Lecluse-van der Bilt et al., 1974; Salamanca-Gomez et al., 1981). Patients have tall stature, dysmorphic facial features, gynecomastia, and hypogonadism. They have moderate to severe mental retardation, with passive but occasionally aggressive behavior and temper tantrums (Cynthia 2005) .

2.6.1.1.4 Diagnosis of KS

The gold standard for diagnosis is karyotyping (study of chromosomes at metaphases from cultured peripheral blood lymphocytes), but it is time-consuming. Analysis of buccal smear to detect Barr bodies is easy method , but does not reach adequate sensitivity to serve for

screening (Pena and Sturzeneker, 2003). FISH (florescence in situ hybridization) has more specificity and sensitivity than karyotype, but FISH requires expensive probes, experienced technologists and imaging software. Quantitative PCR (polymerase chain reaction) which is based on copy number assessment of androgen gene located on X chromosome was found to be easy and sensitive screening method for KS. Q-PCR based on AR gene is more sensitive than Q-PCR based on XIST gene (Ottesen et al., 2007). Array comparative genomic hybridization (array CGH) was also used in diagnosis of KS, the main advantage of this method is the higher resolution of up to or even below 1 kb of altered DNA (Ballif et al., 2006).

2.6.1.2 Other numerical chromosomal abnormalities with infertility issues

2.6.1.2.1 Chromosomal abnormalities with extra Y:

- **47,XYY**

It's incidence is 1 in 1000 males (Nielsen and Wohlert, 1991). It arises from non-disjunction through paternal meiosis II or rarely from post zygotic mitosis. The clinical features are subtle and variable among individuals; they tend to be tall and thin, have an increased risk of learning difficulties and delayed speech and language skills. Most males have normal sexual development and are able to father children, but infertile men with oligospermia were reported (Abdel-Razic et al., 2011; Murakami et al., 1997), and few were azoospermia (Murakami, Baba et al. 1997). 47,XYY males showed a mild significant increase of sex chromosomal aneuploidies but not autosomal aneuploidies in their sperms (Shi and Martin, 2000).

- **48,XYYY**

It is rare; few cases were reported (Hori et al., 1988; Schoepflin and Centerwall, 1972). The origin of 3 Y in the sperm is successive nondisjunction; first in mitosis of spermatocyte, then in meiosis I, then in meiosis II. Affected individuals are tall, have mild mental retardation to low normal intelligence and have azoospermia (Hunter and Quaife, 1973).

2.6.1.2.2 Mosaic 45,X/46,XY

Is associated with a broad spectrum of phenotypes (depending on the percent of 45,X cell line) including:

- Female with gonadal dysgenesis.
- Male with mixed gonadal dysgenesis which characterized by unilateral testis, usually intra-abdominal, a streak gonad on contralateral side, and persistent mullerian structures.
- Male pseudohermaphroditism: the condition in which the gonads are exclusively testes, but the genital ducts and/or external genitalia are incompletely masculinized; the phallus is either very small or there is severe hypospadias; the testes may not have descended in the scrotum.
- Apparently normal male: most commonly with infertility.

One study of 92 prenatally diagnosed cases of 45,X / 46,XY found that 95% had normal male genitalia, from 11 male external genetalia they found 3 (27%) had abnormal gonadal histology (Chang et al., 1990).

Microdeletions of the long arm of the Y chromosome may be associated with Y chromosomal instability, leading to formation of 45,X cell lines (Papadimas et al., 2001). Analysis of sperms from mosaic 45,X / 46,XY showed a significantly higher aneuploidy than control samples but comparable to the rates observed in ICSI candidates with 46,XY oligoasthenoteratozoospermia (OAT) (Giltay et al., 2000). ICSI is possible in azoospermic and oligospermic 45,X / 46,XY men, but PGD should be offered for infertile couple (Kilic et al., 2010).

2.6.1.2.3 46,XX male

Is a rare sex reversal syndrome affecting 1 in 20,000 newborn males (Vilain, 1993). It has a wide spectrum of clinical manifestation:

- 80% have normal male phenotypes, 2 testicles but small, normal penile size, gynecomastia, and sterility resulting from azoospermia.
- 20% have true hermaphroditism with external genital ambiguity, and internal one ovary on one side and a testis on the other, or more commonly one or both gonads is an ovotestis containing both types of tissue (Vilain, 1993).

The causes of XX male syndrome are: (1) translocation of SRY gene (sex-determining region on Y) to X chromosome or autosome (Pepene et al., 2008; Wang et al., 2009), (2) a mutation in X-linked or autosomal gene in the testis-determining pathway (Ergun-Longmire et al., 2005) ; for example: mutations that lead to over expression of SOX3, SOX10 or SOX9 genes lead to XX male in absence of SRY (Polanco et al., 2010; Sutton et al., 2011; Vetro et al., 2011), (3) presence of low-level hidden mosaicism for Y-chromosome derived sequences (Queipo et al., 2002), for example, gonadal SRY mosaicism in which SRY is negative in peripheral blood leukocytes, but positive in gonadal tissue (Hadjiathanasiou et al., 1994).

90% of patients have translocation of SRY on X chromosome. Most of SRY-positive have normal male external genitalia; but, some patients may have ambiguous genitalia and evidence of true hermaphroditism (Grigorescu-Sido et al., 2005). This variability could be the result of differential inactivation of the X chromosome carrying SRY (Kusz et al., 1999).

2.6.1.2.4 46,XX/46,XY

Individuals with 46,XX/46,XY chimerism may present as true hermaphrodites depending on the relative ratio of XX and XY cells; phenotypes may vary from normal male to normal female.

2.6.2 Structural chromosomal disorders leading to male infertility

2.6.2.1 Structural abnormalities of autosome

2.6.2.1.1 Robertsonian translocations

Robertsonian translocations occur when two acrocentric chromosomes (13, 14, 15, 21, 22) fuse together, resulting in a single chromosome with long arms of them and subsequent loss of their short arms. The percentage of Robertsonian translocations carriers in infertile male is about 0.8%, which is up to nine times higher than in the general population (Ferlin et al., 2006). Balanced translocation carriers have only 45 chromosomes, they generally have normal phenotypes, but the translocation can affect fertility and pregnancy outcome. Fertility is affected because presence of 45 chromosomes will lead to disturbance of the meiotic process resulting in various degrees of spermatogenic defects. Anton et al hypothesized that any factor that delays anaphase, such as erratic separation of chromosomes, may arrest

division leading to cell apoptosis (Anton et al., 2004). Pregnancy loss may occur because of production of unbalanced gametes. Studies of the segregation process in Robertsonian carriers showed an increase in unbalanced sperms. One study showed a frequency of unbalanced sperms resulting from adjacent segregation varied between 11.70 and 19.53% and they observed higher frequencies of aneuploidy for sex chromosomes. Furthermore, they observed an interchromosomal effect (sperm aneuploidy not only for the chromosomes involved in the translocation, but also for other chromosomes) (Chen et al., 2007).

So it is very important for infertile couples undergoing ICSI to do cytogenetic analysis, to prevent transmitting of rearrangements in resulting embryos by doing PGD.

2.6.2.1.2 Reciprocal translocations

They consist of a mutual exchange of chromosomal segments between two chromosomes. Its incidence among azoospermic and oligospermic men is seven times more elevated than in newborn series (Ferlin et al., 2006). As Robertsonian translocations, carries of reciprocal translocations are phenotypically normal, but these translocations affect the fertility by altering the spermatogenic process and affect pregnancy outcome. As a general rule reciprocal translocation carriers produce more unbalanced sperm than normal or balanced sperm (Mardesic et al., 2011). Studies also showed a high diploidy rate and disomy for sex chromosomes (Moretti et al., 2009). The proportion of unbalanced forms depends on the characteristics of the rearrangements and it varies widely (from 23% to 81%) (Ferlin et al., 2006).

2.6.2.2 Structural abnormalities of X chromosome leading to male infertility

Males with balanced X; autosome translocations are usually phenotypically normal but almost all are infertile (Ma et al., 2003), in this study, the father had translocation between X and chromosome 20, he had severe oligospermia, the couple underwent ICSI, they conceived a female child, but post natal analysis revealed that the child had the same translocation as her father.

Patients with deletions of X-short arm show contiguous gene syndrome which is characterized by various phenotypes, depending on the length of the deletion. Deletions may cause Kallmann syndrome (See hypogonadotropic hypogonadism).

2.6.2.3 Structural abnormalities of Y chromosome

Individuals with deletions of the short arm of the Y involving band p11.3, the location of *SRY* gene, are usually phenotypic females. Most have streak gonads with Turner syndrome features (Hsu, 1994).

2.6.2.3.1 Y Isochromosomes

Isochromosome is a chromosome that has lost one of its arms and replaced it with an exact copy of the other arm. Y isochromosome is divided into: monocentric isochromosome i(Y) or dicentric isochromosome idic(Y). Idic(Y) is more common than i(Yp) or i(Yq).
Dicentric Y Isochromosomes are the most common Y structural abnormalities (Abdelmoula and Amouri, 2005). Isochromosomes Y mostly are found in mosaic form with another cell line, most often is 45,X, and less often is 46,XY. The presence of a 45,X cell line in addition to isochromosome Y or isodicentric Y leads to many phenotypic features, ranging from male with azoospermia to ambiguous genitalia to females with typical or atypical Turner syndrome (Hsu, 1994). The phenotype of individual with i(Yq) is female, because of the absence of SRY. Males with 46,X, dic(Y p or q) may have azoospermia due to disturbance of spermatogenesis because the presence of two centromeres which disturb the normal segregation of chromosomes in meiosis (Yoshida et al., 1997). Cytogenetic analysis revealing isodicentric Y gives a prognosis of increased risk of gonadoplastoma in males.

2.6.2.3.2 Ring Y chromosome:

Its incidence is 1 every 25,000 newborns; 99% arise de novo while less than 1% of rings are inherited. It may arise through a cytogenetic mechanism involving breaks in chromosome arms and fusion of the proximal broken ends, leading to a loss of distal material. Y ring chromosomes 46,X,r(Y) are present in mosaic karyotype with a 45,X cell line. The clinical spectrum in patients is broad and depends on the percentage of the 45,X cell line in different tissues (Lopez-Valdes et al., 2009). Patients with 45,X/46,X(r)Y karyotype and bilaterally

descended testes varies greatly from males with short stature and azoospermia to males without short stature and oligospermia (Layman et al., 2009). Patients with ring chromosomes can undergo ICSI for reproduction, but PGD should be done before transferring embryos. Transmission of ring y from father to embryo with ICSI was reported (Bofinger et al., 1999; Spinner et al., 2008). These studies strongly recommend the genetic screening before ICSI.

Deletions of heterochromatic region of Yq don't affect normal genital development and sexual differentiation. Deletions involving the euchromatic region of Yq could cause azoospermia.
Microdeletion of a portion of euchromatic region of Y could occur, but it is not large enough to be detected using conventional cytogenetic methods, it is detected by molecular analysis by PCR.

2.6.3 Y chromosome microdeletions and male infertility:

After the Klinefelter syndrome, Y chromosomal microdeletions are the second most frequent genetic cause of male infertility. The prevalence of Y chromosome microdeletions ranges from 4% in oligospermic men to 18% in idiopathic azoospermic men (Foresta et al., 2001).

2.6.3.1 Molecular pathology of Y chromosome:

Y chromosome contains male-specific region of the Y (MSY) and two pseudoautosomal regions (PAR1 and PAR2). MSY does not recombine with the X chromosome. PAR1and PAR2 undergo meiotic exchange with the X chromosome. The MSY contains heterochromatic sequences and three classes of euchromatic sequences: X-transposed (with 99% identity to the X chromosome) , X-degenerate (single-copy genes or pseudogenes homologues of X-linked genes) and ampliconic sequences. The ampliconic sequences which are highly repetitive sequences, composed of eight large palindromes P 1-8 (inverted repeats with very little intervening sequence) (Figure 2.1). A homologous recombination between these identical repeats will cause a deletion and loss of genetic materials between repeats. And this is the mechanism by which microdeletions of Y occur (Noordam and Repping, 2006).

2.6.3.2 Y chromosome microdeletions; AZFa, AZFb, AZFc

Tiepolo and Zufardi in 1976 found a deletion of distal portion of band q11 of the long arm of the Y chromosome in 6 azoospermic men. They suggested that this portion contains factors controlling spermatogenesis (Tiepolo and Zuffardi, 1976). They termed this region the azoospermia factor (AZF). In 1996 Vogt et al subgrouped AZF region into 3 regions; AZFa, AZFb and AZFc (Vogt et al., 1996). After the sequence of the MSY was clarified, a new model of deletions was defined by Repping et al, in which the AZFb and AZFc regions are overlapping. In addition, the AZFb and AZFbc deletions have been suggested to be the consequence of at least three different deletions patterns (Repping et al., 2002).

Nomenclatures of deletions are:

• AZFa

• AZFb (P5/proximal P1),

• AZFbc (P5/distal P1 or P4/distal P1),

• AZFc (b2/b4) (See Fig 2.2).

The AZFa region is about 1100 kb long and contains three single copy genes DFFRY (or USP9Y), DBY and UTY. The complete deletion of AZFb removes 6.2 Mb (including 32 copies of genes and transcription units) and results from homologous recombination between the palindromes P5/proximal P1(Simoni et al., 2004). The candidate gene of AZFb is RBMY1, it has a possible role in RNA processing or translational control during early spermatogenesis. The complete deletion of AZFc (b2/b4) is the most common deletion among other deletions and represents approximately 80% of Y chromosomal microdeletions. It removes 3.5 Mb originates from the homologous recombination between amplicons b2 and b4 in palindromes P3 and P1 respectively, and removes 21 copies of genes and transcription units (Kuroda-Kawaguchi et al., 2001). Deletions of both AZFb and AZFc together occur by two major mechanisms involving homologous recombination between P5/distal P1 (7.7 Mb and 42 copies removed) or between P4/distal P1 (7.0 Mb, 38 copies removed) (Simoni et al., 2004).

Within AZFc region, there is the major AZFc-candidate gene called DAZ gene (deleted in azoospermia), there are 4 copies of DAZ gene, which encodes an RNA-binding protein with a role in spermatogenesis. They exist in two clusters, each comprising an inverted pair of DAZ genes (3' <-- 5'::5' --> 3') (Saxena et al., 2000) (Fig 2.3). DAZ gene has a homolog gene on chromosome 3 called DAZL. Another functional gene in AZFc is CDY1 that encodes a protein with proven histone acetyltransferase activity (Lahn et al., 2002).

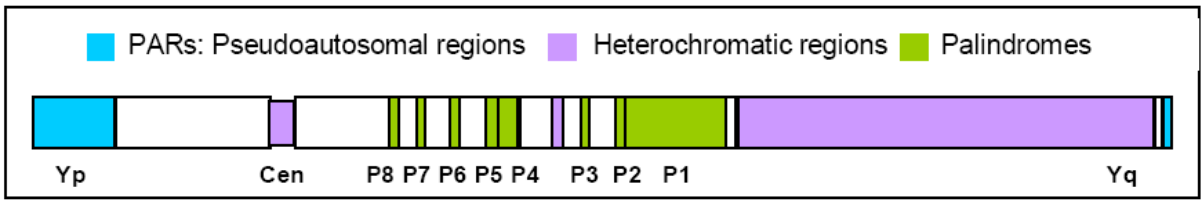

Figure 2.2: The map of Y chromosome palindromes. Adapted from (Shaqalaih, 2007).

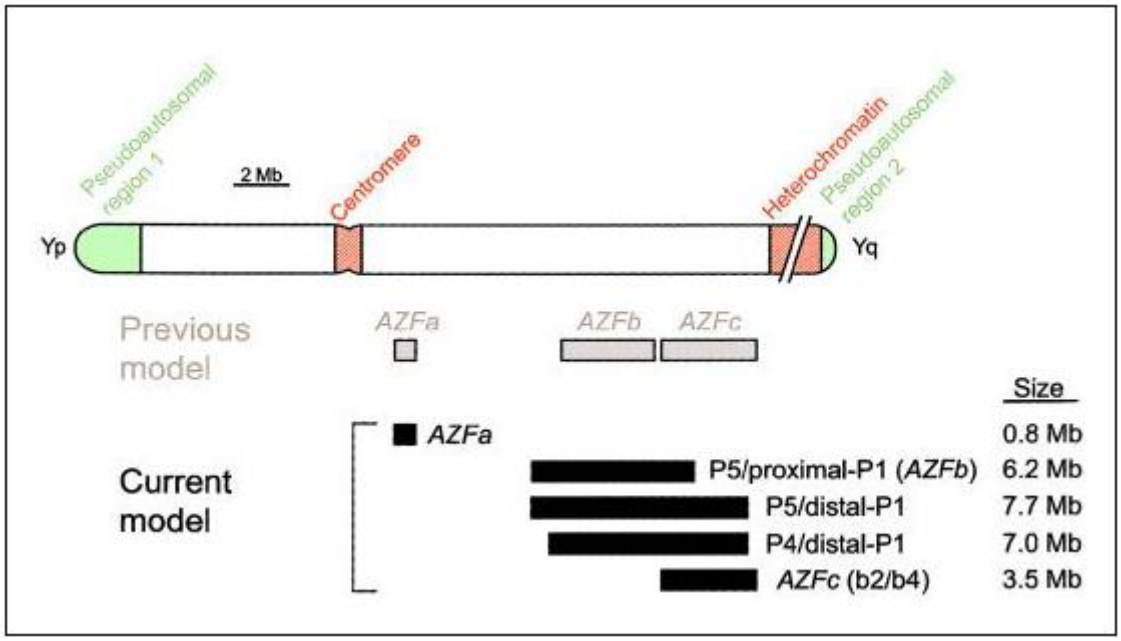

Figure 2.3: Human Y chromosome and previous and current AZF deletions models. The green areas of Y chromosome represent Pseudoautosomal region1 and region2 (PAR1, PAR2). Under Y chromosome, the previous model of deletions mapped by (Vogt et al., 1996) and the current model mapped by (Repping et al., 2002). Adapted from (Repping et al., 2002).

2.6.3.3 Partial deletions of AZFc

2.6.3.3.1 gr/gr deletion

It results from homologous recombination between amplicons g1/r1/r2 and g2/r3/r4 (Figure 2.3). It removes 1.6 Mb of the AZFc region including two copies DAZ gene, but does not remove an entire AZFc gene-family; it reduces the copy number of them. There are subtypes of deletion pattern: DAZ1/DAZ2+CDY1a, DAZ3/DAZ4+CDY1a, and

DAZ3/DAZ4+CDY1b (Giachini et al., 2005). Some men with gr/gr deletions may undergo duplications, which seem to restore gene copy number (Repping et al., 2003).

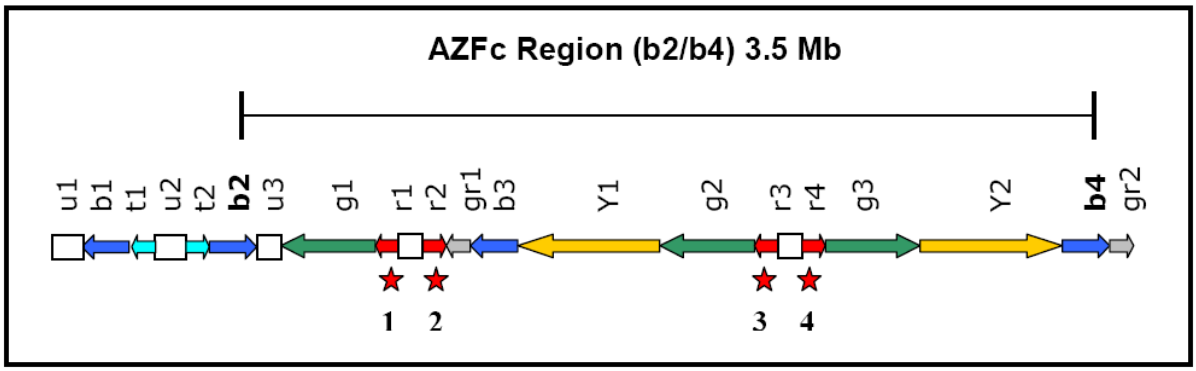

Figure2.3: Ampliconic sequences in AZFc region. It is drawn according to the color code of Kuroda-Kawaguchi et al., 2001. The four copies of DAZ gene are presented with red stars, they exist in two clusters, each comprising an inverted pair of DAZ gene. Adapted from (Shaqalaih, 2007).

2.6.3.3.2 b2/b3

It is called also u3-gr/gr or g1/g3. It is explained by occurring of inversion in AZFc region, then, occurring of deletion (Fig.2.4). It removes 1.8 Mb of AZFc including 12 testes-specific genes or transcripts. These deletions are present almost exclusively in branch N of the Y-chromosome tree (tree constructed on the basis of Y-chromosomal single nucleotide polymorphisms SNPs), which indicates that the founder of branch N was b2/b3 deleted (Repping et al., 2004). Some men with b2/b3 deletions may undergo subsequent duplications, which restore gene copy number.

2.6.3.3.3 b1/b3

It is similar to the gr/gr deletion (1.6 Mb), but it affects a more proximal part of AZFc (Fig.2.5). Its prevalence in the human population is low (Noordam and Repping, 2006).

2.6.3.4 Genotype/phenotype correlation in AZFa, AZFb, AZFc

Deletions of the entire AZFa region cause a histological picture of complete Sertoli cell only (SCO) syndrome in the testes and azoospermia (Kamp et al., 2001). The diagnosis of a complete deletion of the AZFa region implies the virtual impossibility to retrieve testicular sperm for intracytoplasmic sperm injection (ICSI) (Hopps et al., 2003). Complete deletions of AZFb and AZFb+c (P5/proximal P1, P5/distal P1, and P4/distal P1) cause a histological

picture of SCO or spermatogenetic arrest resulting in azoospermia. In the case of diagnosis of complete AZFb deletion, the probability of finding mature spermatozoa is virtually nil. But, partial AZFb deletions are associated with sperm retrieval in approximately 50% (Krausz et al., 2000). Complete deletion of the AZFc region (b2/b4) can cause different degrees of spermatogenic failure, which range from the absence of germ cells in the testis to the presence of spermatozoa in the ejaculate (oligospermia). One of the possible explanation for this phenotypic heterogeneity is the polymorphisms or mutations in the autosomal homologue of DAZ (DAZL), which was found to partially rescue the spermatogenic failure of mice homozygous for a null allele of DAZL, which suggests a possible interplay between DAZL and DAZ in humans (Slee et al., 1999).

2.6.3.5 Genotype/phenotype correlation in Partial AZFc deletions

These partial deletions are associated with extremely variable phenotypes, ranging from normospermia to azoospermia. There is a major controversy between studies on their real impact on male fertility (Ferlin et al., 2005; Hucklenbroich et al., 2005). This may be explained by the type and number of missing gene copies in AZFc region or other unknown Y chromosome related factors (for example duplications or beneficial mutations in other parts of the Y chromosome). Regarding gr/gr deletion; many studies showed that deletions removing DAZ1/DAZ2 are associated with spermatogenic failure, whereas those removing DAZ3/DAZ4 may have no or little effect on fertility (Ferlin et al., 2005; Li et al., 2011). Also, deletion of the CDY1a copy was found to have a strong association with infertility (Giachini et al., 2005; Machev et al., 2004) .

Besides the type and number of missing gene copies that affect phenotype, also, some Y-chromosome haplogroups are predisposing to, or protecting against the effect of AZFc deletions. For example; the gr/gr deletion was shown to be present in haplogroup Db2 (which occurs primarily in Japan) and are phenotypically neutral, despite of its association with the loss of CDY1a + DAZ1/2 (Sin et al., 2010). While in other haplogroups, among Caucasian men, Europe and the Western Pacific region, gr/gr deletion is associated significantly with infertility (Stouffs et al., 2011). b2/b3 microdeletions seems to have no effect on fertility status in Y haplogroup N which is commonly present in northern Eurasian populations (Repping et al., 2004), while b2/b3 microdeletions lead to infertility in other haplogroup (Lu et al., 2009; Xiao et al., 2010). Y-haplogroup N may contain a compensating Y linked

factors. E haplogroup which present in north Italy was shown to be susceptible to complete deletion of AZFc (b2/b3) and infertility (Arredi et al., 2006).

In rare cases, complete AZFc may be present in fertile male and transmitted to infertile sons (Kuhnert et al., 2004; Saut et al., 2000). Also, Partial AZFc deletions may be transmitted from fertile male to infertile sons, and extend to a larger area of deletion (Stuppia et al., 1996; Zhang et al., 2007), which argues that some deletions may not lead to infertility, but make the Y chromosome more liable to a second mutation causing spermatogenesis failure.

The possibility of finding sperms in testicular biopsy of azoospermic AZFc-deleted men is 67% (Oates et al., 2002). So they can undergo ICSI and have biological offspring, but sons will have the same deletion. So AZFc-deleted men have a good chance for retrieval sperms, may be because of presence of autosomal homologues for most of the transcriptional units of AZFc region.

2.6.3.6 The sperm production potential of ICSI-sons of AZFc-deleted men

Many studies confirmed that AZFc microdeletions are transmitted vertically to all male offspring through ICSI (Jiang et al., 1999). The deletion length of AZFc did not increase in some studies (Oates, Silber et al. 2002), but extended to a larger area in other studies (Komori et al., 2002; Lee et al., 2006).

In the case of transmission of AZFc deletions to ICSI-sons, the sons will display a spermatogenic deficiency, but not necessarily as their own fathers, may be less, may be more (Oates et al., 2002). This may be because the genetic and environmental factors that differ between the sons and fathers which strengthen or suppress the deleterious effect of AZFc deletion. For example, most of the transcriptional units in AZFc region have autosomal homologues that serve as sites of allelic variation between fathers and sons. But for sons in reproductive age, many AZFc-deleted men have some small amount of spermatogenesis sufficient for ICSI.

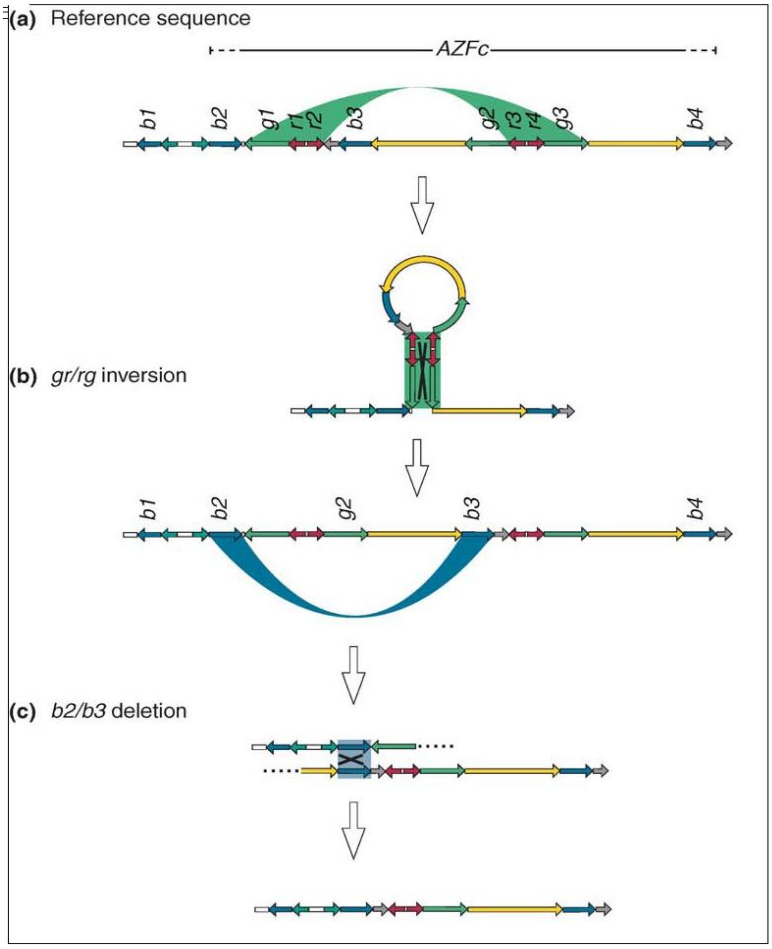

Figure 2.4: b2/b3 deletion. To occur, it should preceded by gr/gr inversion in b step. Adapted from (Noordam and Repping, 2006).

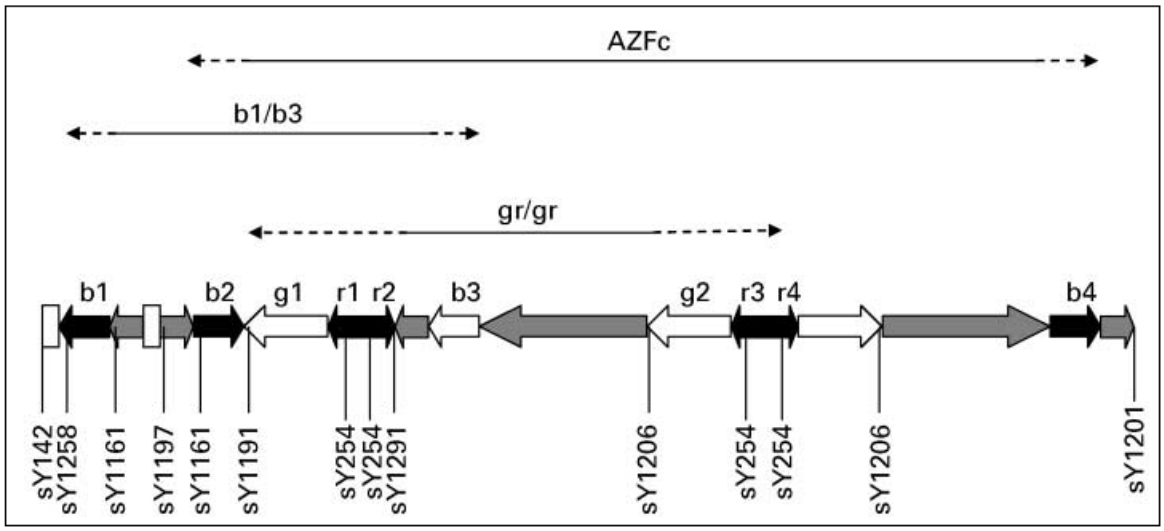

Figure 2.5: b1/b3 microdeletions in relation to gr/gr microdeletions (Hucklenbroich et al., 2005).

2.6.3.7 Risks associated with ICSI for AZFc-deleted men

Siffroi et al showed that some individuals with AZFc microdeletions harbored a significant population of 45,XO cells in both peripheral blood lymphocytes and in germ cells. They hypothesized that presence of a 45,XO/46,XY mosaicism in the father's gonads could lead to the formation of a monosomic X embryo (Siffroi et al., 2000). Their study highlighted an important potential risk for offspring born to fathers carrying Y microdeletions and treated by ICSI. Ferlin et al assessed the sperm aneuploidy rate by FISH in 11 men with the b2/b4 microdeletions and compared with 387 non-deleted severely oligozoospermic men and 103 normozoospermic control men, they found a significant reduction in the percentage of normal Y-bearing spermatozoa with respect to controls and a concomitant increase in nullisomic sperms. Also they found significant increase in XY-disomic sperms than the two groups (Ferlin et al., 2007). On the other hand many studies reported normal karyotyped males born after ICSI for AZFc-microdeleted men (Gambera et al., 2010; Oates et al., 2002).

Many studies emphasized on the importance of Y chromosome microdeletions testing before ICSI, because the test will alter prognosis (AZFc implies the possibility of finding sperms in the testes while AZFb or AZFa implies the virtual impossibility of that), and the transmission of microdeletions to sons should be discussed with infertile couple (Stahl et al., 2010).

2.6.4 The most common Monogenic disorders of male infertility

Several hundred of genes are necessary for normal sexual development, testis determination, testis descent, and spermatogenesis. Few of them have routine clinical importance, such as CFTR gene and androgen receptor gene (AR).

2.6.4.1 Congenital bilateral aplasia of vas deference (CBAVD)

About 75-80% of CBAVD is caused by a mutation in cystic fibrosis transmembrane conductance regulator (CFTR) gene, and in the remaining cases the causes are not related to CF, especially when CBAVD is associated with urinary tract malformations. Congenital unilateral absence of the vas deferens also seems to be associated with CF except when associated with renal abnormalities at the ipsilateral side of the absent vas (Lissens and

Liebaers, 1997). CBAVD accounts for at least 6 percent of cases of obstructive azoospermia and is responsible for 1 to 2 percent of cases of infertility in men (Jequier et al., 1985). CFTR gene is located on chromosome 7 and functions as a chloride channel and controls the regulation of other transport pathways. About 80% of mutations observed in patients with cystic fibrosis (CF) result from deletion of three base pairs causing the loss of the amino acid phenylalanine located at position 508 in the protein (ΔF508) (Ferlin et al., 2006).
More than 1800 mutations have been described, all presenting geographical specificity (Consortium, 2011). Other common mutations exist in most populations, with a frequency of about 1%–2% include: G542X, G551D, R553X, W1282X, and N1303K mutations (Radpour et al., 2008).
Most patients with CBAVD have mutations in one copy of CFTR gene, few have mutations in the two copies, and no mutations have been detected in a third of cases although extensive analysis of the entire coding sequence. This may be explained by the presence of mutations in noncoding sequences, which decreases the level of protein, which is sufficient to cause the obstruction but not the symptoms of cystic fibrosis. For example, Presence of 5T polymorphism in CFTR gene was found in a higher frequency (24%) in CBAVD than the general population (5%)(Chillon et al., 1995). 5T is 1 of the alleles found at the polymorphic Tn locus in intron 8 of the CFTR gene. When a lower number of thymidines are found, less efficient splicing will occur, resulting in transcripts that lack exon 9 sequences. In individuals homozygous for a 5T allele, up to 90% of the CFTR transcripts lack exon 9 (Radpour et al., 2008).
Men with CBAVD have obstructive azoospermia, so they have sperms in their testes, thus they are a candidate for ICSI. Aneuploidy rate is not increased in the sperms of affected patients. Genetic analysis should be performed in the case of obstructive Azoospermia to exclude CFTR gene mutations, because the risk of transmitting cystic fibrosis to offspring when the female partner is heterozygous for CFTR gene mutation (Popli and Stewart, 2007).

2.6.4.2 Androgen receptor gene mutations

The gene encoding AR (NR3C4) is located on the X chromosome (qX11-12) and contains eight exons. The AR belongs to the family of ligand-activated Zn-finger nuclear receptor transcription factors. AR binds all androgens; Testosterone which is the most abundant androgen and 5α-dihydrotestosterone which has the highest affinity for binding of AR (Meaden and Chedrese, 2009).

Mutations in AR gene can result in impaired embryonic sex differentiation in 46,XY genetic individuals despite normal or increased androgen production and metabolism. This disorder is known as androgen insensitivity syndrome (AIS), it may be the most common cause of male pseudohermaphroditism (MPH: presence of female external sex organs and male internal organs like testes). AIS has an incidence of about 1/20,000–1/64,000 male births. It has a wide variation in phenotypic expression and classified into:

- Complete androgen insensitivity (CAIS), which is characterized by female external genitalia, absence of Wolffian duct derived structures and prostate, absent or rudimentary uterus and gynecomastia.
- Partial androgen insensitivity (PAIS), which is characterized by several different phenotypes; predominantly female phenotype with ambiguous genitalia or predominantly male phenotype with micropenis, perineal hypospadias and cryptorchidism, the later group is called Reifenstein syndrome.
- Mild androgen insensitivity (MAIS) which is characterized by usually normal male genitals and internal male structures with impaired sperm production resulting in oligozoospermia or azoospermia (Boehmer et al., 2001).

There are over 500 mutations in androgen receptor gene mutation database (http://androgendb.mcgill.ca/, accessed July, 2011).
Expansion of a CAG trinucleotide repeat in exon 1 which encodes a polyglutamine (polyQ) tract will cause Kennedy disease. The polyQ tract is polymorphic in length; in normal individuals, it ranges between 9 and 36 residues, and expansion over 38 up to 62 residues is pathogenic. Kennedy disease, also known as Spinal and bulbar muscular atrophy (SBMA), a neuromuscular disorder characterized by the loss of lower motor neurons in the brainstem and spinal cord. Some studies showed that severely oligospermic men had a longer CAG repeat than controls (Patrizio et al., 2001). Other studies did not find a significant difference in CAG repeat length of the AR gene in the infertile group than in the control group (Tufan et al., 2005). Elder-Geva reviewed these studies and suggested that the association between CAG repeat and infertility is ethnic dependence (Eldar-Geva et al., 2004).
Oligospermic men are candidate for ICSI, To determine the degree of stability of the paternal AR CAG tract following ICSI, Cram et al compared the CAG repeat number in the AR alleles of 92 men presenting for ICSI and their 99 ICSI-conceived daughters, they found that the AR CAG tracts ranging in size from 15-28 repeats exhibited a stable inheritance in

female offspring, which suggests that the risk of SBMA in second generation sons would be extremely low (Cram et al., 2000).

2.7 References:

Abdel-Razic, M. M., Abdel-Hamid, I. A., Elsobky, E. S., 2011. Nonmosaic 47,XYY syndrome presenting with male infertility: case series. Andrologia.

Abdelmoula, N. B., Amouri, A., 2005. [Dicentric Y chromosome]. Ann Biol Clin (Paris). 63**,** 363-75.

Aboulfotouh, II, Youssef, M. A., Mady, A. F., Abdelhak, A. M., Khattab, S. M., 2011. Non-mosaic Klinefelter syndrome and successful testicular sperm extraction-intracytoplasmic sperm injection procedure: case report. Gynecol Endocrinol.

Akin, H., Onay, H., Turker, E., Ozkinay, F., 2011. Primary male infertility in Izmir/Turkey: a cytogenetic and molecular study of 187 infertile Turkish patients. Journal of Assisted Reproduction and Genetics.

Aksglaede, L., Wikstrom, A. M., Rajpert-De Meyts, E., Dunkel, L., Skakkebaek, N. E., Juul, A., 2006. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. Hum Reprod Update. 12**,** 39-48.

Anton, E., Blanco, J., Egozcue, J., Vidal, F., 2004. Sperm FISH studies in seven male carriers of Robertsonian translocation t(13;14)(q10;q10). Hum Reprod. 19**,** 1345-51.

Arredi, B., Ferlin, A., Speltra, E., Bedin, C., Zuccarello, D., Ganz, F., Marchina, E., Stuppia, L., Krausz, C., Foresta, C., 2006. Y-chromosome haplogroups and susceptibility to azoospermia factor c microdeletion in an Italian population. Journal of Medical Genetics. 44**,** 205-208.

ASRM, Definitions of infertility and recurrent pregnancy loss. Fertil Steril, Vol. 90, 2008, pp. S60.

Bakircioglu, M. E., Erden, H. F., Kaplancan, T., Ciray, N., Bener, F., Bahceci, M., 2006. Aging may adversely affect testicular sperm recovery in patients with Klinefelter syndrome. Urology. 68**,** 1082-1086.

Ballif, B. C., Kashork, C. D., Saleki, R., Rorem, E., Sundin, K., Bejjani, B. A., Shaffer, L. G., 2006. Detecting sex chromosome anomalies and common triploidies in products of conception by array-based comparative genomic hybridization. Prenat Diagn. 26**,** 333-9.

Barr, M. L., Shaver, E. L., Carr, D. H., Plunkett, E. R., 1959. An unusual sex chromatin pattern in three mentally deficient subjects. J Ment Defic Res. 3**,** 78-87.

Bellastella, A., Bizzarro, A., Coronella, C., Bellastella, G., Sinisi, A. A., De Bellis, A., 2003. Lymphocytic hypophysitis: a rare or underestimated disease? Eur J Endocrinol. 149**,** 363-76.

Bergere, M., Wainer, R., Nataf, V., Bailly, M., Gombault, M., Ville, Y., Selva, J., 2002. Biopsied testis cells of four 47,XXY patients: fluorescence in-situ hybridization and ICSI results. Hum Reprod. 17**,** 32-7.

Berkowitz, G. S., Lapinski, R. H., Dolgin, S. E., Gazella, J. G., Bodian, C. A., Holzman, I. R., 1993. Prevalence and natural history of cryptorchidism. Pediatrics. 92**,** 44-9.

Birmingham , A., 2008. Report on varicocele and infertility. Fertil Steril. 90**,** S247-9.

Blanco, J., Egozcue, J., Vidal, F., 2001. Meiotic behaviour of the sex chromosomes in three patients with sex chromosome anomalies (47,XXY, mosaic 46,XY/47,XXY and 47,XYY) assessed by fluorescence in-situ hybridization. Hum Reprod. 16**,** 887-92.

Boehmer, A. L., Brinkmann, O., Bruggenwirth, H., van Assendelft, C., Otten, B. J., Verleun-Mooijman, M. C., Niermeijer, M. F., Brunner, H. G., Rouwe, C. W., Waelkens, J. J., Oostdijk, W., Kleijer, W. J., van der Kwast, T. H., de Vroede, M. A., Drop, S. L., 2001. Genotype versus phenotype in families with androgen insensitivity syndrome. J Clin Endocrinol Metab. 86**,** 4151-60.

Bofinger, M. K., Needham, D. F., Saldana, L. R., Sosnowski, J. P., Blough, R. I., 1999. 45,X/46,X,r(Y) karyotype transmitted by father to son after intracytoplasmic sperm injection for oligospermia. A case report. J Reprod Med. 44**,** 645-8.

Bojesen, A., Juul, S., Gravholt, C. H., 2003. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab. 88**,** 622-6.

Bouligand, J., Ghervan, C., Tello, J. A., Brailly-Tabard, S., Salenave, S., Chanson, P., Lombes, M., Millar, R. P., Guiochon-Mantel, A., Young, J., 2009. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med. 360**,** 2742-8.

Bourne, H., Stern, K., Clarke, G., Pertile, M., Speirs, A., Baker, H. W., 1997. Delivery of normal twins following the intracytoplasmic injection of spermatozoa from a patient with 47,XXY Klinefelter's syndrome. Hum Reprod. 12**,** 2447-50.

Bray, P., Sister, A. J., 1963. An XXXYY sex-chromosome anomaly. Report of a mentally deficient male. JAMA. 184**,** 179-82.

Centeno Malfaz, F., Beltran Perez, A., Ruiz Labarga, C., Centeno Robles, T., Macias Pardal, J., Martin Bermejo, M., 2001. [Chromosome aberrations in malformed newborns]. An Esp Pediatr. 54**,** 582-7.

Chang, H. J., Clark, R. D., Bachman, H., 1990. The phenotype of 45,X/46,XY mosaicism: an analysis of 92 prenatally diagnosed cases. Am J Hum Genet. 46**,** 156-67.

Chen, Y., Huang, J., Liu, P., Qiao, J., 2007. Analysis of meiotic segregation patterns and interchromosomal effects in sperm from six males with Robertsonian translocations. J Assist Reprod Genet. 24**,** 406-11.

Chillon, M., Casals, T., Mercier, B., Bassas, L., Lissens, W., Silber, S., Romey, M. C., Ruiz-Romero, J., Verlingue, C., Claustres, M., et al., 1995. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med. 332**,** 1475-80.

Consortium, C. F. G. A., Cystic fibrosis mutation database. http://www.genet.sickkids.on.ca. 2011.

Cortes, D., Kjellberg, E. M., Breddam, M., Thorup, J., 2008. The true incidence of cryptorchidism in Denmark. J Urol. 179**,** 314-8.

Cowie, V. A., Singh, K. R., Wheater, R., 1986. 49, XXXYY chromosome anomaly in a mentally retarded man. Br J Psychiatry. 148**,** 210-2.

Cram, D. S., Song, B., McLachlan, R. I., Trounson, A. O., 2000. CAG trinucleotide repeats in the androgen receptor gene of infertile men exhibit stable inheritance in female offspring conceived after ICSI. Mol Hum Reprod. 6**,** 861-6.

Cynthia, P., Sex Chromosomes and Sex Chromosome Abnormalities. In: S. Gersen, M. Keagle, Eds.), The Principles of Clinical Cytogenetics. Humana Press, Totowa 2005.

Day, J. W., Ranum, L. P., 2005. RNA pathogenesis of the myotonic dystrophies. Neuromuscul Disord. 15**,** 5-16.

Deng, H. X., Abe, K., Kondo, I., Tsukahara, M., Inagaki, H., Hamada, I., Fukushima, Y., Niikawa, N., 1991. Parental origin and mechanism of formation of polysomy X: an

XXXXX case and four XXXXY cases determined with RFLPs. Hum Genet. 86**,** 541-4.
Dudin, A., Thalji, A., Rambaud-Cousson, A., 2001. CHROMOSOMAL ABERRATIONS DETECTED AT BIRTH. Bulletin of Palestinian Child Society 6**,** 25-28.
Eastmond, D. A., Pinkel, D., 1990. Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence in situ hybridization with chromosome-specific DNA probes. Mutat Res. 234**,** 303-18.
Eldar-Geva, T., Milatiner, D., Halle, D., 2004. [Androgen receptor and male infertility]. Harefuah. 143**,** 432-9, 461.
Ergun-Longmire, B., Vinci, G., Alonso, L., Matthew, S., Tansil, S., Lin-Su, K., McElreavey, K., New, M. I., 2005. Clinical, hormonal and cytogenetic evaluation of 46,XX males and review of the literature. J Pediatr Endocrinol Metab. 18**,** 739-48.
Eskenazi, B., Wyrobek, A. J., Kidd, S. A., Lowe, X., Moore, D., 2nd, Weisiger, K., Aylstock, M., 2002. Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. Hum Reprod. 17**,** 576-83.
Ferlin, A., Arredi, B., Foresta, C., 2006. Genetic causes of male infertility. Reprod Toxicol. 22**,** 133-41.
Ferlin, A., Arredi, B., Speltra, E., Cazzadore, C., Selice, R., Garolla, A., Lenzi, A., Foresta, C., 2007. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab. 92**,** 762-70.
Ferlin, A., Tessari, A., Ganz, F., Marchina, E., Barlati, S., Garolla, A., Engl, B., Foresta, C., 2005. Association of partial AZFc region deletions with spermatogenic impairment and male infertility. J Med Genet. 42**,** 209-13.
Foresta, C., 1999. Analysis of Meiosis in Intratesticular Germ Cells from Subjects Affected by Classic Klinefelter's Syndrome. Journal of Clinical Endocrinology & Metabolism. 84**,** 3807-3810.
Foresta, C., Galeazzi, C., Bettella, A., Stella, M., Scandellari, C., 1998. High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome. J Clin Endocrinol Metab. 83**,** 203-5.
Foresta, C., Moro, E., Ferlin, A., 2001. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev. 22**,** 226-39.
Foss, G. L., Lewis, F. J., 1971. A study of four cases with Klinefelter's syndrome, showing motile spermatozoa in their ejaculates. J Reprod Fertil. 25**,** 401-8.
Fraccaro, M., Kaijser, K., Lindsten, J., 1960. A child with 49 chromosomes. Lancet. 2**,** 899-902.
Fullerton, G., Hamilton, M., Maheshwari, A., 2010. Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009? Hum Reprod. 25**,** 588-97.
Gambera, L., Governini, L., De Leo, V., Luddi, A., Morgante, G., Tallis, V., Piomboni, P., 2010. Successful multiple pregnancy achieved after transfer of frozen embryos obtained via intracytoplasmic sperm injection with testicular sperm from an AZFc-deleted man. Fertil Steril. 94**,** 2330 e1-3.
Giachini, C., Guarducci, E., Longepied, G., Degl'Innocenti, S., Becherini, L., Forti, G., Mitchell, M. J., Krausz, C., 2005. The gr/gr deletion(s): a new genetic test in male infertility? J Med Genet. 42**,** 497-502.
Giltay, J. C., van Golde, R. J., Kastrop, P. M., 2000. Analysis of spermatozoa from seven ICSI males with constitutional sex chromosomal abnormalities by fluorescent in situ hybridization. J Assist Reprod Genet. 17**,** 151-5.
Gonsalves, J., Turek, P. J., Schlegel, P. N., Hopps, C. V., Weier, J. F., Pera, R. A., 2005. Recombination in men with Klinefelter syndrome. Reproduction. 130**,** 223-9.

Gordon, D. L., Krmpotic, E., Thomas, W., Gandy, H. M., Paulsen, C. A., 1972. Pathologic testicular findings in Klinefelter's syndrome. 47,XXY vs 46,XY-47,XXY. Arch Intern Med. 130**,** 726-9.

Gorlov, I. P., Kamat, A., Bogatcheva, N. V., Jones, E., Lamb, D. J., Truong, A., Bishop, C. E., McElreavey, K., Agoulnik, A. I., 2002. Mutations of the GREAT gene cause cryptorchidism. Hum Mol Genet. 11**,** 2309-18.

Greco, E., Iacobelli, M., Rienzi, L., Menchinifabris, G., Tesorio, N., Tesarik, J., 2008. Birth of a healthy boy after fertilization of cryopreserved oocytes with cryopreserved testicular spermatozoa from a man with nonmosaic Klinefelter syndrome. Fertility and Sterility. 89**,** 991.e5-991.e7.

Grigorescu-Sido, A., Heinrich, U., Grigorescu-Sido, P., Jauch, A., Hager, H. D., Vogt, P. H., Duncea, I., Bettendorf, M., 2005. Three new 46,XX male patients: a clinical, cytogenetic and molecular analysis. J Pediatr Endocrinol Metab. 18**,** 197-203.

Hadjiathanasiou, C. G., Brauner, R., Lortat-Jacob, S., Nivot, S., Jaubert, F., Fellous, M., Nihoul-Fekete, C., Rappaport, R., 1994. True hermaphroditism: genetic variants and clinical management. J Pediatr. 125**,** 738-44.

Hall, H., Hunt, P., Hassold, T., 2006. Meiosis and sex chromosome aneuploidy: how meiotic errors cause aneuploidy; how aneuploidy causes meiotic errors. Curr Opin Genet Dev. 16**,** 323-9.

Hardelin, J. P., 2001. Kallmann syndrome: towards molecular pathogenesis. Mol Cell Endocrinol. 179**,** 75-81.

Harris, S., Kandil, H., Niederberger, C. S., Endocrinopathies in Male Infertility. In: Sapanegh.E, (Ed.), Male infertility problems and solutions. Humana press 2011.

Harvey, J., Jacobs, P. A., Hassold, T., Pettay, D., 1990. The parental origin of 47,XXY males. Birth Defects Orig Artic Ser. 26**,** 289-96.

Hennebicq, S., Pelletier, R., Bergues, U., Rousseaux, S., 2001. Risk of trisomy 21 in offspring of patients with Klinefelter's syndrome. Lancet. 357**,** 2104-5.

Hinney, B., Guttenbach, M., Schmid, M., Engel, W., Michelmann, H. W., 1997. Pregnancy after intracytoplasmic sperm injection with sperm from a man with a 47,XXY Klinefelter's karyotype. Fertil Steril. 68**,** 718-20.

Hopps, C. V., Mielnik, A., Goldstein, M., Palermo, G. D., Rosenwaks, Z., Schlegel, P. N., 2003. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod. 18**,** 1660-5.

Hori, N., Kato, T., Sugimura, Y., Tajima, K., Tochigi, H., Kawamura, J., 1988. A male subject with 3 Y chromosomes (48, XYYY): a case report. J Urol. 139**,** 1059-61.

Hsu, L. Y., 1994. Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. Am J Med Genet. 53**,** 108-40.

Hucklenbroich, K., Gromoll, J., Heinrich, M., Hohoff, C., Nieschlag, E., Simoni, M., 2005. Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. Hum Reprod. 20**,** 191-7.

Huleyuk, N., Zastavna, D., Tyrkus, M., Makukh, H., Gavrylyshyn, S., Kurpisz, M., 2010. Complex cytogenetic and molecular-genetic analysis of males with spermatogenesis failure. Tsitol Genet. 44**,** 51-6.

Hunt, P. A., Worthman, C., Levinson, H., Stallings, J., LeMaire, R., Mroz, K., Park, C., Handel, M. A., 1998. Germ cell loss in the XXY male mouse: altered X-chromosome dosage affects prenatal development. Mol Reprod Dev. 49**,** 101-11.

Hunter, H., Quaife, R., 1973. A 48,XYYY male: a somatic and psychiatric description. J Med Genet. 10**,** 80-3.

Hutson, J. M., Balic, A., Nation, T., Southwell, B., 2010. Cryptorchidism. Semin Pediatr Surg. 19**,** 215-24.

Imbeaud, S., Carre-Eusebe, D., Rey, R., Belville, C., Josso, N., Picard, J. Y., 1994. Molecular genetics of the persistent mullerian duct syndrome: a study of 19 families. Hum Mol Genet. 3**,** 125-31.

Irvine, D. S., 1998. Epidemiology and aetiology of male infertility. Hum Reprod. 13 Suppl 1**,** 33-44.

Issa, Y., Sallmen, M., Nijem, K., Bjertness, E., Kristensen, P., 2010. Fecundability among newly married couples in agricultural villages in Palestine: a prospective study. Hum Reprod. 25**,** 2132-8.

Jacobs, P. A., Hassold, T. J., Whittington, E., Butler, G., Collyer, S., Keston, M., Lee, M., 1988. Klinefelter's syndrome: an analysis of the origin of the additional sex chromosome using molecular probes. Ann Hum Genet. 52**,** 93-109.

Jayasena, C. N., Dhillo, W. S., Bloom, S. R., 2009. Kisspeptins and the control of gonadotropin secretion in humans. Peptides. 30**,** 76-82.

Jequier, A. M., Ansell, I. D., Bullimore, N. J., 1985. Congenital absence of the vasa deferentia presenting with infertility. J Androl. 6**,** 15-9.

Jiang, M. C., Lien, Y. R., Chen, S. U., Ko, T. M., Ho, H. N., Yang, Y. S., 1999. Transmission of de novo mutations of the deleted in azoospermia genes from a severely oligozoospermic male to a son via intracytoplasmic sperm injection. Fertil Steril. 71**,** 1029-32.

Kamp, C., Huellen, K., Fernandes, S., Sousa, M., Schlegel, P. N., Mielnik, A., Kleiman, S., Yavetz, H., Krause, W., Kupker, W., Johannisson, R., Schulze, W., Weidner, W., Barros, A., Vogt, P. H., 2001. High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. Mol Hum Reprod. 7**,** 987-94.

Katherine, L., O'Flynn O'Brien, B. A., Varghese, A. C., Agarwal, A., 2010. The genetic causes of male factor infertility: A review. Fertility and Sterility. 93**,** 1-12.

Kilic, S., Yukse, B., Tasdemir, N., Dogan, M., Ozdemir, E., Yesilyurt, A., Keskin, I., 2010. Assisted reproductive treatment applications in men with normal phenotype but 45,X/46,XY mosaic karyotype: clinical and genetic perspectives. Taiwan J Obstet Gynecol. 49**,** 199-202.

Kitamura, M., Matsumiya, K., Koga, M., Nishimura, K., Miura, H., Tsuji, T., Matsumoto, M., Okamoto, Y., Okuyama, A., 2000. Ejaculated spermatozoa in patients with non-mosaic Klinefelter's syndrome. Int J Urol. 7**,** 88-92; discussion 93-4.

Kleczkowska, A., Fryns, J. P., Van den Berghe, H., 1988. X-chromosome polysomy in the male. The Leuven experience 1966-1987. Hum Genet. 80**,** 16-22.

Komori, S., Kato, H., Kobayashi, S., Koyama, K., Isojima, S., 2002. Transmission of Y chromosomal microdeletions from father to son through intracytoplasmic sperm injection. J Hum Genet. 47**,** 465-8.

Koşar, P. A., Özçelik, N., Koşar, A., 2010. Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia. Journal of Assisted Reproduction and Genetics. 27**,** 17-21.

Krausz, C., Quintana-Murci, L., McElreavey, K., 2000. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod. 15**,** 1431-4.

Kremer, H., Kraaij, R., Toledo, S. P., Post, M., Fridman, J. B., Hayashida, C. Y., van Reen, M., Milgrom, E., Ropers, H. H., Mariman, E., et al., 1995. Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. Nat Genet. 9**,** 160-4.

Kuhnert, B., Gromoll, J., Kostova, E., Tschanter, P., Luetjens, C. M., Simoni, M., Nieschlag, E., 2004. Case report: natural transmission of an AZFc Y-chromosomal microdeletion from father to his sons. Hum Reprod. 19**,** 886-8.

Kumtepe, Y., Beyazyurek, C., Cinar, C., Ozbey, I., Ozkan, S., Cetinkaya, K., Karlikaya, G., Karagozoglu, H., Kahraman, S., 2009. A genetic survey of 1935 Turkish men with severe male factor infertility. Reprod Biomed Online. 18**,** 465-74.

Kuroda-Kawaguchi, T., Skaletsky, H., Brown, L. G., Minx, P. J., Cordum, H. S., Waterston, R. H., Wilson, R. K., Silber, S., Oates, R., Rozen, S., Page, D. C., 2001. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet. 29**,** 279-86.

Kusz, K., Kotecki, M., Wojda, A., Szarras-Czapnik, M., Latos-Bielenska, A., Warenik-Szymankiewicz, A., Ruszczynska-Wolska, A., Jaruzelska, J., 1999. Incomplete masculinisation of XX subjects carrying the SRY gene on an inactive X chromosome. J Med Genet. 36**,** 452-6.

Lahn, B. T., Tang, Z. L., Zhou, J., Barndt, R. J., Parvinen, M., Allis, C. D., Page, D. C., 2002. Previously uncharacterized histone acetyltransferases implicated in mammalian spermatogenesis. Proc Natl Acad Sci U S A. 99**,** 8707-12.

Lanfranco, F., Kamischke, A., Zitzmann, M., Nieschlag, E., 2004. Klinefelter's syndrome. The Lancet. 364**,** 273-283.

Laron, Z., Dickerman, Z., Zamir, R., Galatzer, A., 1982. Paternity in Klinefelter's syndrome--a case report. Arch Androl. 8**,** 149-51.

Layman, L. C., Tho, S. P., Clark, A. D., Kulharya, A., McDonough, P. G., 2009. Phenotypic spectrum of 45,X/46,XY males with a ring Y chromosome and bilaterally descended testes. Fertil Steril. 91**,** 791-7.

Lecluse-van der Bilt, F. A., Hagemeijer, A., Smit, E. M., Visser, H. K., Vaandrager, G. J., 1974. An infant with an XXXYY karyotype. Clin Genet. 5**,** 263-70.

Lee, S. H., Ahn, S. Y., Lee, K. W., Kwack, K., Jun, H. S., Cha, K. Y., 2006. Intracytoplasmic sperm injection may lead to vertical transmission, expansion, and de novo occurrence of Y-chromosome microdeletions in male fetuses. Fertil Steril. 85**,** 1512-5.

Li, Y., Pan, K. J., Wang, L., Ren, J., 2011. [Association of gr/gr deletion in the AZFc region of Y chromosome with male infertility: a meta-analysis]. Zhonghua Nan Ke Xue. 17**,** 546-52.

Linden, H., Overzier, C., 1956. [True agonadism (anorchism) in siblings]. Gynaecologia. 142**,** 215-33.

Linden, M. G., Bender, B. G., Robinson, A., 1995. Sex chromosome tetrasomy and pentasomy. Pediatrics. 96**,** 672-82.

Lissens, W., Liebaers, I., 1997. The genetics of male infertility in relation to cystic fibrosis. Baillieres Clin Obstet Gynaecol. 11**,** 797-817.

Lopez-Valdes, J. A., Nieto, K., Najera, N., Cervantes, A., Kofman-Alfaro, S., Queipo, G., 2009. Mix gonadal dysgenesis associated with ring Y chromosome mosaics in a phenotypic male. Sex Dev. 3**,** 177-82.

Lorda-Sanchez, I., Binkert, F., Maechler, M., Robinson, W. P., Schinzel, A. A., 1992. Reduced recombination and paternal age effect in Klinefelter syndrome. Hum Genet. 89**,** 524-30.

Lu, C., Zhang, J., Li, Y., Xia, Y., Zhang, F., Wu, B., Wu, W., Ji, G., Gu, A., Wang, S., Jin, L., Wang, X., 2009. The b2/b3 subdeletion shows higher risk of spermatogenic failure and higher frequency of complete AZFc deletion than the gr/gr subdeletion in a Chinese population. Hum Mol Genet. 18**,** 1122-30.

Ma, S., Yuen, B. H., Penaherrera, M., Koehn, D., Ness, L., Robinson, W., 2003. ICSI and the transmission of X-autosomal translocation: a three-generation evaluation of X;20 translocation: case report. Hum Reprod. 18**,** 1377-82.

Machev, N., Saut, N., Longepied, G., Terriou, P., Navarro, A., Levy, N., Guichaoua, M., Metzler-Guillemain, C., Collignon, P., Frances, A. M., Belougne, J., Clemente, E., Chiaroni, J., Chevillard, C., Durand, C., Ducourneau, A., Pech, N., McElreavey, K., Mattei, M. G., Mitchell, M. J., 2004. Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. J Med Genet. 41**,** 814-25.

Mardesic, T., Kosarova, M., Zudova, D., Jelinkova, L., Sobotka, V., Gregor, V., 2011. [Preimplantation genetic diagnosis (PGD) in carriers of chromosomal translocations: possibilities and results]. Ceska Gynekol. 76**,** 100-3.

Mariotti, A., Di Carlo, L., Orlando, G., Corradini, M. L., Di Donato, L., Pompa, P., Iezzi, R., Cotroneo, A. R., Romani, G. L., Merla, A., 2011. Scrotal thermoregulatory model and assessment of the impairment of scrotal temperature control in varicocele. Ann Biomed Eng. 39**,** 664-73.

Meaden, C., Chedrese, P., Androgens—Molecular Basis and Related Disorders. In: P. J. Chedrese, (Ed.), Reproductive Endocrinology. Springer Science+Business Media, 2009, pp. 205-214.

Miller, N. L., Wevrick, R., Mellon, P. L., 2009. Necdin, a Prader-Willi syndrome candidate gene, regulates gonadotropin-releasing hormone neurons during development. Hum Mol Genet. 18**,** 248-60.

Morel, F., Bernicot, I., Herry, A., Le Bris, M. J., Amice, V., De Braekeleer, M., 2003. An increased incidence of autosomal aneuploidies in spermatozoa from a patient with Klinefelter's syndrome. Fertil Steril. 79 Suppl 3**,** 1644-6.

Moretti, E., Pascarelli, N. A., Giannerini, V., Geminiani, M., Anichini, C., Collodel, G., 2009. 18, X, Y aneuploidies and transmission electron microscopy studies in spermatozoa from five carriers of different reciprocal translocations. Asian J Androl. 11**,** 325-32.

Mroz, K., Carrel, L., Hunt, P. A., 1999a. Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. Dev Biol. 207**,** 229-38.

Mroz, K., Hassold, T. J., Hunt, P. A., 1999b. Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. Hum Reprod. 14**,** 1151-6.

Murakami, J., Baba, K., Minagawa, N., Kono, S., Yajima, M., Iwamoto, T., 1997. [A case of 47XYY syndrome presenting with male infertility]. Hinyokika Kiyo. 43**,** 433-6.

Murao, K., Imachi, H., Muraoka, T., Fujiwara, M., Kushida, Y., Haba, R., Ishida, T., 2008. Isolated follicle-stimulating hormone (FSH) deficiency without mutation of the FSHbeta gene and successful treatment with human menopausal gonadotropin. Fertil Steril. 90**,** 2012 e17-9.

Nef, S., Parada, L. F., 1999. Cryptorchidism in mice mutant for Insl3. Nat Genet. 22**,** 295-9.

Nielsen, J., Wohlert, M., 1991. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet. 87**,** 81-3.

Nodar, F., De Vincentiis, S., Olmedo, S. B., Papier, S., Urrutia, F., Acosta, A. A., 1999. Birth of twin males with normal karyotype after intracytoplasmic sperm injection with use of testicular spermatozoa from a nonmosaic patient with Klinefelter's syndrome. Fertil Steril. 71**,** 1149-52.

Noordam, M. J., Repping, S., 2006. The human Y chromosome: a masculine chromosome. Current Opinion in Genetics & Development. 16**,** 225-232.

Oates, R. D., Silber, S., Brown, L. G., Page, D. C., 2002. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod. 17**,** 2813-24.

Ottesen, A. M., Aksglaede, L., Garn, I., Tartaglia, N., Tassone, F., Gravholt, C. H., Bojesen, A., Sorensen, K., Jorgensen, N., Rajpert-De Meyts, E., Gerdes, T., Lind, A. M., Kjaergaard, S., Juul, A., 2010. Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. Am J Med Genet A. 152A**,** 1206-12.

Ottesen, A. M., Garn, I. D., Aksglaede, L., Juul, A., Rajpert-De Meyts, E., 2007. A simple screening method for detection of Klinefelter syndrome and other X-chromosome aneuploidies based on copy number of the androgen receptor gene. Molecular Human Reproduction. 13**,** 745-750.

Palermo, G. D., Schlegel, P. N., Sills, E. S., Veeck, L. L., Zaninovic, N., Menendez, S., Rosenwaks, Z., 1998. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with nonmosaic Klinefelter's syndrome. N Engl J Med. 338**,** 588-90.

Papadimas, J., Goulis, D. G., Giannouli, C., Papanicolaou, A., Tarlatzis, B., Bontis, J. N., 2001. Ambiguous genitalia, 45,X/46,XY mosaic karyotype, and Y chromosome microdeletions in a 17-year-old man. Fertil Steril. 76**,** 1261-3.

Patrizio, P., Leonard, D. G., Chen, K. L., Hernandez-Ayup, S., Trounson, A. O., 2001. Larger trinucleotide repeat size in the androgen receptor gene of infertile men with extremely severe oligozoospermia. J Androl. 22**,** 444-8.

Pena, S. D., Sturzeneker, R., 2003. Molecular barr bodies: methylation-specific PCR of the human X-linked gene FMR-1 for diagnosis of Klinefelter syndrome. J Androl. 24**,** 809; author reply 810.

Pepene, C. E., Coman, I., Mihu, D., Militaru, M., Duncea, I., 2008. Infertility in a new 46, XX male with positive SRY confirmed by fluorescence in situ hybridization: a case report. Clin Exp Obstet Gynecol. 35**,** 299-300.

Pfeiffer, R. A., Sanger, R., 1973. Origin of 48,XXXY: the evidence of the Xg blood groups. J Med Genet. 10**,** 142-3.

Pitteloud, N., Boepple, P. A., DeCruz, S., Valkenburgh, S. B., Crowley, W. F., Jr., Hayes, F. J., 2001. The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab. 86**,** 2470-5.

Polanco, J. C., Wilhelm, D., Davidson, T. L., Knight, D., Koopman, P., 2010. Sox10 gain-of-function causes XX sex reversal in mice: implications for human 22q-linked disorders of sex development. Hum Mol Genet. 19**,** 506-16.

Popli, K., Stewart, J., 2007. Infertility and its management in men with cystic fibrosis: review of literature and clinical practices in the UK. Hum Fertil (Camb). 10**,** 217-21.

Poulakis, V., Witzsch, U., Diehl, W., de Vries, R., Becht, E., Trotnow, S., 2001. Birth of two infants with normal karyotype after intracytoplasmic injection of sperm obtained by testicular extraction from two men with nonmosaic Klinefelter's syndrome. Fertil Steril. 76**,** 1060-2.

Queipo, G., Zenteno, J., Pe, x000F, a, R., x000Ed, Nieto, K., Radillo, A., Dorantes, L., Era, a, L., Lieberman, E., derlund, D., Jim, x000E, nez, A., Ram, n, G., Kofman-Alfaro, S., 2002. Molecular analysis in true hermaphroditism: demonstration of low-level hidden mosaicism for Y-derived sequences in 46,XX cases. Human Genetics. 111**,** 278-283.

Radpour, R., Gourabi, H., Dizaj, A. V., Holzgreve, W., Zhong, X. Y., 2008. Genetic investigations of CFTR mutations in congenital absence of vas deferens, uterus, and vagina as a cause of infertility. J Androl. 29**,** 506-13.

Rapaport, F. T., Sampath, A., Kano, K., McCluskey, R. T., Milgrom, F., 1969. Immunological effects of thermal injury. I. Inhibition of spermatogenesis in guinea pigs. J Exp Med. 130**,** 1411-25.

Repping, S., Skaletsky, H., Brown, L., van Daalen, S. K., Korver, C. M., Pyntikova, T., Kuroda-Kawaguchi, T., de Vries, J. W., Oates, R. D., Silber, S., van der Veen, F., Page, D. C., Rozen, S., 2003. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet. 35**,** 247-51.

Repping, S., Skaletsky, H., Lange, J., Silber, S., Van Der Veen, F., Oates, R. D., Page, D. C., Rozen, S., 2002. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet. 71**,** 906-22.

Repping, S., van Daalen, S. K., Korver, C. M., Brown, L. G., Marszalek, J. D., Gianotten, J., Oates, R. D., Silber, S., van der Veen, F., Page, D. C., Rozen, S., 2004. A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. Genomics. 83**,** 1046-52.

Reubinoff, B. E., Abeliovich, D., Werner, M., Schenker, J. G., Safran, A., Lewin, A., 1998. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. Hum Reprod. 13**,** 1887-92.

Romero Tovar, S., Juarez Espinosa, B., Galindo Garcia, C. G., Mendoza Romo, M., Sanchez Usabiaga, R. A., 2009. [Prevalence of chromosomal alterations in infertile patients studied in a clinic of assisted reproduction]. Ginecol Obstet Mex. 77**,** 128-35.

Ron-el, R., Friedler, S., Strassburger, D., Komarovsky, D., Schachter, M., Raziel, A., 1999. Birth of a healthy neonate following the intracytoplasmic injection of testicular spermatozoa from a patient with Klinefelter's syndrome. Hum Reprod. 14**,** 368-70.

Ron-El, R., Raziel, A., Strassburger, D., Schachter, M., Bern, O., Friedler, S., 2000a. Birth of healthy male twins after intracytoplasmic sperm injection of frozen-thawed testicular spermatozoa from a patient with nonmosaic Klinefelter syndrome. Fertil Steril. 74**,** 832-3.

Ron-El, R., Strassburger, D., Gelman-Kohan, S., Friedler, S., Raziel, A., Appelman, Z., 2000b. A 47,XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome: case report. Hum Reprod. 15**,** 1804-6.

Rosenlund, B., Hreinsson, J. G., Hovatta, O., 2002. Birth of a healthy male after frozen thawed blastocyst transfer following intracytoplasmic injection of frozen thawed testicular spermatozoa from a man with nonmosaic Klinefelter's syndrome. J Assist Reprod Genet. 19**,** 149-51.

Salamanca-Gomez, F., Cortes, R., Sanchez, J., Armendares, S., 1981. A 49,XXXYY male. Am J Med Genet. 10**,** 351-5.

Saut, N., Terriou, P., Navarro, A., Levy, N., Mitchell, M. J., 2000. The human Y chromosome genes BPY2, CDY1 and DAZ are not essential for sustained fertility. Mol Hum Reprod. 6**,** 789-93.

Saxena, R., de Vries, J. W., Repping, S., Alagappan, R. K., Skaletsky, H., Brown, L. G., Ma, P., Chen, E., Hoovers, J. M., Page, D. C., 2000. Four DAZ genes in two clusters found in the AZFc region of the human Y chromosome. Genomics. 67**,** 256-67.

Schara, U., Schoser, B. G., 2006. Myotonic dystrophies type 1 and 2: a summary on current aspects. Semin Pediatr Neurol. 13**,** 71-9.

Schiff, J. D., Palermo, G. D., Veeck, L. L., Goldstein, M., Rosenwaks, Z., Schlegel, P. N., 2005. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab. 90**,** 6263-7.

Schoepflin, G. S., Centerwall, W. R., 1972. 48,XYYY: a new syndrome? J Med Genet. 9**,** 356-60.

Sciurano, R. B., Luna Hisano, C. V., Rahn, M. I., Brugo Olmedo, S., Rey Valzacchi, G., Coco, R., Solari, A. J., 2009. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. Hum Reprod. 24**,** 2353-60.

Seminara, S. B., Acierno, J. S., Jr., Abdulwahid, N. A., Crowley, W. F., Jr., Margolin, D. H., 2002. Hypogonadotropic hypogonadism and cerebellar ataxia: detailed phenotypic characterization of a large, extended kindred. J Clin Endocrinol Metab. 87**,** 1607-12.

Shaqalaih, A. J., Genetic Causes of Male Infertility A Combined Cytogenetic andY Chromosome Microdeletions Study. Faculty of Science, Vol. master. The Islamic University, Gaza strip, 2007, pp. 93.

Shaqalaih, A. J., Abu Halima, M. S., Ashour, M. J., Sharif, F. A., 2009. Screening for Y-chromosome microdeletions in a population of infertile males in the Gaza Strip. J Exp Clin Assist Reprod. 6**,** 7.

Shenker, N. S., Huynh, J., Farmer, P. J., Hutson, J. M., 2006. A new role for androgen in testicular descent: permitting gubernacular cell proliferation in response to the neuropeptide, calcitonin gene-related peptide. J Pediatr Surg. 41**,** 407-12.

Shi, Q., Martin, R. H., 2000. Multicolor fluorescence in situ hybridization analysis of meiotic chromosome segregation in a 47,XYY male and a review of the literature. Am J Med Genet. 93**,** 40-6.

Shiraishi, K., Naito, K., 2003. Fertile eunuch syndrome with the mutations (Trp8Arg and Ile15Thr) in the beta subunit of luteinizing hormone. Endocr J. 50**,** 733-7.

Siffroi, J. P., Le Bourhis, C., Krausz, C., Barbaux, S., Quintana-Murci, L., Kanafani, S., Rouba, H., Bujan, L., Bourrouillou, G., Seifer, I., Boucher, D., Fellous, M., McElreavey, K., Dadoune, J. P., 2000. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. Hum Reprod. 15**,** 2559-62.

Simoni, M., Bakker, E., Krausz, C., 2004. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. Int J Androl. 27**,** 240-9.

Sin, H. S., Koh, E., Shigehara, K., Sugimoto, K., Maeda, Y., Yoshida, A., Kyono, K., Namiki, M., 2010. Features of constitutive gr/gr deletion in a Japanese population. Hum Reprod. 25**,** 2396-403.

Slee, R., Grimes, B., Speed, R. M., Taggart, M., Maguire, S. M., Ross, A., McGill, N. I., Saunders, P. T., Cooke, H. J., 1999. A human DAZ transgene confers partial rescue of the mouse Dazl null phenotype. Proc Natl Acad Sci U S A. 96**,** 8040-5.

Sobel, V., McGinley, J., <Hypergonadotropic Hypogonadism.pdf>. Encyclopedia of Endocrine Diseases Vol. 2. Elsevier Inc, 2004.

Sousa, M., Cremades, N., Alves, C., Silva, J., Barros, A., 2002. Developmental potential of human spermatogenic cells co-cultured with Sertoli cells. Hum Reprod. 17**,** 161-72.

Spinner, N. B., Saitta, S. C., Delaney, D. P., Colliton, R., Zderic, S. A., Ruchelli, E., Zackai, E., Kolon, T. F., 2008. Intracytoplasmic sperm injection (ICSI) with transmission of a ring(Y) chromosome and ovotesticular disorder of sex development in offspring. Am J Med Genet A. 146A**,** 1828-31.

Staessen, C., Tournaye, H., Van Assche, E., Michiels, A., Van Landuyt, L., Devroey, P., Liebaers, I., Van Steirteghem, A., 2003. PGD in 47,XXY Klinefelter's syndrome patients. Hum Reprod Update. 9**,** 319-30.

Stahl, P. J., Masson, P., Mielnik, A., Marean, M. B., Schlegel, P. N., Paduch, D. A., 2010. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. Fertility and Sterility. 94**,** 1753-1756.

Stemkens, D., Roza, T., Verrij, L., Swaab, H., van Werkhoven, M. K., Alizadeh, B. Z., Sinke, R. J., Giltay, J. C., 2006. Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. Clin Genet. 70**,** 43-8.

Stouffs, K., Lissens, W., Tournaye, H., Haentjens, P., 2011. What about gr/gr deletions and male infertility? Systematic review and meta-analysis. Hum Reprod Update. 17**,** 197-209.

Stuppia, L., Calabrese, G., Franchi, P. G., Mingarelli, R., Gatta, V., Palka, G., Dallapiccola, B., 1996. Widening of a Y-chromosome interval-6 deletion transmitted from a father to his infertile son accounts for an oligozoospermia critical region distal to the RBM1 and DAZ genes. Am J Hum Genet. 59**,** 1393-5.

Sutton, E., Hughes, J., White, S., Sekido, R., Tan, J., Arboleda, V., Rogers, N., Knower, K., Rowley, L., Eyre, H., Rizzoti, K., McAninch, D., Goncalves, J., Slee, J., Turbitt, E., Bruno, D., Bengtsson, H., Harley, V., Vilain, E., Sinclair, A., Lovell-Badge, R., Thomas, P., 2011. Identification of SOX3 as an XX male sex reversal gene in mice and humans. J Clin Invest. 121**,** 328-41.

Tapanainen, J. S., Aittomaki, K., Min, J., Vaskivuo, T., Huhtaniemi, I. T., 1997. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet. 15**,** 205-6.

Terzoli, G., Lalatta, F., Lobbiani, A., Simoni, G., Colucci, G., 1992. Fertility in a 47,XXY patient: assessment of biological paternity by deoxyribonucleic acid fingerprinting. Fertil Steril. 58**,** 821-2.

Thomas, N. S., Hassold, T. J., 2003. Aberrant recombination and the origin of Klinefelter syndrome. Hum Reprod Update. 9**,** 309-17.

Thonneau, P., Marchand, S., Tallec, A., Ferial, M. L., Ducot, B., Lansac, J., Lopes, P., Tabaste, J. M., Spira, A., 1991. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod. 6**,** 811-6.

Thorup, J., McLachlan, R., Cortes, D., Nation, T. R., Balic, A., Southwell, B. R., Hutson, J. M., 2010. What is new in cryptorchidism and hypospadias--a critical review on the testicular dysgenesis hypothesis. J Pediatr Surg. 45**,** 2074-86.

Tiepolo, L., Zuffardi, O., 1976. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet. 34**,** 119-24.

Torresani, T., Biason-Lauber, A., 2007. Congenital adrenal hyperplasia: Diagnostic advances. Journal of Inherited Metabolic Disease. 30**,** 563-575.

Tufan, A. C., Satiroglu-Tufan, N. L., Aydinuraz, B., Satiroglu, M. H., Aydos, K., Bagci, H., 2005. No association of the CAG repeat length in exon 1 of the androgen receptor gene with idiopathic infertility in Turkish men: implications and literature review. Tohoku J Exp Med. 206**,** 105-15.

Tuttelmann, F., Gromoll, J., 2010. Novel genetic aspects of Klinefelter's syndrome. Molecular Human Reproduction. 16**,** 386-395.

Van Assche, E., Bonduelle, M., Tournaye, H., Joris, H., Verheyen, G., Devroey, P., Van Steirteghem, A., Liebaers, I., 1996. Cytogenetics of infertile men. Hum Reprod. 11 Suppl 4**,** 1-24; discussion 25-6.

Veld, P. A., Weber, R. F., Los, F. J., den Hollander, N., Dhont, M., Pieters, M. H., Van Hemel, J. O., 1997. Two cases of Robertsonian translocations in oligozoospermic males and their consequences for pregnancies induced by intracytoplasmic sperm injection. Hum Reprod. 12**,** 1642-4.

Venkateshwari, A., Srilekha, A., Begum, A., Sujatha, M., Rani, P. U., Sunitha, T., Nallari, P., Jyothy, A., 2010. Clinical and behavioural profile of a rare variant of Klinefelter syndrome-48, XXXY. The Indian Journal of Pediatrics. 77**,** 447-449.

Vernaeve, V., Staessen, C., Verheyen, G., Van Steirteghem, A., Devroey, P., Tournaye, H., 2004. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? Hum Reprod. 19**,** 1135-9.

Vetro, A., Ciccone, R., Giorda, R., Patricelli, M. G., Della Mina, E., Forlino, A., Zuffardi, O., 2011. XX males SRY negative: a confirmed cause of infertility. J Med Genet.

Vilain, E. J., 1993. 46,XX Testicular Disorder of Sex Development.

Vincent, M. C., Daudin, M., De, M. P., Massat, G., Mieusset, R., Pontonnier, F., Calvas, P., Bujan, L., Bourrouillout, G., 2002. Cytogenetic investigations of infertile men with low sperm counts: a 25-year experience. J Androl. 23**,** 18-22; discussion 44-5.

Visootsak, J., Graham, J. M., 2003. klinefelter syndrome and its varients. Orphanet Journal of Rare Diseases. 1**,** 42.

Visootsak, J., Rosner, B., Dykens, E., Tartaglia, N., Graham, J. M., 2007. Behavioral phenotype of sex chromosome aneuploidies: 48,XXYY, 48,XXXY, and 49,XXXXY. American Journal of Medical Genetics Part A. 143A**,** 1198-1203.

Vogt, P. H., Edelmann, A., Kirsch, S., Henegariu, O., Hirschmann, P., Kiesewetter, F., Kohn, F. M., Schill, W. B., Farah, S., Ramos, C., Hartmann, M., Hartschuh, W., Meschede, D., Behre, H. M., Castel, A., Nieschlag, E., Weidner, W., Grone, H. J., Jung, A., Engel, W., Haidl, G., 1996. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet. 5**,** 933-43.

Wang, T., Liu, J. H., Yang, J., Chen, J., Ye, Z. Q., 2009. 46, XX male sex reversal syndrome: a case report and review of the genetic basis. Andrologia. 41**,** 59-62.

Warburg, E., 1963. A fertile patient with Klinefelter's syndrome. Acta Endocrinol (Copenh). 43**,** 12-26.

Wikström, A. M., Dunkel, L., 2011. Klinefelter syndrome. Best Practice & Research Clinical Endocrinology & Metabolism. 25**,** 239-250.

Wikstrom, A. M., Hoei-Hansen, C. E., Dunkel, L., Rajpert-De Meyts, E., 2007. Immunoexpression of androgen receptor and nine markers of maturation in the testes of adolescent boys with Klinefelter syndrome: evidence for degeneration of germ cells at the onset of meiosis. J Clin Endocrinol Metab. 92**,** 714-9.

Wikstrom, A. M., Painter, J. N., Raivio, T., Aittomaki, K., Dunkel, L., 2006. Genetic features of the X chromosome affect pubertal development and testicular degeneration in adolescent boys with Klinefelter syndrome. Clin Endocrinol (Oxf). 65**,** 92-7.

Xiao, X. S., Jiang, L. Q., Wang, T. Y., Wang, Y. Q., Lu, L. H., He, R., 2010. [Study on the relationship between partial deletions in the azzospermia factor C region of Y chromosome and male infertility]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 27**,** 294-8.

Yarali, H., Bozdag, G., 2006. An ongoing pregnancy after frozen thawed embryo transfer in a patient with Klinefelter's syndrome. Gynecol Obstet Invest. 62**,** 165-7.

Yoshida, A., Nakahori, Y., Kuroki, Y., Motoyama, M., Araki, Y., Miura, K., Shirai, M., 1997. Dicentric Y chromosome in an azoospermic male. Mol Hum Reprod. 3**,** 709-12.

Zeger, M., Zinn, A., Lahlou, N., Ramos, P., Kowal, K., Samangosprouse, C., Ross, J., 2008. Effect of Ascertainment and Genetic Features on the Phenotype of Klinefelter Syndrome. The Journal of Pediatrics. 152**,** 716-722.

Zhang, F., Lu, C., Li, Z., Xie, P., Xia, Y., Zhu, X., Wu, B., Cai, X., Wang, X., Qian, J., Jin, L., 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. J Med Genet. 44**,** 437-44.

Zhang, L., Rothman, N., Wang, Y., Hayes, R. B., Bechtold, W., Venkatesh, P., Yin, S., Dosemeci, M., Li, G., Lu, W., Smith, M. T., 1996. Interphase cytogenetics of workers exposed to benzene. Environ Health Perspect. 104 Suppl 6**,** 1325-9.

Zhang, Q. S., Li, D. Z., 2009. A case of 48,XXYY syndrome detected prenatally by QF-PCR. J Matern Fetal Neonatal Med. 22**,** 1214-6.

Zhang, X. Y., Li, Y. Q., Zheng, K. Q., Zhou, R. B., Chen, X. P., Liao, X., 2006. [Analysis on genetic defects of patients with azoospermia and severe oligospermia--report of 2 novel abnormal karyotypes]. Yi Chuan. 28**,** 646-51.

Zinn, A. R., Ramos, P., Elder, F. F., Kowal, K., Samango-Sprouse, C., Ross, J. L., 2005. Androgen receptor CAGn repeat length influences phenotype of 47,XXY (Klinefelter) syndrome. J Clin Endocrinol Metab. 90**,** 5041-6.

Zitzmann, M., Depenbusch, M., Gromoll, J., Nieschlag, E., 2004. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab. 89**,** 6208-17.