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Master’s Thesis

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“An investigation of the genetic and non-genetic causes of Male Factor Infertility in the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos”

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Date: ____________

Kaunas
2016/17
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1. SUMMARY

Author: Orwa Majzoub
Title: An investigation of the genetic and non-genetic causes of Male Factor Infertility in patients of the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos.

Research Aim: To investigate and analyze the most prevalent genetic and non-genetic causes of male factor infertility in patients consulted at the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos.

Research Objectives: To inspect demographic, anamnestic and clinical data in infertile men, consulted at the Department of Genetics and Molecular Medicine of the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos for the period of 2010-2016; to analyze the genetic tests of infertile men (Karyotype, Y Chromosome microdeletion, CFTR gene mutation); to elucidate the significance of the collected data, regarding the most prevalent genetic and non-genetic causes in primarily and secondarily infertile males.

Method(s): Search engines were consulted for the most relevant papers published pertaining to male factor infertility using the keywords “genetics” and “male infertility,” for an extensive review of the causes of infertility that should be looked for in the studied patients. Retrospective analysis and data collection from patients’ clinical case histories were done to identify the most prevalent genetic and non-genetic causes of infertility, in males consulted by the Department of Genetics and Molecular Medicine of the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos.

Research Result(s): The average age of infertile males was 35 ± 5 years; they had a median duration of infertility of 3 (min 1 – max 20) years. Of those infertile men, 66% had primary infertility and 11% had secondary infertility. A normal sperm analysis was found in 28% of them, while 78% had abnormal sperm parameters. Karyotype analysis revealed that 88% of the males had normal male karyotype (46,XY), while 12% exhibited variations. Secondarily infertile men were older than those with primary infertility (40 ± 4.54 years vs. 35 ± 4.72 years, respectively). Azoospermia was only present in primarily infertile males (26%); teratozoospermia however, was more common in those with secondary infertility (25% vs. 5%, respectively; p< 0.05). Urinary tract infections were more common in patients with secondary infertility than those with primary infertility (13% vs. 2%). Genetic infertility was only present in primarily infertile males, who exhibited chromosomal aberrations, Y-Chromosome microdeletion in the AZFc subregion and CFTR gene mutation of ΔF 508 region.

Research Conclusion(s): Clinical factors such as, azoospermia, teratozoospermia and urinary tract infections were the most common causes of infertility, respectively. The most common genetic cause of infertility was chromosomal aberrations. Genetic infertility due to chromosomal aberrations, Y Chromosome microdeletions and CFTR gene mutations was only found in primarily infertile males. Men with secondary infertility were older and had better sperm quality. Urinary tract infections, in secondarily infertile men, could have decreased their ability to reproduce. Other sperm analysis parameters and medical conditions did not remotely differ in their effect on infertility in both groups.
2. CONFLICTS OF INTEREST

The author reported no conflicts of interest.
3. CLEARANCE ISSUED BY THE ETHICS COMMITTEE

Permission to conduct the research was obtained from Lithuanian University of Health Sciences, Bioethics Center, under the title “An investigation of the genetic and non-genetic causes of Male Factor Infertility in the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos”, No. BEC-MF-417, 2017-04-28.
4. ABBREVIATIONS

ART - Assisted reproductive technology
CF - Cystic fibrosis
CHARGE syndrome - Coloboma, heart defects, atresia of the nasal choanae, retardation of growth and development, genital and urinary abnormalities, and ear abnormalities and deafness.
CHD7 - Chromodomain-helicase-DNA-binding protein 7
FGF8 - Fibroblast growth factor 8
FGFR1 - Fibroblast growth factor receptor 1
FSH - Follicle-stimulating hormone
GnRH - Gonadotropin-releasing hormone
KAL1 - Kallmann syndrome 1 gene
KS - Kallmann syndrome
LH - Luteinizing hormone
NS - Noonan syndrome
PG - Pituitary gland
PTPN11 - Tyrosine-protein phosphatase non-receptor type 11
SOS1 - Son of sevenless homolog 1
SCOS - Sertoli-cell only syndrome
T - Testosterone
5. INTRODUCTION

Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up like ours, with a strong emphasis on childbearing. According to the International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO), infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility can be:

1. Primary – Failure of couple to conceive and carry a pregnancy to a live birth after 12 months of regular intercourse without the use of contraception in women <35 years; and after 6 months of regular intercourse without the use of contraception in women ≥35 years [1].
2. Secondary – When a woman is unable to bear a child, either due to the inability to become pregnant or the inability to carry a pregnancy to a live birth following either a previous pregnancy or a previous ability to carry a pregnancy to a live birth. Thus those who repeatedly spontaneously miscarry or whose pregnancy results in a stillbirth, or following a previous pregnancy or a previous ability to do so, are then not unable to carry a pregnancy to a live birth would present with secondarily infertile.

About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility [2], and genetic abnormalities are thought to account for 15%–30% of male factor infertility [3].

Genetics contributes to infertility by influencing a variety of physiological and clinical processes including hormonal homeostasis, spermatogenesis and sperm quality. For instance, chromosomal abnormalities, Y Chromosome microdeletions and CFTR gene mutations can impact sperm quality producing abnormalities ranging from severe oligozoospermia to azoospermia.

Retrospective analysis and data collection from patients’ case histories was done to identify the most relevant genetic and non-genetic causes of male factor infertility and determine their prevalence.

Therefore, an understanding of the basis of reproductive failure is essential to appropriately manage an infertile couple. Considered as one of the most perplexing disorders in the reproductive field, male factor infertility is prevalent, and its incidence is rising while its etiology remains elusive. This retrospective analysis will discuss the genetic and non-genetic causes of male factor infertility that are considered most relevant today.
6. AIM AND OBJECTIVES OF THE THESIS

The general aim of this thesis is to investigate and analyze the most prevalent genetic and non-genetic causes of male factor infertility in patients consulted at the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos.

The research objectives are:

1. to determine the clinically important demographic, anamnestic and clinical data from a set of parameters taken from case histories of infertile men, consulted at the Department of Genetics and Molecular Medicine of the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos for the period of 2010-2016;

2. to analyze the genetic tests of infertile men (Karyotype, Y Chromosome microdeletion, CFTR gene mutation) and to evaluate the relationship between genetics and infertility;

3. to elucidate the significance of the collected data, regarding the most prevalent genetic and non-genetic causes in primarily and secondarily infertile males.
7. LITERATURE REVIEW

7.1 Infertility

7.1.1 Definition and prevalence – Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year, World Health Organization (WHO) [2]. About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounter problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child [2].

In developed countries, female factor infertility was reported in 37% of infertile couples, male factor infertility in 8%, and both male and female factor infertility in 35%. 5% of couples had unexplained infertility and 15% became pregnant during a study performed by The World Health Organization (WHO) task force on Diagnosis and Treatment of Infertility of 8500 infertile couples and utilized standard diagnostic criteria to determine the medical conditions contributing to infertility. This study illustrates that infertility should not be assumed to result primarily from disorders in the female partner.

7.1.2 Etiology of male factor infertility – Infertility affects both men and women. In 50% of involuntarily childless couples, a male-infertility-associated factor is found together with abnormal semen parameters. A fertile partner may compensate for the fertility problem of the man and thus infertility usually manifests if both partners have reduced fertility [2]. Categories of male infertility — The causes of male infertility can be divided into the following four main areas:

1. Endocrine and systemic disorders
   - Hypothalamic pituitary disease (secondary hypogonadism) – 1 to 2 percent (table 1)

<table>
<thead>
<tr>
<th>Congenital disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital GnRH deficiency (Kallmann syndrome)</td>
</tr>
<tr>
<td>Hemochromatosis</td>
</tr>
<tr>
<td>Multiorgan genetic disorders (Prader-Willi syndrome, Laurence-Moon-Beidl syndrome, familial cerebellar ataxia)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary and hypothalamic tumors (pituitary macroadenoma, craniopharyngioma)</td>
</tr>
<tr>
<td>Infiltrative disorders (sarcoidosis, histiocytosis, tuberculosis, fungal infections)</td>
</tr>
<tr>
<td>Lymphocytic infundibulitis or hypophysitis</td>
</tr>
</tbody>
</table>

Table 1: Hypothalamic-pituitary disorders.
Trauma, postsurgery, postirradiation
Vascular (pituitary infarction, aneurysm)
Hormonal (hyperprolactinemia, androgen excess, estrogen excess, cortisol excess)
Drugs (exogenous androgens, opioids and psychotropic drugs, GnRH agonists or antagonists)

**Systemic disorders**

Chronic illnesses
Nutritional deficiencies
Obesity

- Testicular disease (primary testicular defects and other genetic causes) – 30 to 40 percent (table 2)

### Table 2: Testicular defects in sperm/hormone production.

<table>
<thead>
<tr>
<th>Congenital disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klinefelter's syndrome (XXY) and its variants (XXY/XY; XXXY)</td>
</tr>
<tr>
<td>Cryptorchidism</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
</tr>
<tr>
<td>Functional prepubertal castrate syndrome (congenital anorchia)</td>
</tr>
<tr>
<td>Androgen insensitivity syndromes</td>
</tr>
<tr>
<td>5-alpha-reductase deficiency</td>
</tr>
<tr>
<td>Estrogen receptor or synthesis disorders</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections - Viral orchitis (mumps, echovirus, arbovirus); Granulomatous orchitis (leprosy, tuberculosis); Epididymo-orchitis (gonorrhea, chlamydia)</td>
</tr>
<tr>
<td>Drugs - Alkylating agents, alcohol, marijuana, antiandrogens, ketoconazole, spironolactone, histamine2 receptor antagonists, ionizing radiation</td>
</tr>
<tr>
<td>Environmental toxins - Dibromochloropropane, carbon disulfide, cadmium, lead, mercury, environmental estrogens and phytoestrogens; smoking; hyperthermia</td>
</tr>
<tr>
<td>Immunologic disorders, including polyglandular autoimmune disease and anti-sperm antibodies</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
<tr>
<td>Testicular torsion</td>
</tr>
</tbody>
</table>

### Systemic illness

Renal failure, hepatic cirrhosis, cancer, sickle cell disease, amyloidosis, vasculitis, celiac disease

2. Genetic disorders of spermatogenesis (including Y chromosome microdeletions) – 10 to 20 percent (table 3)

### Table 3: Genetic disorders.

| Y chromosome microdeletions and related disorders |
| Autosomal and X chromosome defects |
| Mutations causing severe defects in sperm morphology |
3. Post-testicular defects (disorders of sperm transport) – 10 to 20 percent (table 4)

4. Idiopathic – 40 to 50 percent (table 4)

Idiopathic infertility should be distinguished from unexplained infertility, which refers to couples in whom no apparent cause can be found and the male has normal semen analyses.

**Table 4: Developmental sperm transport disorders, and idiopathic male infertility.**

<table>
<thead>
<tr>
<th>Developmental and sperm transport disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicocele</td>
</tr>
<tr>
<td><strong>Sperm transport disorders</strong></td>
</tr>
<tr>
<td>Epididymal dysfunction (drugs, infection)</td>
</tr>
<tr>
<td>Abnormalities of the vas deferens (congenital absence, Young's syndrome, infection, vasectomy)</td>
</tr>
<tr>
<td>Seminal vesicles and prostate</td>
</tr>
<tr>
<td>Ejaculatory ducts disorders</td>
</tr>
<tr>
<td><strong>Sexual dysfunction</strong></td>
</tr>
<tr>
<td>Ejaculatory dysfunction (spinal cord disease, autonomic dysfunction); premature ejaculation; erectile dysfunction</td>
</tr>
<tr>
<td><strong>Idiopathic male infertility</strong></td>
</tr>
</tbody>
</table>

### 7.2 Endocrine and systemic disorders

#### 7.2.1 Hypothalamic-pituitary disease

Any hypothalamic or pituitary disease can cause gonadotropin-releasing hormone (GnRH) or gonadotropin deficiency (hypogonadotropic hypogonadism) and therefore infertility. These conditions can be subdivided into congenital, acquired, or systemic disorders. It is important to diagnose secondary hypogonadism, because gonadotropin treatment is often successful. All of these disorders are discussed in detail separately.

#### 7.2.1.1 Congenital disorders

Congenital idiopathic hypogonadotropic hypogonadism is characterized by isolated gonadotropin deficiency resulting in eunuchoidism (sexual infantilism and eunuchoidal body habitus) and sometimes impaired olfactory function (anosmia). In addition, many of the men have midline facial defects, color blindness, hearing difficulties, renal malformations, and cryptorchidism (Kallmann's syndrome). The underlying cause of the hypogonadism is a defect in GnRH secretion. Some men have gonadotropin subunit mutations causing hypogonadotropic hypogonadism. In studies of a population of Estonian men, a single nucleotide polymorphism in the follicle-stimulating hormone (FSH) beta gene promoter correlated with serum FSH concentrations and reproductive parameters [5-7]. Men with the T allele had smaller testis volume, lower sperm concentration, and lower serum FSH levels. It is
possible that they would respond well to exogenous FSH therapy. A rare cause of male infertility is an inactivating mutation in the FSH receptor gene [8,9]. One report described five men who were homozygous for an inactivating mutation of the FSH receptor [8]. These men had variably low sperm counts and serum inhibin B concentrations and high serum FSH concentrations. Other genetic disorders of gonadotropin secretion include multiorgan genetic syndromes such as the Laurence-Moon-Biedl syndrome, Prader-Willi syndrome, Lowe oculocerebral syndrome, and familial cerebellar ataxia [9].

7.2.1.2 Acquired diseases

**Tumors** – Tumors associated with hypogonadotropic hypogonadism include pituitary macroadenomas (macroprolactinomas and nonfunctioning adenomas) or surgical and radiation therapy of either macroadenomas or craniopharyngiomas and other sellar masses.

**Infiltrative diseases** – Infiltrative diseases include sarcoidosis, histiocytosis, tuberculosis, fungal infections, transfusion-related hemosiderosis, and hemochromatosis (a genetic disorder of mucosal iron transport that results in increased deposition of iron in many tissues including the pituitary gland). Hemochromatosis-associated hypogonadotropic hypogonadism is nearly always of postpubertal onset.

Lymphocytic hypophysitis or infundibulitis — This is an autoimmune condition that affects the pituitary and/or the infundibulum, resulting in enlargement of the pituitary gland that may spontaneously involute or require pharmacologic treatment of the inflammation.

**Vascular lesions** – Vascular lesions include pituitary infarction and carotid aneurysm.

**Endocrinopathies** – Functional hypogonadotropic hypogonadism and infertility can be induced by hyperprolactinemia, estrogen excess, glucocorticoid excess, and androgen excess.

- Lactotroph adenomas are the most likely cause of hyperprolactinemia. Less common causes are medications and hypothyroidism.
- Estrogen excess may be due to estrogen therapy or to estrogen production by a testicular tumor.
• Chronic glucocorticoid therapy or other causes of Cushing’s syndrome in men result in lower serum testosterone concentrations and inappropriately normal serum gonadotropins, suggesting an alteration of hypothalamic GnRH secretion.

• Androgen overproduction due to congenital adrenal hyperplasia or to tumors of the testis or adrenal glands suppresses gonadotropin secretion.

**Drugs** – Opioid-like or other central nervous system-activating drugs, including many psychotropic drugs, can inhibit GnRH or gonadotropin secretion, resulting in secondary hypogonadism and infertility. GnRH analogues (agonists and antagonists) usually are given to suppress gonadotropin secretion, as in men with prostatic carcinoma; infertility is an expected effect of this treatment.

Androgen excess may also be due to the administration of either exogenous testosterone or other anabolic steroids. Anabolic steroid use should be suspected in men with low sperm counts, low serum luteinizing hormone (LH) concentrations, and a well-androgenized phenotype.

While some men are being prescribed testosterone for true hypogonadism, others with vague symptoms such as fatigue and sexual function but normal serum testosterone concentrations are also being prescribed testosterone. Exogenous testosterone use suppresses spermatogenesis and causes infertility. In a review of 1540 men evaluated for infertility, 110 (7.1 percent) were taking supplemental testosterone. Median sperm concentration 4.5 months after stopping testosterone increased from 0 to 6.3 million/mL, but some men remained azoospermic. Pregnancy rates were not addressed.

**Systemic illnesses** – Any serious systemic illness or chronic nutritional deficiency can cause hypogonadotropic hypogonadism, primary hypogonadism, and infertility.

**Obesity** – Obesity in women appears to be associated with subfertility and poor reproductive outcomes. Obesity in men results in hypogonadotropic hypogonadism with total testosterone, free testosterone, and low or inappropriately normal gonadotropin concentrations. The obesity-associated decrease in serum sex hormone-binding globulin (SHBG) contributes to the low serum total testosterone concentrations. In addition, serum free testosterone concentrations appear to be inversely related to body weight and body mass index (BMI), independent of changes in SHBG. Other factors contributing to the hypogonadotropic hypogonadism seen with obesity include an increase in estrogens through aromatization in adipose tissue, insulin resistance, metabolic syndrome, diabetes mellitus, and sleep apnea.
The relationship between obesity and semen parameters is less clear, with some studies reporting lower sperm concentrations/motility and volume with increasing BMI and others finding no association. Sperm quality may also be inversely related to BMI. A cross-sectional study of over 500 men found no association between BMI and semen parameters [10], but a meta-analysis of 21 trials reported a higher risk of oligozoospermia with increasing BMI [11]. Although these data are conflicting, we still advise weight loss to obese men seeking infertility treatment, given the known negative effects of obesity on serum SHBG and testosterone concentrations.

7.2.2 Primary testicular defects in sperm production

7.2.2.1 Congenital and developmental disorders

**Klinefelter’s syndrome** – One of the most common causes of primary hypogonadism, and therefore male infertility, is Klinefelter's syndrome, which may occur in up to 1 out of 500 to 700 phenotypic males and in up to 10 to 15 percent of infertile men with azoospermia and small testes [12]. It is characterized by sex chromosome aneuploidy, with an extra X (XXY) chromosome being the most frequent. These patients often have very small testes and almost always have azoospermia.

**Androgen receptor or biosynthesis disorders** – Men with congenital androgen insensitivity due to androgen receptor or postreceptor abnormalities and those with 5-alpha-reductase deficiency are nearly always infertile. Men with partial androgen insensitivity (Reifenstein's syndrome) have varying degrees of ambiguous external genitalia, hypogonadism, and infertility. Mild androgen insensitivity can cause infertility alone. Normal sexual differentiation and spermatogenesis require androgen and a normal functioning receptor. Polymorphisms of the androgen receptor gene may also be associated with male infertility. The number of trinucleotide (CAG) repeats in exon 1 of the androgen receptor is inversely correlated with the transcriptional activity of the androgen target gene. In a study of normal fertile men, those with short CAG repeats had the highest sperm output [13]. Reports of CAG repeat lengths in men with idiopathic infertility have been inconsistent. In some but not all reports, a modest association of longer CAG repeat length with male infertility and/or abnormal semen quality has been observed. In a meta-analysis of 33 studies of men with idiopathic infertility and fertile controls, those with infertility had significantly longer CAG repeat lengths than controls. While androgen receptor CAG repeat length may be a valuable tool for epidemiological studies and pharmacogenomic evaluation of efficacy in treatment trials, it is not a practical tool for assessment of individual patients.
Men with 5-alpha-reductase deficiency have pseudohermaphroditism but partially virilize at puberty. Infertility in this disorder may be due to mechanical problems associated with the small phallus, severe hypospadias, cryptorchidism, and poor prostatic secretions.

**Disorders of estrogen receptor or estrogen synthesis** – In mice lacking a functional estrogen receptor alpha, fluid absorption is impaired in the efferent tubules, resulting in excess accumulation of fluid in the seminiferous tubules and impaired spermatogenesis [14]. In a man with an inactivating mutation of estrogen receptor alpha, sperm count was normal but sperm motility was decreased [15]. Furthermore, aromatase gene knockout older adult mice are infertile because of impaired spermatogenesis. The generation of estrogen receptor beta knockout mice should provide additional information on the effect of estrogen on fertility in men. Polymorphisms of the promoter region (variable TA tandem repeats) of the estrogen receptor gene have been shown to be related to sperm production. Men with higher numbers of TA repeats have lower sperm counts. Other polymorphisms of the estrogen receptor may have different effects in different populations [16].

**Myotonic Dystrophy** – Myotonic dystrophy is an autosomal disorder with delayed onset (age 30 to 40 years) of impaired motor function, cataracts, premature frontal balding, mild mental retardation, and infertility due to impaired spermatogenesis. Only 20 percent of men with myotonic dystrophy have low serum testosterone concentrations.

### 7.2.2.2 Acquired disorders of the testes

**Infection** – Viral orchitis, especially mumps, is a well-recognized cause of infertility. Among those with mumps, clinical orchitis is rare in prepubertal males but occurs in 15 to 25 percent of adult men. Some, but perhaps not all, of these men become infertile, due either to germinal cell damage, ischemia, or the immune response to the infection. In mumps and other viral causes of orchitis (echovirus and arbovirus), germ cell failure is much more common than androgen deficiency.

Other infectious causes of orchitis and infertility include tuberculosis and leprosy; the former may also cause epididymal obstruction. Sexually transmitted diseases (STDs) such as gonorrhea and chlamydia can also cause orchitis. Human immunodeficiency virus (HIV)-infected men may have relative normal semen parameters, while others may have low sperm motility and infertility. White blood cells may be present in the semen, especially if the HIV was associated with other STDs such as gonorrhea.
Drugs and radiation – Many drugs are associated with impaired spermatogenesis and/or Leydig cell dysfunction. Among them, the most important are the alkylating drugs (cyclophosphamide and chlorambucil). Antiandrogens (flutamide, cyproterone, bicalutamide, spironolactone), ketoconazole, and cimetidine cause testicular dysfunction by inhibiting testicular androgen production or action.

Ionizing radiation impairs spermatogenesis. Doses as low as 0.015 Gy (15 rads) may transiently suppress spermatogenesis, while doses above 6 Gy (600 rads) usually cause irreversible azoospermia and infertility.

Environmental factors, smoking and hyperthermia – Environmental toxins may be an underappreciated cause of infertility. The pesticide dibromochloropropane is a well-known cause, as are lead, cadmium, and mercury. The possibility that chemicals with estrogenic or antiandrogenic activity (“endocrine disruptors”), including insecticides and fungicides, may lower sperm counts has attracted much attention lately, although direct proof of an effect in men is lacking.

RNAs and epigenetics in spermatogenesis have led to the identification of environmental toxins as causes of male infertility. Review of data of men exposed to pesticides indicates that changes in semen quality may be multifactorial, including effects on spermatogenesis, DNA damage, abnormal sperm morphology and the above-mentioned estrogenic or anti-androgenic compounds such as metabolites of dichlorodiphenyltrichloroethane (DDT). Occupational and environmental exposure has been associated with lower quality semen analyses; limited data suggest that consumption of fruits and vegetables with high pesticide residues may also be associated with lower semen quality.

Smoking – Data on cigarette smoking and its possible effect on sperm counts are inconsistent. However, in a meta-analysis of 20 observational studies, men who smoked cigarettes were more likely to have low sperm counts [17].

The possibility that in utero exposure to smoking may have a detrimental effect on sperm count in adulthood was studied in 1770 young, healthy, potential military recruits and the results showed the possibility of a small effect. Exposure to smoking in utero (after adjusting for some confounding factors, eg, the man's present smoking habits, but not others, eg, alcohol intake) was associated with mean sperm concentrations that were 20 percent lower (95% CI 7 to 34 percent) when compared with unexposed men. The fertility implication of this small difference is not known. In a second study, there were no significant differences in mean sperm concentrations in men whose mothers either smoked or did not smoke during pregnancy. However, men whose mothers had smoked ≥10 cigarettes per day while pregnant were at higher risk of having oligozoospermia (sperm concentration <20 x 106/mL). Smoking has been shown to
change microRNA content in spermatozoa. These microRNAs are associated with cell death and apoptosis. The significance of this finding, if any, on infertility and progeny is not known [18].

**Hyperthermia** – Hyperthermia has long been thought to impair spermatogenesis. Prolonged high testicular temperature may explain the infertility associated with spinal cord injuries, varicocele, and chronic sauna and Jacuzzi exposure. Studies in men have shown that small increases in testicular temperature accelerate germ cell loss through apoptosis [19]. Similarly, febrile illness, prolonged sitting during work or truck driving, welding, baking, tight fitting underwear, and laptop use with increased heat to the testes have been proposed to adversely affect male fertility. The data to support these associations are inconsistent and may be a very weak risk factor for infertility.

**Antisperm antibodies** – Some infertile men have antisperm antibodies in serum or semen and both presumably could impair spermatogenesis. Presence of sperm agglutination in the semen should trigger the laboratory to test for anti-sperm antibodies. Whether antibodies occur spontaneously or only after some testicular injury is not known. Primary hypogonadism occasionally occurs in men with type 2 autoimmune polyglandular syndrome [20].

**Systemic disorders** – Men with debilitating illnesses such as chronic renal insufficiency, cirrhosis, or malnutrition of any cause may have primary as well as secondary hypogonadism. Infertility is common in men with sickle cell anemia, presumably due to intratesticular ischemia.

### 7.3 Genetic disorders of spermatogenesis only

Although male infertility is a common disorder, a number of genetic causes have only recently been identified by a number of techniques, including genome-wide association studies (GWAS). Genetic disorders affecting spermatogenesis only can be identified in about 10 to 20 percent of cases of male infertility. With the widespread availability of assisted reproductive technologies (ART), men can have children, but there are genetic risks for the offspring.

### 7.3.1 Y chromosome and related defects

Y chromosome microdeletions and substitutions are increasingly recognized as genetic causes of azoospermia and severe oligozoospermia [21]. Up to 20 percent of infertile men have microdeletions in the long arm of the Y chromosome, many of which map to
the Yq11 region of the chromosome, that is named azoospermic factor (AZF). The AZF region of Yq11 contains three regions: AZFa, AZFb, and AZFc.

- Deletion of the AZFa and AZFb regions results in severe spermatogenesis defects and azoospermia. Testicular biopsies in these men may show germinal cell maturation arrest or Sertoli cell-only syndrome.
- Deletions of AZFc that cause infertility have a variable phenotype ranging from oligozoospermia to azoospermia and represent the largest well-defined recurrent deletions in the human genome [22,23].

The AZFb and AZFc regions contain large sections of duplicate sequences allowing for rearrangements and partial deletions. The gr/gr deletion removes a large segment of the AZFc gene and represents a significant risk factor for oligozoospermia in some, but not all, populations.

The focus on candidate genes has been on the AZFa region because this region, unlike the AZFb and AZFc, does not have repeat sequences. DDX3Y (the DEAD [Asp-Glu-Ala-Asp] box polypeptide 3, Y-linked gene) and USP9Y are genes located in the AZFa region of the Y chromosome. USP9Y has been considered to be a candidate gene for male infertility, as deletions in the gene have been observed in men with azoospermia or severe oligozoospermia. However, deletions in USP9Y have also been reported in two men with normal fertility (a normospermic male and his father), suggesting that USP9Y does not have an important independent role in spermatogenesis. When both USP9Y and DDX3Y are deleted, azoospermia is consistently seen, suggesting either that DDX3Y has a critical role in the regulation of spermatogenesis or that the two adjacent genes are necessary for normal sperm development [24].

Y chromosome deletions may be detectable not only in men with idiopathic “oligozoospermia” or azoospermia, but also in men with identifiable other causes of testicular dysfunction. As an example, in a study of 131 infertile men, Y chromosome deletions were found in 16 of 85 men (19 percent) with idiopathic oligo- or azoospermia and 3 of 46 men (7 percent) with disorders such as cryptorchidism, varicocele, and obstructive lesions of the vas deferens [27]. Similar results were seen in a second report.

7.3.2 Autosomal and X chromosome defects – A number of autosomal and X-linked genes have been identified as regulators of spermatogenesis. Gene mutations that have been associated with possible male infertility or increased risk of infertility include:
• Polymorphisms of DAZL (T54A), an autosomal homolog of the DAZ (deleted in azoospermia) gene [28].
• In a genome-wide association study in Han Chinese men [29] and follow-up validation study of three single nucleotide polymorphisms identified as risk loci for azoospermia, the SOX5 gene was significantly associated with nonobstructive azoospermia.
• X-linked TEX11 mutations appear to be an important cause of meiotic arrest and azoospermia in infertile men. In a report of 289 patients with azoospermia and 384 controls, hemizygous TEX11 mutations on chromosome Xq13.2 were identified in 7 of 289 men with azoospermia (2.4 percent) [29]. Five of the mutations were in 33 men (15 percent) with azoospermia and meiotic arrest. In testes from normal men, immunohistochemical analysis showed TEX11 expression in late spermatocytes and in round and elongated spermatids. Testes from patients with azoospermia and TEX11 mutations had meiotic arrest and no TEX11 expression.
• Copy number variants (CNVs) in the X chromosome – Using high-resolution array-comparative genomic hybridization, CNVs in a large number of infertile men and controls showed that recurrent deletions (CNV67, 64, and 69) were more frequent in infertile men with lower sperm counts versus controls [30]. More recent studies indicated that recurrent duplications resulting in X chromosome gene gains in infertile men might also be related to lower sperm counts and new genes that regulate spermatogenesis [31].

7.3.3 Epigenetics in male infertility – Epigenetics in male infertility has only recently been studied. Sperm DNA methylation, histone acetylation, and noncoding RNAs (including microRNAs, long non-coding RNAs, and sRNAs) may contribute to defective embryogenesis and idiopathic male infertility. Both hypo- and hyper-DNA methylation have been reported with imprinted genes in men with infertility [32]. The epigenetic changes in sperm DNA methylation, histone acetylation, or non-coding RNAs are believed to be related to offspring’s outcome in aging men, obesity, and environmental toxicants [33].

7.3.4 Severe defects of sperm morphology – Gene mutations have been identified in men with severe defects of sperm morphology. Globozoospermia (sperm with a round head and no acrosome that are unable to fertilize oocytes) is most commonly caused by mutations in the DPY19L2 gene [34] (found in about 70 percent of men with this condition). AURKC mutations have been identified in men with macrocephalic sperm (sperm with round heads and an abnormal acrosome).
7.4 Developmental and sperm transport disorders

7.4.1 Cryptorchidism – Men with a history of undescended testes have lower sperm counts, sperm of poorer quality, and lower fertility rates than men with normally descended testes. Impaired spermatogenesis in the undescended testis is probably related to underlying genetic, hormonal, and developmental abnormalities, some of which may be partially reversible through early surgical intervention. Sperm counts in adulthood are directly related to prepubertal germ cell counts and type of cell at the time of orchiopexy. The degree of germ-cell dysfunction with cryptorchidism is correlated with the duration of suprascrotal location of the testes. Serum follicle-stimulating hormone (FSH) concentrations are often high, but serum luteinizing hormone (LH) concentrations are usually normal, indicating normal Leydig cell function. Formerly cryptorchid men with low serum inhibin B and high FSH concentrations may be at particularly high risk for infertility. Bilateral cryptorchidism must be distinguished from the functional bilateral castrate syndrome, in which the testes are not detectable in the abdomen or other location; the latter condition is not associated with an increased risk of testicular tumors.

7.4.2 Testicular cancer – There is evidence of an increased incidence of testicular cancer in men presenting with infertility (even in the absence of a history of cryptorchidism). As an example, in one observational study of 3847 men with oligozoospermia (using previously published rather than current World Health Organization [WHO] criteria for normal semen parameters [35], defined as sperm concentration less than 20 million/mL with concomitant defects in total motility [less than 50 percent]), 10 cases of testicular cancer were seen (8 of 10 with no history of cryptorchidism) [36]. When compared with a control population, this represented approximately an 18-fold greater incidence of testicular cancer (standardized incidence ratio 18.3, 95% CI 18.0-18.8). In a study in United States fertility centers, 34 cases of germ cell tumors were found in 22562 male partners of the couples seeking infertility treatment, giving a hazard ratio (HR) of 2.8 (CI 1.5-2.8) compared with men without male infertility [37]. However, these studies are limited by the small number of cases, and thus, routine screening for testicular cancer in men who present with infertility is not warranted at this time.

7.4.3 Varicocele – Varicocele is a dilatation of the pampiniform plexus of the spermatic veins in the scrotum. Left-sided varicoceles are 10 times more common than right-sided ones, perhaps because of anatomic variations that lower blood flow in the left spermatic vein. The mechanisms by which a varicocele might cause infertility and the reversibility of infertility after varicocele surgery are
controversial. Varicoceles are found in about 10 to 15 percent of normal men and an even higher percentage of infertile men; the former finding has led many clinicians to question whether a varicocele alone can cause infertility.

7.4.4 Disorders of sperm transport – The epididymis is an important site for sperm maturation and an essential part of the sperm transport system. The vas deferens then transports sperm from the epididymis to the urethra, where they are diluted by secretions from the seminal vesicles and prostate. Abnormalities at any of these sites, particularly the epididymis and vas deferens, can cause infertility. Finally, sperm must be ejaculated.

Abnormalities of the epididymis – Absence, dysfunction, or obstruction of the epididymis leads to infertility even though testicular sperm production is normal. Intrauterine exposure to estrogens may cause epididymal dysfunction. Little is known about functional abnormalities of the epididymis, but some drugs used in other countries (eg, triptolide) and chemical toxins (chlorohydrin) affect the function of metabolism of spermatozoa within the epididymis. While poorly documented, it is presumed that some men with isolated asthenospermia (impaired motility) have defects of epididymal function.

Abnormalities of the vas deferens – Male infertility can result from acquired or congenital abnormalities of the vas deferens. Bilateral obstruction, ligation, or altered peristalsis of the vas deferens results in infertility. Obstruction may result from infection (gonorrhea, chlamydia, tuberculosis), while ligation of the vas deferens (vasectomy) is an intentional, medically-induced cause of infertility. It may be reversible, but some men have an immune response to sperm granulomas that form on the proximal side of the ligation and remain infertile after adequate reanastomosis of the vas. One to 2 percent of infertile men have bilateral congenital absence of the vas deferens. Most have mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Many infertile men with mutations of CFTR present with infertility in the absence of many of the other findings (eg, respiratory and pancreatic disease). A primary ciliary dyskinesia is a genetically heterogeneous disease that affects cilia function and structure. The clinical presentations include recurrent sinopulmonary infections, bronchiectasis, situs inversus, and male infertility (with asthenozoospermia or oligozoospermia [38]). Genetic mutations of dynein proteins or thioredoxin-nucleoside diphosphate kinase have been implicated to cause primary ciliary dyskinesia.
A similar genetic defect that may lead to abnormal transport of sperm is Young's syndrome, in which inspissated secretions within the vas and epididymis interfere with transport of sperm, leading to obstructive azoospermia; impaired axonemal structures are sometimes found [39].

**Ejaculatory duct obstruction** – Patients with ejaculatory duct obstruction present with a low ejaculate volume and seminal fructose with no sperm count and/or very low sperm motility. Ejaculatory duct obstruction is uncommon but can be diagnosed and treated surgically with minimally invasive techniques.

**Seminal vesicles and prostate** – It is not known if abnormal function of the seminal vesicles and prostate contributes to infertility, but chronic infection of the accessory glands may result in leukospermia and may be a possible cause of infertility.

7.4.5 **Sexual dysfunction** – Spinal cord disease or trauma, sympathectomy, or autonomic disease (eg, diabetes mellitus) all can interfere with normal ejaculation and lead to decreased fertility.

Erectile dysfunction, mechanical obstruction (condoms and diaphragm use), premature ejaculation, and infrequency of intercourse also may be contributing factors.

7.5 **Idiopathic male infertility** – Male infertility of unknown origin is a condition in which fertility impairment occurs spontaneously or due to an obscure or unknown causes. It includes, unexplained male infertility and idiopathic male infertility. The dividing line between them is semen analysis, which is normal in unexplained category and abnormal in idiopathic infertility.

Despite careful assessment of all possible causal mechanisms, a cause of abnormal sperm number, morphology, or function cannot be identified in a substantial proportion of infertile men. There are also men who have repeatedly normal semen analyses but cannot impregnate an apparently normal female partner.

7.6 **Evaluation of male factor infertility**

Guidelines for male factor infertility investigation are numerous. The most popular ones that are widely used are those created by the European Association of Urology (EAU) and American Society of
Reproductive Medicine (ASRM). However, generally, all guidelines used should include both genetic and non-genetic tests represented in figures 1 and 2.

**Figure 1: Algorithm 1 of male factor infertility evaluation.**

No pregnancy within 1 year of unprotected intercourse obtain reproductive history and physical exam → Sperm analysis

No pregnancy within 1 year of unprotected intercourse obtain reproductive history and physical exam → Sperm analysis

**Figure 1**

1. **No pregnancy within 1 year of unprotected intercourse**
   - Obtain reproductive history and physical exam → Sperm analysis

2. **Reduced sperm count, often with abnormal morphology and <50 percent motility**
   - Karyotyping for all males presenting with infertility (for chromosomal morphological alterations)

3. **Normal sperm count with abnormal morphology or decreased motility**
   - After 3 months, if no natural pregnancy achieved.

4. **Normal, with no abnormality in female**
   - Repeat semen analysis

5. **Abnormal**
   - Specialized tests of sperm function
   - Measure serum T, FSH, LH

6. **↓ T ↑ FSH ↑ LH**
   - Primary panhypogonadism
     - Karyotype (XXY); Genetic testing: NS (PTPN11, SOS1, RAF1), Androgen insensitivity (AR gene)

7. **↑ FSH Normal LH**
   - Germinal epithelium failure

8. **Normal FSH Normal LH**
   - Hypogonadotropic hypogonadism

9. **↓ T ↓ FSH ↓ LH**
   - Partial androgen resistance

10. **Normal T Normal FSH Normal LH**
    - See next figure

11. **↓ T FSH LH**
    - Azoo- or severe oligozoospermia: Karyotype and Y chromosome microdeletions

12. **↑ T FSH LH**
    - Prolactin; Imaging of PG; Genetic testing: KS (KAL, FGFR1, FGF8), CHARGE syndrome (CHD7).
Figure 2: Algorithm 2 of male factor infertility evaluation.
8. RESEARCH METHODOLOGY AND METHODS

8.1 Studied patients’ sample selection

The Department of Genetics and Molecular Medicine conducted the research planning. Infertile males consulted by clinical geneticists were the objects of the study and participant selection involved the Lithuanian population for the duration of 2010 – 2016. A total of 241 clinical case histories were analyzed.

Retrospective analysis and data collection from these histories were done to identify the prevalence of the reviewed causes of male factor infertility, in patients of the Department of Genetics and Molecular Medicine of the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos. The selected patients were narrowed down to only those who had full and complete history recorded, as presented in Figure 3. 74 patients (31%) were then selected for conducting the research; 50 patients (68%) had a complete history excluding the Y Chromosome microdeletion test and 24 (32%) including it. Such data was collected from these case histories:

- age;
- type and period of infertility;
- laboratory and genetic tests (sperm analysis, karyotype, Y-chromosome microdeletion test, CFTR gene mutation);
- anamnesis and medical history;
- smoking.
8.2 Methods for collection of infertility causes

For the genetic and non-genetic causes of male factor infertility, an exhaustive literature review was performed in PubMed and UpToDate using the keywords “genetics” and “male infertility.” The results were filtered by limiting the search to English manuscripts published within the last 10 years that discussed studies of human subjects. This initial search produced 646 associated articles. Subsequent searches were performed using the keywords “Y chromosome,” “epigenetics,” “genomics,” “proteomics,” and “metabolomics” to further supplement the information obtained. After careful review of the abstracts, 40 articles were selected for inclusion in the manuscript. This group of articles consisted of one meta-analysis, 19 original articles, and 20 review articles. The results of the review were collected based on the most relevant causes.
8.3 Statistical analysis

Frequencies are presented in percentages; parametric results are presented as mean ± SD and non-parametric results as median (range). Means were compared by using t-test using SPSS software and a $p \leq 0.05$ was taken as statistically significant.
9. RESULTS

9.1 Non-genetic factors

The average age of infertile males that presented at the Department of Genetic and Molecular Medicine of Lithuanian University of Health Sciences, Kauno Klinikos between 2010 and 2016 was 35 ± 5 years with a median age of 34 (26-50) years. These males had a median duration of infertility of 3 (1-20) years. 89% of them men had primary infertility, whereas, 11% were secondarily infertile. A normal sperm analysis (normozoospermia) was found in 28% of them, while 78% had abnormal sperm parameters, most of which were azoospermia – 23%. Infertility-causing medical conditions were found in 22% of the patients, where mumps, urogenital disorders and urinary tract infections were the most common.

9.2 Genetic factors

Karyotype analysis revealed that 88% of males had normal male karyotype (46,XY), while 12% exhibited variations. These variations were chromosomal aberrations, aneuploidies and mosaicism respectively. Chromosomal aberrations with a prevalence of 5% consisted of the following karyotypes: 46,XY,9qh-; 46,XY,add(16)(q13); 46,XY,inv(9)(p11;q13); and 46,XY,qh+. Aneuploidies of 4% only included those for KFS (47, XXY), and mosaicism with a 2% prevalence consisted of mos46,X,idic(Y)(p11.3)[35]/45,X[5] and mos45,X[46]/46,XY[15]. 8% of these infertile males had Y Chromosome microdeletions of the AZFc subregion and 3% had CFTR gene mutations of ΔF 508 region.

9.3 Impact of the above mentioned genetic and non-genetic factors on males with primary and secondary infertility

9.3.1 Non-genetic factors

9.3.1.1 Demographic and clinical factors:

The detailed results are presented in Table 5.
Secondarily infertile men were older (40 ± 4.54) than those with primary infertility (35 ± 4.72), \( p=0.0059 \). Normospermia was not significantly different between primarily and secondarily infertile men, \( p>0.1 \). Azoospermia was more common in primarily infertile males than those with secondary infertility, \( p=0.1 \). Oligozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Asthenozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Oligoasthenozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Teratozoospermia was significantly more common in secondarily infertile men than those with primary infertility, \( p=0.041 \). Oligoasthenoteratozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Asthenoteratozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Necrozoospermia was not significantly different between patients with primary and secondary infertility, \( p>0.1 \).

9.3.1.2 Medical history:

The detailed results are presented in Table 5.

Insignificant medical history was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Mumps was not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Urogenital disorders (cryptorchidism, varicocele, SCOS) were not significantly different between patients with primary and secondary infertility, \( p>0.1 \). Urinary tract infections, however, were more common in patients with secondary infertility than those with primary infertility, \( p=0.098 \).

9.3.1.3 Smoking:

Smoking had no significant effect on infertility group (Table 5), \( p>0.1 \).
Table 5: Results of the patients’ demographic, anamnestic and clinical data (non-genetic factors).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary infertility n=66 (89%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD in years</td>
<td>35 ± 4.72</td>
</tr>
<tr>
<td>Median (range) in years</td>
<td>34 (26-50)</td>
</tr>
<tr>
<td>Period of infertility</td>
<td></td>
</tr>
<tr>
<td>Median (range) in years</td>
<td>3 (1-20)</td>
</tr>
<tr>
<td>Sperm analysis</td>
<td></td>
</tr>
<tr>
<td>Normozoospermia, n(%)</td>
<td>18 (27)*</td>
</tr>
<tr>
<td>Sperm abnormalities, n (%):</td>
<td></td>
</tr>
<tr>
<td>Azoospermia, n(%)</td>
<td>17 (26)</td>
</tr>
<tr>
<td>Oligozoospermia, n(%)</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Asthenozoospermia, n(%)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Oligoasthenozoospermia, n(%)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Teratozoospermia, n(%)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia, n(%)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Asthenoteratozoospermia, n(%)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Necrozoospermia, n(%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
</tr>
<tr>
<td>Insignificant medical history, n(%)</td>
<td>52 (78)*</td>
</tr>
<tr>
<td>Infertility-causing conditions, n (%):</td>
<td></td>
</tr>
<tr>
<td>Mumps (epidemic parotitis), n(%)</td>
<td>7 (11)</td>
</tr>
<tr>
<td>Urogenital disorders, n(%)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Urinary tract infections, n(%)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Smokers, n(%)</td>
<td>18 (27)</td>
</tr>
<tr>
<td>Non-smokers, n(%)</td>
<td>48 (73)</td>
</tr>
<tr>
<td>Partner’s pregnancy history (1 or multiple)</td>
<td></td>
</tr>
<tr>
<td>No pregnancy, n(%)</td>
<td>44 (67)</td>
</tr>
<tr>
<td>Spontaneous miscarriage, n(%)</td>
<td>18 (27)</td>
</tr>
<tr>
<td>Missed abortion, n(%)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Ectopic pregnancy, n(%)</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

*Percentages were calculated for total patients within each group of infertility.
9.3.2 Genetic factors:

The detailed results are presented in Table 6.

Patients with primary infertility had karyotype variations as shown in Table 6. The chromosomal aberrations with a prevalence of 6% were: 46,XY,9qh-; 46,XY,add(16)(q13); 46,XY,inv(9)(p11;q13); and 46,XY,qh+. Aneuploidy of 5% only included that for KFS (47, XXY), and mosaicism with a 3% prevalence consisted of mos46,X, dic(Y)(p11.3)[35]/45,X[5] and mos45,X[46]/46,XY[15]. However, men with secondary infertility did not have any karyotype variations. From a total of 24 patients analyzed, 2 patients that had primary infertility also had AZFc subregion deletion (9%); on the contrary, those with secondary infertility (n=1) did not have any Y Chromosome microdeletion. From a total of 74 patients only 2 patients were tested for CFTR gene mutation and revealed ΔF 508 region deletion, had primary infertility, whereas, no patients with secondary infertility were indicated for testing.

Table 6: Results of the patients’ genetic tests.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary infertility n=66 (89%)</td>
<td>Secondary infertility n=8 (11%)</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (46,XY), n(%)</td>
<td>57 (86)*</td>
<td>8 (100)*</td>
</tr>
<tr>
<td><strong>Variation of Karyotype, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosomal aberrations, n(%)</td>
<td>9 (14)*</td>
<td>0*</td>
</tr>
<tr>
<td>Aneuploidy, n(%)</td>
<td>4 (6)</td>
<td>0*</td>
</tr>
<tr>
<td>Mosaicism, n(%)</td>
<td>3 (5)</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td>2 (3)</td>
<td>0*</td>
</tr>
<tr>
<td><strong>Y Chromosome microdeletion test</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, n(%)</td>
<td>21 (91)*</td>
<td>1 (100)*</td>
</tr>
<tr>
<td>AZFc subregion deletion, n (%)</td>
<td>2 (9)*</td>
<td>0*</td>
</tr>
<tr>
<td><strong>CFTR gene mutation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFTR ΔF508, n (%)</td>
<td>2 (3)*</td>
<td>0*</td>
</tr>
</tbody>
</table>

*Percentages were calculated for total patients within each group of infertility.
10. DISCUSSION OF THE RESULTS

Secondarily infertile men of the present thesis were older than those with primary infertility. Azoospermia was more common in primarily infertile males; teratozoospermia however, was more common in those with secondary infertility. The rest of sperm analysis parameters were not significantly different in both groups. Urinary tract infections were more common in patients with secondary infertility than those with primary infertility; however, other medical conditions were not significantly different among these groups. Patients with primary infertility had karyotype variations; however, those with secondary infertility did not have any. Patients with primary infertility had Y-Chromosome microdeletion in the AZFc subregion, while; those with secondary infertility did not have any. Men indicated for CF testing were primarily infertile and had CFTR gene mutation; whereas, no patients with secondary infertility were tested.

10.1 Discussion of the non-genetic factors

According to the results of the present study, the average age of infertile men was 35 years; the median duration of infertility was 4 years. The prevalence of azoospermia in these men was 23%, while the frequency of other abnormalities of sperm parameters was 49%. Urogenital disorders (cryptorchidism, varicocele, SCOS) were found in 8% of these men. Some of these parameters were relatively close to those found by Ho K et al. [41]; which stated in their study that the average age of infertile men was 37 years, median duration of infertility was 3 years, and other abnormalities of sperm parameters was 48%. In contrast, they also found that prevalence of azoospermia and urogenital disorders were relatively higher (36% and 25% respectively). 74 men with complete data were presented in this study, while a bigger cohort of 387 patients (5 times bigger) in that by Ho K et al [41].

10.2 Discussion of the genetic factors

Comparison of Karyotype variations between patients of the present thesis and those of a study done in Southern India, and another one done by the European Association of Urology (EAU):

Among the 74 patients of the present thesis, 4 men (5%) exhibited chromosomal aberrations of: 46,XY,9qh-; 46,XY,add(16)(q13); 46,XY,inv(9)(p11;q13); and 46,XY,qh+. 3 of these patients (4%) had aneuploidy only of Klinefelter syndrome (47, XXY), and 2 had mosaicism (2%) that consisted of
mos46,X,idic(Y)(p11.3)[35]/45,X[5] and mos45,X[46]/46,XY[15]. Jaganathan S. et al [43] found in their study of 180 infertile males that chromosomal analysis revealed an abnormal karyotype in seven men (4%). Four men showed Klinefelter syndrome (47,XXY). Reciprocal translocations involving autosomes t(1;11)(p22.3;p13) and t(1;10)(q25;q24) were seen in two cases and one individual exhibited a structurally abnormal chromosome 17,der(17)t(17;?) (p12;?) [43]. In a study of the EAU by Johnson MD [44], which included 9,766 infertile men, the incidence of chromosomal (karyotype) abnormalities was 5.8% [44]. The prevalence of karyotype variations (abnormal karyotypes) was similar in all the three studies, but these variations found were individual to each selected population.

Comparison of Y Chromosomal microdeletion between patients of the present thesis and those of studies done in Europe, Central and East Asia, and Africa:

In 24 patients of the studied thesis who have undergone a Y Chromosome microdeletion analysis, no AZFa and AZFb subregion deletions were found; while, AZFc subregion deletions, being the most common Y-Chromosome microdeletions, were found in 2 patients. Maurer B et al. [45] presented in their study, that most of the microdeletions were observed in the AZFc region (15/19). Two patients presented with microdeletions in AZFb, and one patient was found to have a microdeletion in AZFa. Both subregions AZFb and AZFc were deleted in one patient [45]. In a study done by Birowo P et al. [46] the following was presented: partial deletion of AZFa subregion was found in 11 men, complete deletion of AZFb was found in 1 man and that of AZFc in another one [46]. Ambulkar P et al. [47] presented in their study that 19 had microdeletions in different AZF regions. Deletions in AZFa region were found in 3 patients, AZFb in 5 patients, whereas high frequencies of deletions in AZFc were recorded in 9 patients. In two azoospermic males were shown microdeletions in AZFb+c loci [47]. Hammami W et al. [48] presented in their study that no microdeletions were detected in the men with severe oligozoospermia. In the azoospermic group, 2/74 patients had Y chromosome microdeletions. Both had complete deletion of the AZFc region. No microdeletion was identified in the AZFa region or in the AZFb region [48].

Comparison of the CFTR gene mutations between patients of the present thesis and those of a study of the European Association of Urology:

According to the present thesis, 3% of patients had CFTR gene mutations, and consequently congenital bilateral absence of vas deferens (CBAVD). Similarly, in a study of the EAU of men with obstructive azoospermia attending a clinic in Edinburgh, UK [49], Donat R, et al. stated that congenital bilateral
absence of the vas deferens (CBAVD) is associated with CFTR gene mutations and was found in ~2% of men [49].

10.3 Discussion of the genetic and non-genetic factors in primarily and secondarily infertile men

Among the 74 patients of the present thesis, 66 males had primary infertility and 8 had secondary infertility. Azoospermia was far more common in primarily infertile men with an incidence of 26% (17 patients). Secondarily infertile men, on the other hand, were older (mean age 40 vs. 35 years), more commonly teratozoospermic – 25% with a higher incidence of normozoospermia (38%) than primarily infertile men. 9 men with primary infertility had karyotype variations; 4 of them (6%) exhibited chromosomal aberrations of: 46,XY,9qh-; 46,XY,add(16)(q13); 46,XY,inv(9)(p11;q13); and 46,XY,qh+. 3 men (5%) had aneuploidy only of Klinefelter syndrome (47, XXY), and 2 had mosaicism (3%) that consisted of mos46,X,idic(Y)(p11.3)[35]/45,X[5] and mos45,X[46]/46,XY[15]. Moreover, AZFc subregion deletions were found in 2/23 primarily infertile men (9%) who underwent a Y Chromosome microdeletion test. 2 of these men with primary infertility were indicated for CF testing, and tested positive for CFTR gene mutation. Walsh T et al. [42] found in a study of the differences in the clinical characteristics of primarily and secondarily infertile men with varicocele, that from 295 patients, 205 had primary infertility and 90 had secondary infertility. As with the clinical characteristics of patients of the present thesis, secondarily infertile men were older (mean age 39.6 vs. 35 years), but had better semen parameters than primarily infertile men [42]. The incidence of genetic factors in primarily and secondarily infertile men could not be compared due to lack of relevant studies in males of these groups.

Therefore, analysis of the genetic and non-genetic factors that impact male factor infertility will provide valuable insights into the creation of targeted treatments for patients and the determination of the causes of idiopathic infertility. Novel technologies that analyze the influence of genetics from a global perspective may lead to further developments in the understanding of the etiology of male factor infertility through the identification of specific infertile phenotype signatures.
11. CONCLUSIONS

11.1 The average age of infertile men consulted at the Department of Genetic and Molecular Medicine of the Lithuanian University of Health Sciences, Kauno Klinikos, was 35 years. Clinical factors that indicate absence of spermatozoa from their ejaculate, such as, azoospermia were found in about one third of these patients. Those that lead to decreased viability of sperm cells, such as, teratozoospermia and urinary tract infections were less common.

11.2 Two thirds of the studied males had normal karyotype (46,XY). The most common karyotype variation found was chromosomal aberrations with a normal phenotype, such as: 46,XY,9qh-; 46,XY,add(16)(q13); 46,XY,inv(9)(p11;q13); and 46,XY,qh+. Y Chromosome microdeletions of AZFc subregion only were present in one tenth of these males, while CFTR gene mutations (ΔF 508 region) on the other hand, were very rare and found in 3% of them.

11.3 About one fourth of males with primary infertility had no spermatozoa in their ejaculate (azoospermia). About one sixth of males with primary infertility had karyotype variations, such as, chromosomal aberrations, aneuploidies and mosaicism. One tenth of these men had Y-Chromosomal microdeletions in the AZFc subregion. CFTR gene mutations of ΔF 508 region were found in all primarily infertile men that were tested. Men with secondary infertility were older; one fourth of them had morphologically abnormal spermatozoa (teratozoospermia) but about one third had better sperm quality. One eighth of secondarily infertile men suffered urinary tract infections that could have decreased their ability to reproduce. Other sperm analysis parameters and medical conditions did not remotely differ in their effect on infertility in both groups.
12. PRACTICAL RECOMMENDATIONS

These research findings suggest that accurate transmission of genetic information is essential for fertility. Concerning genetic evaluation, the following genetic tests should be taken into account by general practitioners, infertility specialists and clinical geneticists for a better understanding of infertility as a multifactorial condition:

- karyotype, Y Chromosome microdeletion and CFTR gene mutation tests.

Using this knowledge, clinicians will be better able to diagnose and treat infertile patients and make knowledgeable decisions about the use of ART.
13. LITERATURE LIST


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