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Male infertility in context of testicular cancer

Neplodnost mužů v kontextu rakoviny varlat

Bachelor thesis

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Poděkování

Děkuji za poskytnutí cenných rad a vstřícnost při vypracovávání této bakalářské práce své školitelce paní RNDr. Kateřině Hortové, Ph.D. a konzultantovi panu prof. Ing. Jiřímu Neužilovi, CSc.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 10. 5. 2018

Podpis

Abstrakt

Tato bakalářská práce se zabývá mužskou neplodností ve vztahu k rakovině varlat. Rakovina varlat je nejčastější maligní onemocnění mladých mužů v době reprodukční zralosti a celosvětově vzrůstá míra incidence. Mnoho pozornosti je jí věnováno také kvůli stále narůstající neplodnosti. Z uvedených důvodů je cílem této práce objasnit změny probíhající v těle muže, jako jsou měnící se hladiny hormonů, modifikace procesu tvorby samčích pohlavních buněk zvaném spermatogeneze a specifických parametrů spermií, které mají vliv na početí vlastního potomka. K těmto změnám dochází vlivem samotného onemocnění, ale i léčbou. V této bakalářské práci bude také řešena problematika kryoprezervace spermatu jakožto vhodného řešení pro zachování plodnosti pacienta a poskytnutí možnosti početí pro páry potýkající se s mužským faktorem neplodnosti a rozhodnou se k řešení tohoto stavu pomocí asistované reprodukce.

Klíčová slova: rakovina varlat, léčba rakoviny, neplodnost mužů, parametry spermií, mitochondrie, asistovaná reprodukce

Abstract

This bachelor thesis focuses on male infertility in a connection to testicular cancer. Testicular cancer is the most common malignancy among young men in a reproductive age and the worldwide incidence of testicular cancer is on the rise. A lot of attention is also given to an increasing rate of infertility in a context of testicular cancer. For these reasons, the aim of this thesis is to clarify several non-physiological changes, such as hormone levels, spermatogenesis and sperm parameters, which take place in a male body and influence the chance to become a biological father. These pathological changes can be due to the disease itself but also due to the treatment. A cryopreservation of semen is also going to be discussed as the way to preserve male fertility and as an option for couples with the infertility problems due to male factor which rely on help of the assisted reprodiction.

Kye words: testicular cancer, cancer treatment, male infertility, sperm parameters, mitochondria, assisted reproduction

List of abbreviations

AFP α-fetoprotein

ALH amplitude of lateral head

ART assisted reproduction treatment

ATP adenosine triphosphate

CIS carcinoma *in situ*

DOG 2-Deoxy-D-glucose

FSH follicle-stimulating hormone

GAPDH glyceraldehyde 3-phosphate dehydrogenase

GCT germ cell tumour

hCG human chorionic gonadotropin

chemo chemotherapy

ICSI intracytoplasmic sperm injection

ICSI-FER ICSI-frozen embryo replacement

IUI intrauterine insemination

IVF in vitro fertilisation

LDH lactate dehydrogenase

LH luteinizing hormone

MMP mitochondrial membrane potential

MSC motile sperm count

mtDNA mitochondrial DNA

Onco-TESE oncologic testicular sperm extraction

Pesc swimming force

PLAP placental alkaline phosphatase

ROS reactive oxygen species

RT radiation therapy

SIR standardized-incidence ratio

TC testicular cancer

TE transrectal electroejaculation

TESE testicular sperm extraction

TGCT testicular germ cell tumour

VCL curvilinear velocity

WHO World Health Organization

WT wild type

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1 Introduction

Nowdays, a problem of infertility is commonly disscussed as it is one of the major reproductive health problem which affects 8 to 12 % of couples worldwide. Infertility may be caused by female or male factor. The male factor is caused of approximately 40 to 50 % of infertility for many reasons as a combination of low sperm concentration, poor sperm motility or abnormal morphology (Kumar and Singh, 2015). Good sperm parameters predict a success of conceiving. In the context of motility, sperm-type mitochondria play a significant role. Their function lies in a production of energy in the form of adenosine triphosphate (ATP) important for sperm motility. A source of ATP for sperm motility might be glycolysis or oxidative phosphorylation, in a dependence of animal species (Piomboni et al., 2012).

Testicular cancer is a malignancy which deserves attention and that for many different reasons. The first reason is that testicular cancer is the most common malignancy among young men in reproductive age, frequently in the age 20 to 34. The second reason is a rapid worldwide increase in incidence in the last 40 years in a context of rising fertility (Huyghe et al., 2003). Rising cancer include many changes, e.g. hormone levels, declining semen quality or metabolic pathways changes. In the second half of 20th century, Otto Warburg discovered that there are some modifications in metabolic pathways in cancer cells. These are dependent on aerobic glycolysis as on a source of ATP (Warburg, 1956).

Germ cell tumours represent a majority of testicular cancer cases. There are many epidemiological risk factors for developing testicular germ cell tumours (TGCTs), such as a history of TGCT in a contralateral testis, cryptorchidism, hypospadias, various perinatal factors or hormone-disrupting chemicals (Fukawa and Kanayama, 2018). A big importance is also laid on familial risk of testicular cancer (TC), especially among siblings (Forman et al., 1992). With the increased incidence of TC was noted that the number of men with infertility and abnormal semen analysis was also increasing. Developing of TC is almost three times more likely in men diagnosed with male factor infertility in comparison with men without male factor infertility. These results could indicate common etiologic factors for infertility and TC (Walsh et al., 2009).

Current medicine offers revolutionary approach in management and treatment of different diseases and this is also the case for TC. In the mid-1970s improvement began in systematic

treatment cisplatin-based combination chemotherapy (chemo). It results in mortality decline observed in all regions, especially among European and Oceanic countries (Van Hemelrijck et al., 2013). Sperm parameters at diagnosis of germ cell tumour (GCT) are frequently already abnormal and due to an effect of cancer treatment also goes to impaired of fertility. For this reason, is usually carried out a semen cryopreservation before treatment because it is believed that pre-treatment freezing is the best way to preserve fertility in testicular cancer patients. And despite the declining sperm quality after thawing. Cryopreserved semen is used for assisted reproduction technologies, earlier only for intrauterine insemination (IUI), in the present era rather for in vitro fertilisation (IVF) and intracytoplasmatic sperm injection (ICSI) which have very successful rates of achieving parenthood in cancer survivals. But it is not excluded that patients after cancer treatment cannot reach of spontaneous pregnancy with their partners (van Casteren et al., 2008).

2 Infertility

The clinical definition of infertility is defined as one year of unprotected intercourse in the fertile phase of the menstrual cycle. But most of the couples, which look for fertility treatment, are not infertile (sterile) but they only have decrease fertility called subfertility. For this reason, the above definition is usually used just for subfertility, while infertility is an absolute inability of conceiving (Evers, 2002). A subfertility affects one in 20 men. Male subfertility may have various causes as a raised level of reactive oxygen species (ROS) in semen, abnormal semen morphology or testicular diseases and abnormalities. For assessment of a male fertile problem is used semen analysis (Hirsh, 2003).

In 2010 it was published by World Health Organization (WHO) *Laboratory Manual for the Examination and Processing of Human Semen, 5th edition* in which there was published a set results of reference values for semen analyses based on the evaulation of 4 500 men from 14 countries and four continets made by Cooper and his colleagues. The outcome for one-sided lower reference limits semen sample had these values - semen volume: 1.5 ml; total sperm number: 39 million per ejaculate; sperm concentration: 15 million per ml; vitality: 58 % live; progressive motility: 32 %; total motility: 40 %; morphologically normal forms: 4.0 %. These values serve as a hallmark for the assessment of semen quality and fertility under a light of clinical data (Cooper et al., 2010).

3 Sperm mitochondria

3.1 Mitochondrial functionality in sperm cells

The flagellum of human sperm, as in many other mammalian types of sperm, is separated into four major parts – the connecting piece, the midpiece in which mitochondria are located, the principal piece and the end piece. Sperm-type mitochondria show some differences when compared to somatic mitochondria. These organelles in sperm are tightly wrapped around the axoneme. Specific spermatic glycolytic enzymes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and hexokinase 1 (Welch et al., 1992; Travis et al., 1998) are associated with the fibrous sheath in the principal piece of the flagellum (Eddy et al., 2003). In another mammalian species, including humans, a homologue of GAPDH was detected. Comparison of the deduced human GAPDH and mouse GAPDH proteins by sequence alignment showed that they share about 83 % amino acid identity. The difference between the human and mouse proteins is caused by the presence of more proline-residues in the amino-terminal segment of mouse GAPDH, which is therefore some 30 amino acids longer. The proline-rich domains at the N-terminus are not present in the GAPDH of somatic cells. A higher degree of identity exists between the mouse and human GAPDH proteins in humans and mice than between the somatic cell enzyme and the spermatogenic cell enzyme of the same species (Welch et al., 2000).

There are two types of physiological motility of the sperm – the activated motility detected in the ejaculated spermatozoa and the hyperactivated motility observed in sperm recovered from the site of fertilization. Both types of motility require optimal flagellar function propelled by energy in the form of ATP that is used by the flagellar dynein ATPase, the motor protein of the axoneme (Suaréz and Osman, 1987). Sperm cells are specialized in the conversion of free energy in the form of ATP into mechanical work. Two pathways participate in ATP production in mammalian sperm, i. e. mitochondrial respiration and glycolysis (Fig. 1). The main source of energy for sperm motility has been a subject for discussion, since ATP generated by sperm mitochondria located at the anterior end of the flagellum needs to diffuse aacross in order to supply the needs of the axonemal dyneins located in the distal segments of the flagellum. Work on the presumption that this distance is too great to deliver the ATP in a timely fashion to the distal part it has been suggested that

another regions of the sperm flagellum must be able to produce ATP (Mukai and Okuno, 2004).

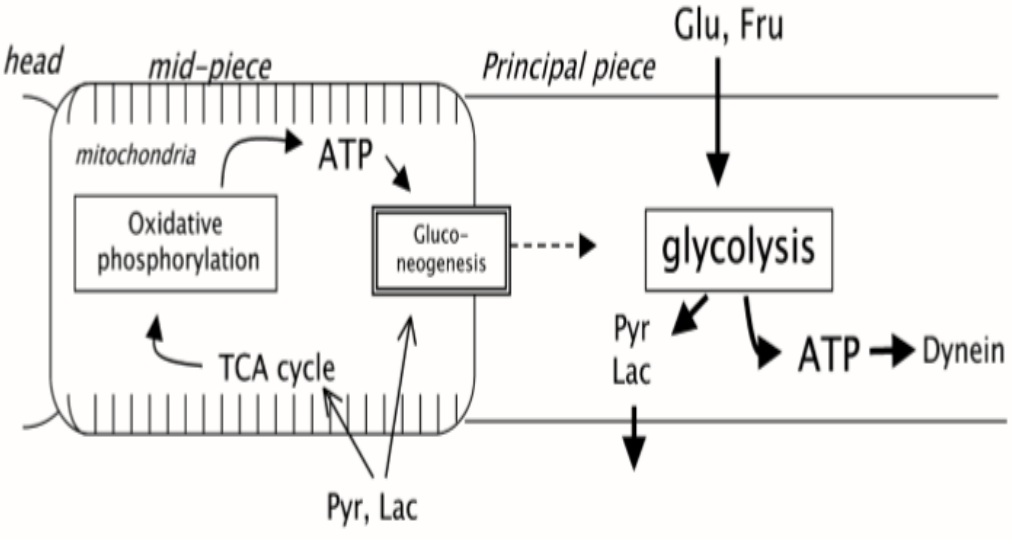

Figure 1 *Simplified diagram of mouse sperm with a location of energy metabolism (adapted from Mukai and Okuno, 2004).*

The role of oxidative phosphorylation and glycolysis in sperm motility was studied in dog and human sperm. At the same time it was shown that glucose plays a significant role in sperm motility using parametres such as curvilinear velocity (VCL) and swimming force (Pesc) in the context of mitochondrial membrane potential (MMP). It was found that there is no relationship between VCL/Pesc and MMP, which would signify a lack of relationship between sperm motility and mitochondrial respiration. The effect of specific agents, such as that of glucose and 2-Deoxy-D-glucose (DOG), an inhibitor of glycolysis, as well as inhibitors of oxidative phosphorylation inhibitors (antimycin A and rotenone) on VCL, Pesc and MMP was studied. Glucose added to human sperm at differrent concentrations (0, 5.55 and 2.78 mM) affected neither sperm motility (VCR and Pesc) mor MMP when given in two different media. However, sperm incubated in media lacking glucose showed a significant decrease in VCL and Pesc. Addition of DOG to sperm in media lacking glucose did not affect their motility. On the other hand, the addition of antimycin A or rotenone suppressed sperm motility. It would appear that the level of energy produced by oxidative phosphorylation in human sperm is lower in comparison to glycolysis (Nascimento et al., 2008). The results of another experiment, where sperm glycolysis in mice was blocked by targeted deletion of the GAPDH enzyme, revealed that most of the energy required for the motility of sperm is generated by glycolysis, not by oxidative phosphorylation. GAPDH

spositioned in the glycolytic pathway before both ATP-generating steps. This means that elimination of GAPDH would block most ATP production in sperm (Fig. 2). The experiment also showed that male mice with mutant GAPDH were infertile, although they were

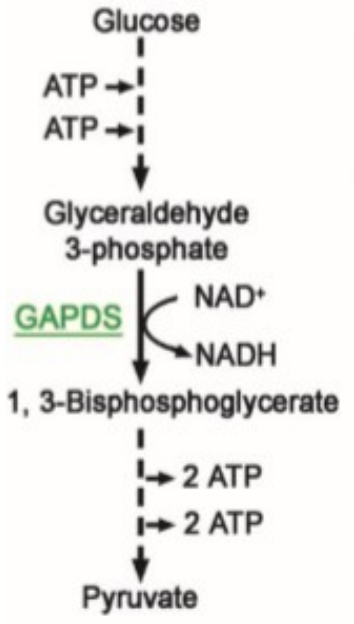

Figure 2 *The scheme of sperm glycolysis showing the role of GAPDH for production of ATP (adapted from Miki et al., 2004).*

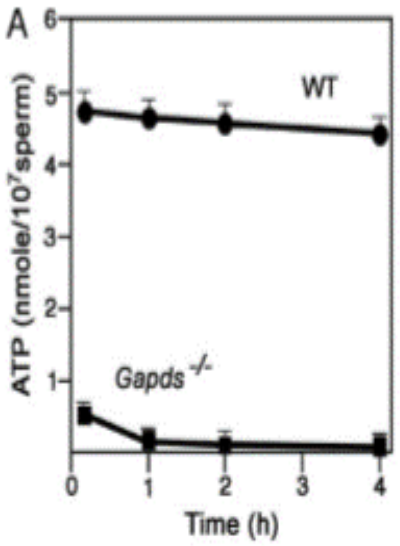

Figure 3 *The ATP levels in GAPDH mutants mouse sperm were markedly lower than in wild type (WT) mice (adapted from Miki et al., 2004).*

comparable to wild type males (similar body weight, testis histology and the number of sperm). The greatest difference was detected in the level of the ATP in mice with mutant GAPDH, which was only 10.4% when comparred with wild type mice (Fig. 3; Miki et al., 2004). Opposite results follow from a study, where sperm mitochondria respiratory efficicacy in connection with oxygen consumption was assessed, showing that higher mitochondrial respiration was associated with higher sperm motility. This was interpreted such that mitochondrial oxidative phosphorylation is the principal source of ATP, providing energy for spermatozoa. It cannot be ruled out, though, that glycolisis is an important source of ATP for sperm motility, pointing to both pathways, i. e. oxidative phosphorylation and glycolysis, being important for sperm motility (Ferramosca et al., 2012).

3.2 Mitochondrial DNA and pathology of the organelle

Mitochondria have their own circular genome, mitochondrial DNA (mtDNA), which in mammals encodes 13 polypeptides, all being subunits of respiratory complexes associated

with the inner mitochondrial membrane. Mitochondrial DNA also encodes 22 tRNAs and 2 rRNAs with a role in the translation of mtDNA-encoded mRNAs. Defects in mtDNA are known to cause physio-pathological disorders including infertility and reproductive pathology (Spiropoulos et al., 2002). Many studies have focused on uncovering a relationship between the qualitative abnormalities of mtDNA and infertility. In view of the fact that mtDNA mutations are often present in patients with fertility problems, there is a possibility that mitochondrial respiration defects may be linked with male infertility (Ruiz-Pesini et al., 1998). The experiment using a transmitochondrial mouse model carrying wild type mtDNA and mutant mtDNA with a pathogenic deletion showed that mice with more than 73 % pathogenic mtDNA are not capable of reproduction caused by systematic mitochondrial respiration defects and mitochondrial myopathy. Studies with mice comprising more than 68 % of pathogenic mtDNA revealed that the main causes of male infertility were asthenozoospermia[1] and oligozoospermia[2]. In addition to these results, histological changes in testis of infertile mice with pathogenic mtDNA indicate that mitochondrial respiration defects may affect the transformation of diploid spermatocytes to haploid spermatids during spermatogenesis (Nakada et al., 2006). Oligoasthenozoospermia[3] was identified in patients having specific mutations and multiple mitochondrial deletions (Lestienne et al., 1997). Long PCR analysis indicated higher numbers of mtDNA mutations in patients suffering from oligoasthenoteratozoospermia[4]. More than one-third of these patients had multiple deletions in tDNA (St John et al., 2001).

Quality and quantity of mtDNA determine sperm quality and their capability of fertilizing an egg. Analysis of semen from men diagnosed with infertility and men with normal semen parameters revealed that the integrity and copy number of mtDNA significantly correlated with sperm count. Significant differences were evaluated in the average mtDNA copy number among the groups of infertile men. Oligoasthenoteratozoospermia patients featurred higher mtDNA copy number than oligozoospermia and asthenozoospermia patients, and then men with normal semen parameters. The increase of mtDNA copy number negatively correlated with sperm count, and on the other hand, a significant decrease in mtDNA

[1] reduced sperm motility

[2] low concentration of sperm

[3] changes in count and motility of sperm

[4] low sperm concentration and important abnormalities in sperm morfology and motility

integrity was shown in men with poor semen quality (Song and Lewis, 2008). Another study supports the notion that a high copy number of mtDNA is associated with poor sperm function and male infertility (Gabriel et al., 2012). The decrease of mtDNA copy number is regulated during spermatogenesis by downregulation of the mammalian mitochondrial transcription factor A (Tfam; Rantanen et al., 2001).

One of the sperm count problems is caused by abnormal spermatogenesis during which a marked increase of mtDNA copy number per cell was detected (May-Panloup et al., 2003; Díes-Sánchez, 2003). In other cases of infertile men with alterations in mtDNA copy number in sperm production of extensive levels of reactive oxygen species were detected (Kumar et al., 2009).

4 Testicular cancer

Testicular cancer is ranked among relatively rare men's diseases. It is estimated that there were 55 000 new cases in 2012, which accounts for 0.7 % of male cancers; worldwide mortality linked to testicular cancer is around 10 000 deaths per year. The highest incidence of testicular cancer is in Europe, while it is low in Asia and Africa (Ferlay et al., 2015). On the other hand, testicular cancer is the most common cause of cancer in men aged 15 to 44 years, accounting for 11.5 % of new cancer cases among men of this age in developed countries (Parkin et al., 1999). The incidence of testicular cancer has been on the increase over the last few years (Adami et al., 1994). However, effective treatment combining surgery and radiation with modern chemotherapy led to decline testicular cancer mortality (Bray et al., 2006). From 1990 – 1994 to 2000 – 2004 the decrease in mortality was about 26 % in patients of all ages living in European Union. However, the number of deaths in eastern and central European countries (Hungary, Latvia, Romania, Bulgaria, the Czech Republic) differed in 2000-2004 from that west European countries, where the mortality was on average more than three times lower (La Vecchia et al., 2010). The problem of higher mortality in Eastern Europe was linked to unsatisfactory treatment of testicular tumours (Levi et al., 2003). The same problem was noticed over the period of 1980 – 2003 in Latin America, where testicular cancer mortality was considerably higher than in North America (Bertuccio et al., 2007).

With the growing incidence of testicular tumours in the second half of the 20th century, a decline in semen quality closely connected to male infertility was observed. It has been

specilated that there is a link between the increase of testicular tumours and the decrease of important semen parameters (Carlsen et al., 1992). The analysis showed the association between the elevated risk of testicular tumours and changes of specific semen characteristics such as low semen concentration, poor motility of the spermatozoa and high proportion of morphologically abnormal spermatozoa. Analysis of semen from more than 32 000 men done at the Sperm Analysis Laboratory in Copenhagen during 1963 – 1995 and the subsequent study showed that patients with fertility problems were more likely to develop testicular cancer than other men. It was shown by 50 cases of seminomas, 37 non-seminomas and two unspecified testicular cancer (Jacobsen et al., 2000). In comparison to the general population, infertile men with abnormal semen have 20-fold greater incidence of testicular cancer (Raman et al., 2005). Males with azoospermia[5] have almost 3-fold increased risk of cancer in comparison with the general population (Eisenberg et al., 2013).

4.1 Classification and types of testicular tumours

The latest classification of tumours of the testis was formulated at the WHO Consensus Conference in Zurich, Switzerland, in March 2015. The tumour types are divided into six major categories – germ cell tumours derived from germ cell neoplasia *in situ*, germ cell tumours unrelated to germ cell neoplasia *in situ*, sex cord-stromal tumours, miscellaneous tumours of the testis, haematolymphoid tumours and tumours of the collecting duct and rete testis. More details are provided in the chapter 4.1.1 (Moch et al., 2016).

4.1.1 Main histological types of testicular tumours based on the latest WHO classification

- **germ cell tumours derived from germ cell neoplasia *in situ***
  - non-invasive germ cell neoplasia *(germ cell neoplasia in situ, specific forms of intratubular germ cell neoplasia)*
  - tumours of a single histological type
    - seminoma
    - non-seminomatous germ cell tumours *(embryonal carcinoma, yolk sac tumour –postpubertal type, trophoblastic tumours, teratoma – postpubertal type and many more types)*
    - non-seminomatous germ cell tumours of more than one histological type

[5] absence of sperm in ejacuate

    - germ cell tumours of unknown type
- **germ cell tumour unrelated to germ cell neoplasia *in situ***
    - spermatocytic tumour
    - teratoma – prepubertal type
    - mixed teratoma and yolk sac tumour – prepubertal type
    - yolk sac tumour – prepubertal type
- **sex cord-stromal tumours**
    - pure tumours *(Leydig cell tumour, Sertoli cell tumour, granulosa cell tumour)*
- **miscellaneous tumours of the testis**
    - ovarian epithelial-type tumours
    - haemangioma
- **haematolymphoid tumours**
- **tumours of the collecting duct and rete testis**

4.1.2 Testicular germ cell tumours

Testicular germ cell tumours account for most of the testicular cancers, being classified into two main groups based on their histology: pure seminoma and a group known collectively as non-seminoma. Non-seminomas comprise four subgroups: embryonal carcinoma, teratoma, choriocarcinoma and yolk sac carcinoma (Gori et al., 2005). The characteristics of these four types of tumours are stated below in Table 1 (Sesterhenn and Davis, 2004). In case of non-seminoma, higher incidence of a metastatic spread than in seminoma patients is expected (Cremerius et al., 1999).

The main factors linked to the risk of TGCTs are undescended testis (cryptorchidism), contralateral testicular tumour and familial testicular cancer. Nevertheless, infertility, twin-ship and testicular atrophy are considered as other risk factors, albeit with lower incidence (Dieckmann and Pichlmeier, 2004). In 1972 it was clarified for the first time that the common precursor of germ cell tumours is carcinoma *in situ* (CIS), also known as intratubular germ cell neoplasia (Gondos et al., 1983). A pre-invasive CIS lesion could progress to a tumour via two pathways. In the case of non-seminoma, the CIS cells behave

Table 1 *The main groups of germ cell tumours derived from germ cell neoplasia in situ and their characteristics; adapted from Sesterhenn and Davis, 2004.*

	type	% of GCTs in the age group	age	surface of tumour	aspects of cells	aspects of the nuclei
Seminoma	pure seminoma	about 50 %	between 30 and 50	grayish-white, bulging and glistening	large with abundant pale or amphophilic cytoplasm (depending on the glycogen content)	coarse chromatin distibution, prominent nucleoli
Non-seminoma	embryonal carninoma	3 - 4 %, present in tumours of more than one histological type – 40 % of cases	between 20 and 40, less often in adolescent (15 to 20 years of age)	grayish-white with foci of hemorrhage and necrosis	large and embryonic in appearance, having pale, amphophilic or eosinophilic cytoplasm	nuclei are vesicular with a see-through appearance, necleoli are prominent with common mitoses
	yolk sac tumor	65 %, present in tumours of more than one histological type – 42 % of cases	infants and children	soft, homogenous, grayish-yellow and not encapsulated	small, ranging from cuboidal to flattened endothelial	variable sizes, frequent mitoses, hyaline globules
	trophoblastic tumours	less than 1 %	patients with disseminated disease	small and extensively hemorrhagic, small rim of grayish-white tissue	syncytiotrophoblastic (large with eosinophilic cytoplasm), cytotrophoblastic and intermediate cells	multinucleated, large and hyperchromatic, one or two nucleoli
	teratoma	35 %	infants and prepubertal children	cystic areas, sometimes containing mucoid or gelatinous material	complex,representing the three germ cell layers: endoderm, ectoderm and mesoderm	no available data

as totipotent, because CIS cells maintain the features of embryonic stem cells in young men. In the second case in relatively older men, the CIS cells and the seminoma cells have a similar phenotype, which indicates that seminoma originates from CIS cells having little or no stem cell characteristics (Skakkebæk et al., 1998). The CIS is usually an asymptomatic condition. It is important that patients with increased risk of developing cancer, undergo careful medical screenings, including testicular ultrasound and biopsies of one or both testes. If the CIS is diagnosed relevant treatment, such as orchidectomy or radiotherapy, ensues. Treatment of patients with CIS who want to have children may be postponed while being under medical surveillance, because there is a relatively long time between the diagnosis of the CIS and the development of TGCT (Hoei-Hansen et al., 2005). It is important to remember that an early diagnose of testicular cancer may preserve the reproductive function.

4.2 Changes in sperm parameters and hormones in patients diagnosed with testicular cancer

It was found that malfunctions of spermatogenesis are already present in males with germ cell tumours before onset of any treatment, such as orchiectomy or radiotherapy. Neoplasic pathology can disturb the process of spermatogenesis for many dissimilar reasons. Environmental factors as well as genetic factors can play a role. For functional spermatogenesis and good semen quality, certain nutritional trace elements that can be affected by cancer are important. An essential cofactor for many metalloenzymes is zinc, which plays a key role in normal testicular development and in spermatogenesis. There are other vitamins and minerals that are important for normal sperm motility (selenium, vitamin E) or semen quality (vitamin C and D; Wong et al., 2000). A germ cell tumour may cause non-specific damage to the seminiferous epithelium, which then results in poor seminal parameters in the pre-treatment period. Moreover, defects in testosterone production and feedback to the hypothalamic-pituitary-gonadal axis are common (Costabile and Spevak, 1998).

A poor semen quality is probably caused by pre-existing impairment of spermatogenesis, which correlates with an increased risk of male reproductive abnormalities in TGCT patients. This is examplified by testicular atrophy, cryptorchidism and testicular dysgenesis. The testicular dysgenesis syndrome which follows from male reproduction malfunctions, such as impaired spermatogenesis, TC, cryptorchidism or hypospadias, may be a result of faulty embryonal programming and development of gonads during fetal life just as male infertility can have an origin in fetal life (Skakkebæk et al., 2001). It could suggest the presence of common etiological factors for TC and male infertility (Walsh et al., 2009). Patients with testicular cancer show significantly lower sperm concentration, lower total sperm count and lower volume of ejaculate before orchiectomy than healthy men. Also, differences have been observed in hormonal levels. Testicular cancer patients have higher levels of follicle-stimulating hormone (FSH) and lower levels of luteinizing hormone (LH), and patients with increased human chorionic gonadotropin (hCG) levels have lower LH but higher testosterone and estradiol levels than men with normal levels of hCG. The likely reason is direct hCG stimulation of Leydig cells (Petersen et al., 1999). The level of inhibin B before onset of cancer treatment is significantly lower in the men with TGCT, which points to

decreased semen quality. TGCT patients also feature a decreased gonadal function (van Casteren et al., 2010).

On the other hand, a testicular cancer does not necessarily mean that spermatogenesis cannot be normal. In many patients with testicular cancer, complete spermatogenesis was found, however its rate was influenced by the size of the tumour. Increased size of testicular tumour was found to correlate with decreasing count of mature spermatozoa. The main reason for pathophysiological spermatogenesis could be a local tumour-suppressive effect. The relationship between semen parameters and the number of mature spermatozoa is still a question of debate (Shoshany et al., 2016).

4.3 The effect of cancer treatment on the sperm parameters and spermatogenesis

The first step in the treatment of almost all TGCTs begins with orchiectomy, which is applied in 46 % of cases of stage I and II seminoma. Some patients suffering from seminoma (stage I and II) need, in addition, another treatment. In 31 % of cases, they receive radiation therapy (RT), in 22 % chemotherapy. Seminoma stages III and IV are usually treated by means of surgery and chemotherapy (Fig. 4). Patients with stage I and II non-seminomas need to undergo retroperitoneal lymph node dissection, and patients with stage III and IV of the

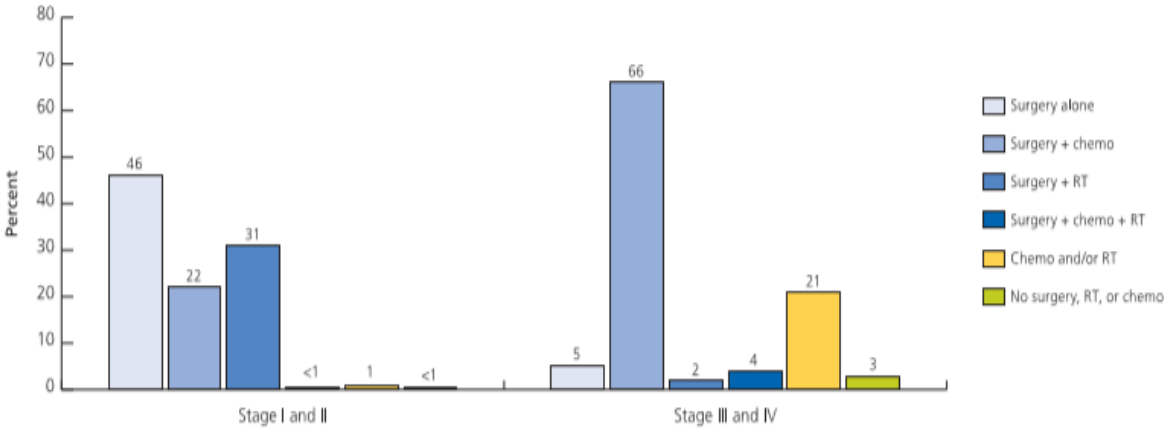

Figure 4 *The graph showing a proportional representation of treatment methods in patients suffering from seminoma in all stages, 2009 – 2013 (adapted from Miller et al., 2016).*

disease are treated with surgery and chemotherapy (Miller et al., 2016). The defect of spermatogenesis was observed in the testicular cancer patients after unilateral orchiectomy but before cytotoxic treatments, which can be explained by about 50 % decrease of germinal epithelium (Botchan et al., 1997). One study showed that there is no significant difference

in fertility between seminoma and non-seminoma patients after orchiectomy and before the onset of radiotherapy. In both groups patients suffered from subfertility in more than 50 % of cases. In this study, the participants were divided into two groups according to doses of radiation, i. e. a high-dose group (≥0.79 Gy) and a low-dose group (<0.79 Gy). The aim was to clarify the effect of the radiation dose on sperm concentration, and the time of fertility recovery and the increase of hormonal levels. A significant increased in LH was noticed in connection with higher radiation dose, while serum testosterone concentration did not change. The level of FSH was most effected by radiation, regardless the dose. The level of FSH peaked at about 6 months in both dose group and its decrease in the low-dose group ocurred 12 months after orchiectomy. These findings correlated with the changes in sperm concentration. The level of FSH and sperm concentration behaved reversely, with one parameter decreasing and the other increasing. The sperm count in the low-dose radiation group returned into normal after 12 months, in the high-dose group at least after 24 months (Gordon et al., 1997). Exposure of immatur cells to the dose 0.1 Gy may cause morphological and quantitative changes of spermatogonia, doses of 2 – 3 Gy lead to damage to spermatocytes, which results in the reduction of spermatid number. The doses of 4 – 6 Gy resulted in decreased number of spermatozoa. Doses of up to 3 Gy caused a decline in sperm number in following 60 – 70 days, which was more rapid at doses of 4 and more Gy.

Complete recovery of spermatogenesis is based on the surviving stem cells. Also, the time of recovery is shorter at lower doses of radiation. However, doses of 1.2 Gy and above show a reduced risk of recovery. The doses of more than 6 Gy may end up in permanent azoospermia. A number of drugs that are applied in chemotherapy are rather gonadotoxic. Their negatively effect the synthesis of DNA, RNA and proteins and disturbe the function of microtubule. All these processes play an important role in cell division. Gonadotoxicity effect has been shown for alkylating agents (cyclophosphamide, chlorambucil, mustine, melphalan, busulfan, carmustine, lomustine), antimetabolites (cytarabine), vinca alkaloids (vinblastine) and their combinations. Testicular cancer patients treated with cisplatin/carboplatin-based chemotherapy may suffer from temporary azoospermia. The recovery of spermatogenesis is possible after 2 years of treatment cessation with about 50% probability and after 5 years with 80% probability (Howell and Shalet, 2005).

4.4 Glycolysis in context of testicular cancer

As tumour size increases, there is greater alternation in energy metabolism in comparison with that of the original, non-malignant tissue. During the malignant transformation, the Warburg effect takes place, which is one of the most fundamental metabolic alternations exhibited by most cancer cells. In these cells ATP is generated as the main source of energy by the elevated aerobic glycolysis. In most cases, cancer cells convert most glucose to lactate irrespective of the presence of oxygen, but its efficacy is lower than oxidative phosphorylation (4 molekules of ATP per molecule of glucose vs. 36 molecules of ATP per molecule of glucose). In the presence of oxygen, differentiated tissue metabolizes glucose to pyruvate via glycolysis; subsequently, pyruvate is oxidased to CO_2 in mitochondria by oxidative phosphorylation. In case of hypoxia, pyruvate generated by glycolysis can be redirected away from oxidative phosphorylation to generate lactate, which leads to the continuation of glycolysis with minimal production of ATP. In comparison, two molecules of ATP per molecule of glucose are generated by anaerobic glycolysis while by oxidative

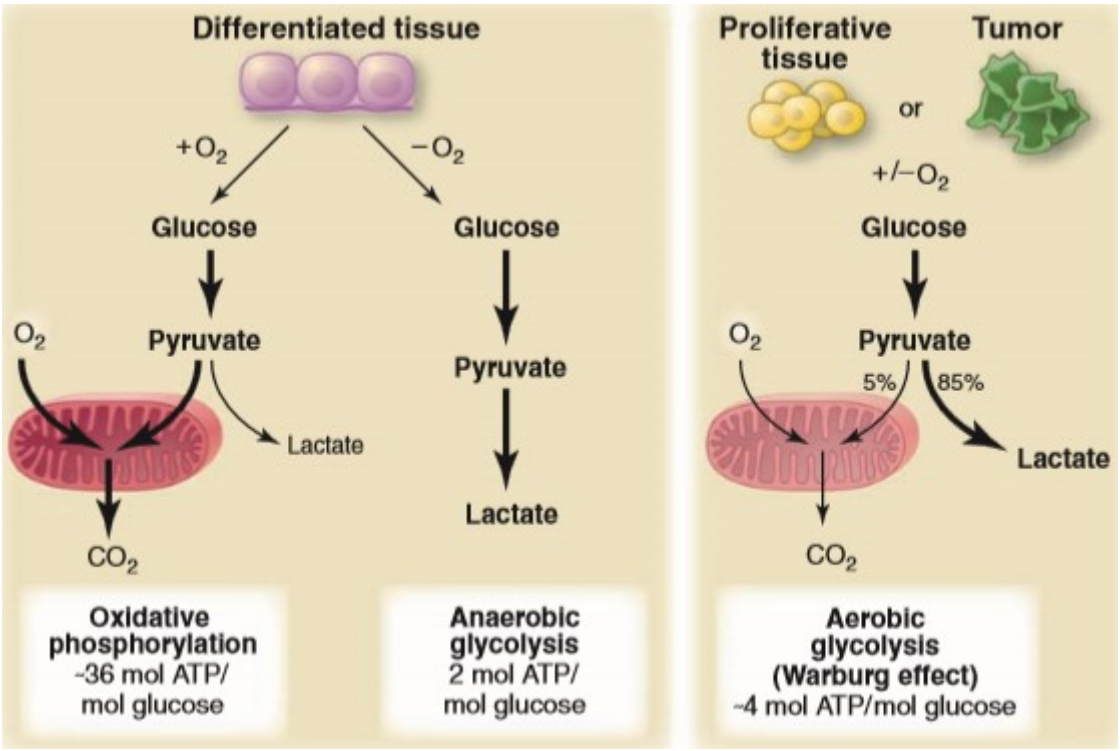

Figure 5 *Simplified diagrams of the differences between oxidative phosphorylation and glycolysis – anaerobic and aerobic (Warburg effect; adapted from Vander Heiden et al., 2009).*

phosphorylation it is 36 ATP per molecule of glucose (Vander Heiden et al., 2009). Figure 5 shows simplified diagrams of the above mentioned processes.

There are diverse mechanisms that can lead to the Warburg effect. One of them is linked to mitochondrial defects, since the mitochondrial genome encodes proteins of the respiratory chain and mtDNA mutations may change the function of the respiratory chain (Pelicano et al., 2006). Increased glycolysis in cancer cells may be caused by various mechanisms such as increased expression and translation of relevant genes or their amplification. It was found that some types of cancer are feature considerable overexpression of glycolysis genes. Importantly, testicular tumour is one of the about 20 types of cancer, in which this mechanism was discovered. Hexokinase 1, glucose phosphate isomerase, aldolase A and glyceraldehyde-3-phosphate dehydrogenase are examples of ubiquitously overexpressed genes coding for enzymes of the glycolytic pathway (Altenberg and Greulich, 2004).

4.5 Sperm DNA in context of testicular cancer

Good sperm DNA quality is one of the major sperm characteristics important for a successful fertilization. High level of sperm DNA fragmentation may be a reason for unexplained recurrent pregnancy loss in some couples (Carrell et al., 2009). Relevant to this, increased level of DNA fragmentation has been shown in testicular cancer. Thus, testicular cancer patients with normal and abnormal semen parameters have, at the time of diagnosis, high levels of single- and double-stranded DNA breaks in spermatozoa compared to the control group. The alkaline Comet assay was used to show that cancer patients have three times more DNA damage compared to the control group. DNA fragmentation level is higher in patients with abnormal semen parameters than in normozoospermic men (Kumar et al., 2018). Figure 6 shows the use of the

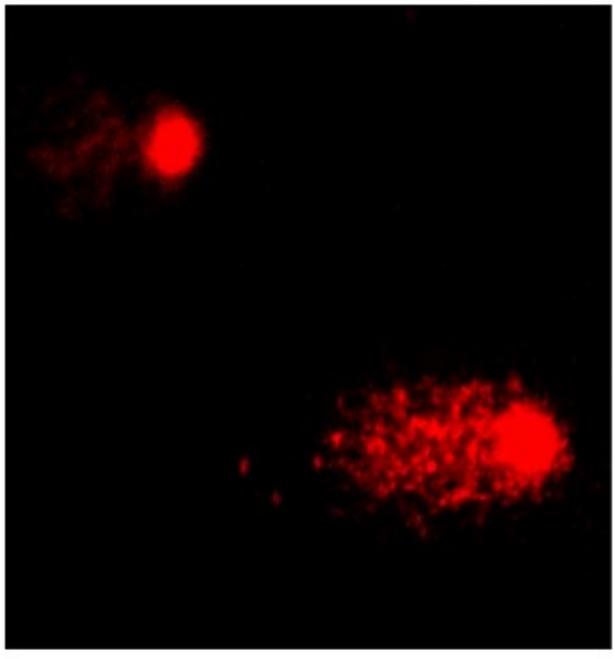

Figure 6 *Picture showing DNA damage in the male germ line with the use of Comet assay (adapted from Lewis and Aitken, 2005).*

Comet assay for the detection of DNA damage. The spermatozoon in the upper part of the picture has no significant DNA damage. The bottom spermatozoon appears like a "comet", which indicates a cell with DNA damage (Lewis and Aitken, 2005).

A very sensitive and specific test for detection of sperm double-stranded breaks is based on the γH2AX analysis, which seems like a good predictive parameter for successful assisted reproduction treatment in cancer survivors (Garolla et al., 2015). The percentage of sperm DNA fragmentation does not differ statistically between non-seminoma and seminoma tumour type before treatment onset (Meseguer et al., 2008). Certain alternations in DNA quality may be linked to cancer treatment. Surgery affects sperm DNA integrity, however, radiation therapy results in increase in sperm cells with DNA damage that continues rising over 1-2 years post-therapy, after which it normalises within 3-5 years. In contrast, during 1-2 years after more than two cycles of chemotherapy, the level of sperms with impaired DNA integrity declines (Ståhl et al., 2006). An effect on cryopreservation of DNA integrity of spermatozoa in infertile men has also been shown, resulting in the loss of DNA integrity after freeze-thawing. On the other hand, cryopreserved spermatozoa from fertile patients show no significant alternations of DNA integrity (Donnelly et al., 2001). Sperm with satisfactory DNA integrity from patients should be frozen in large aliquots that can be used for intrauterine insemination. Semen samples with high level of DNA damage can be stored in multiple small aliquots, which can be used for ICSI (Said et al., 2009).

Various types of tumour markers, such as α-fetoprotein (AFP), hCG, placental alkaline phosphatase (PLAP) or lactate dehydrogenase (LDH) has been used for diagnosis of cancer (Trigo et al., 2000). For testicular tumour patients relatively novel analysis of mtDNA levels has been, based on elevated presence of cell-free circulating mtDNA. Testicular germ cell cancer patients, regardles eheter they belong to the seminoma or non-seminoma group, show increased levels of short (79 bp) and large (220 bp) mtDNA fragments compared to the control group. It may frequently occur that patients have normal conventional tumour markers discovered by a single analysis, but that they feature increased levels of mtDNA. This marker is less sensitive than combined analysis of mtDNA levels and conventional tumour makers, which could be beneficial for managing marker-negative testicular cancer patients (Ellinger et al., 2009). Interestingly, increased mtDNA copy number has been detected in different types of human cancers (Yu, 2011).

4.6 Familial risk of testicular cancer

Genetic predisposition may be one of the major reasons for the origin of testicular germ cell tumour. Having a family history of GCT increases a chance for cancer development, with 3-fold higher relative risk in patients having first-degree relatives (a father or brothers) suffering from testicular cancer. Lower incidence of family GCTs was established among second- or third-degree relatives, such as cousins, nephews or grandfathers (Dieckmann and Pichlmeier, 1997).

A comparison of mean age at the time of testicular cancer diagnosis in familial cancer cases and non-familial cases shows that patients with a familial cancer history are younger at diagnosis than those without such history. At diagnosis, a mean age for seminoma in familial cases is 32.5 years, while it is 26 years for other germ cell tumours, for non-familial cases it is 35.5 and 28.5 years, respectively (Forman et al., 1992). Results of analysis, including cases of more than one million common tumours from 1958 up to 2000, from the largest population-based database on familial cancer – Swedish Family-Cancer Database – show that standardized incidence ratio (SIR – a ratio of observed to expected cases) of testicular cancer for offspring with a parental disease history regardless of age was 4.26, while SIR for offsprings whose sibling have cancer history was 9.28. Thus, if certain family members, in particular siblings, have been diagnosed with testicular cancer, other relatives should be very cautious about the symptoms (Hemminki et al., 2004). The SIR for non-seminoma tumour type is higher (9.59) than for seminoma (5.44). The notion that there is a stronger heritable component in non-seminoma than in seminoma cases is supported by the hierarchical frailty model (Valberg et al., 2014). Testicular cancer may be also associated with other types of cancers in the family, including close female relatives. Increased risk of seminoma cancer was found when parents suffered from colorectal, pancreatic, breast and lung cancer and non-Hodkin's lymphoma and Hodkin's lymphoma or when siblings are diagnosed with melanoma. Teratomas may be associated with parental lung cancer and melanoma (Hemminki and Li, 2004).

5 Paternity of men diagnosed and treated for testicular cancer

The chance to become a father in case of men diagnosed with testicular cancer is increased in the context of advancement in assisted reproduction techniques in recent years, but it is not always necessary. The study using data from the Norwegian long-term survivors treated

for unilateral testicular cancer during 1980 – 1994 showed that 90 % of these men had children before the testicular cancer diagnosis. To conceive a child following treatment was attemted by 39 % of men, of which 65 % of them succeeded in fathering biological children without the need of cryopreserved of the semen. The rates of paternity in patients treated by radiotherapy, low-dose chemotherapy and retroperitoneal lymph node dissection were similar, and the cumulative paternity rate 15 years after orchiectomy was 71 % and at 20 years 76 %. However, there was a difference in case of high-dose chemotherapy patients, where the paternity rates were significantly lower, showing that 25 % of all men had become post-treatment fathers and 72 % of them had biological children either before or after treatment (Brydøy et al., 2005).

5.1 Semen cryopreservation

Semen cryopreservation, also known as sperm banking, is a procedure which testicular cancer patients should undergo to preserve their future fertility potential. Given the fact that patients suffering from testicular cancer may become subfertile or even azoospermic after treatment, cryopreservation is carried out before initiation of treatment regardless of the clinical tumour stage or histologic features because of these parameters do not significantly influence the semen quality. The process of cryopreservation has influence on the semen quality, such that some of the changes are similar in testicular tumour patients and control donors. Men with cancer have lower pre-freeze and post-thaw motile sperm count (MSC), motility, curvilinear velocity (VCL) and linearity, which characterise the overall poor semen quality in testicular cancer patients. The only sperm parameter, amplitude of lateral head displacement (ALH), remains unchanged after cryopreservation (Hallak et al., 1999). A decrease in viability after freezing and thawing, caused probably by loss of vitality, correlates with an increase in immotile spermatozoa. The most probable reason for a decrease in viability of sperm cells is the chemical and physical nature of the environment surrounds them, as well as formation of crystal ice outside the cells (Ozkavukcu et al., 2008). The difference of viability between spermatozoa before and after freezing is shown in Figure 7. A significant increase in the motility of sperm after thawing is dependent on the elapsed time. An recent study showed that a total number of motile sperm was higher after 40 minutes of post-thawing interval than after 20 minutes (Oberoi et al., 2014). Sperm

parameters such as motility, total motile sperm count and viability may be affected by the choice of a suitable cryopreservation protocol.

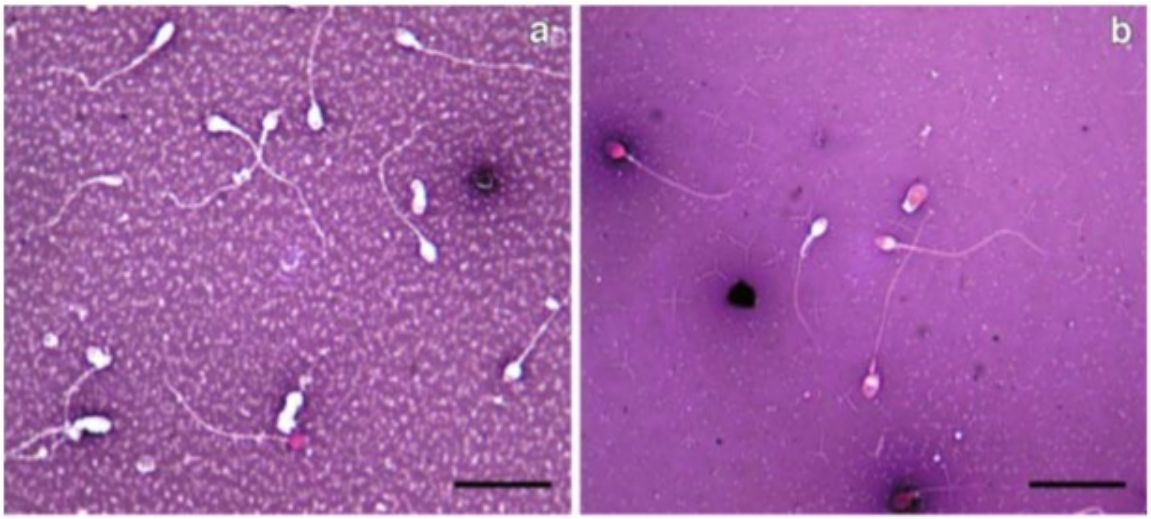

Figure 7 *Pictures showing a difference between the same samples before freezing (a) and after thawing (b). A decrease of mean percentage of viability after thawing is evident. It was used eosin-nigrosin staining. Scale bars indicate 20 µm (adapted from Ozkavukcu et al., 2008).*

An organelle which is most predisposed to freezing and thawing damage is the acrosome because of its fragile membrane, which is prone to physical and chemical effects and to ionic changes. Scanning electron microscopy are distinguishable acrosomal abnormalities as cracks or peelings. Not only increased rates of acrosomal changes but also a significant increase in the widening of the subacrosomal regions were found (Ozkavukcu et al., 2008). Cryopreservation also brings about alternations in mitochondria. A relationship between mitochondrial activity and certain sperm characteristics (morphology, viability, sperm motility parameters) could be detected before and after cryopreservation. The rate of damage to the plasma membrane caused by freeze-thawing are similar to the tevel of the number of sperm with functional mitochondria. Low motility of sperm can be thus explained by the impairment of mitochondrial activity, which is linked to the fact that integrity of both plasma and mitochondrial membranes is necessary for sperm motility. The extent of mitochondrial damage after cellular trauma such as cryopreservation can be detected using the Rhodamine 123 assay (O'Connell et al., 2002).

5.2 Assisted reproductive technology

Couples in which male factor infertility due to cancer was indicated may use assisted reproductive treatment as an option to become parents. Men diagnosed with testicular cancer may have impaired spermatogenesis already at the time of diagnosis, but many cancer survivors have problems with fertility after treatment. Majority of patients use the possibility of semen cryopreservation before treatment, which is more covered in chapter 5. 1. There are many techniques which are used for fertility treatment for such couples. The main techniques are in vitro fertilisation, intracytoplasmic sperm injection, intra-uterine insemination and sometimes ICSI-frozen embryo replacement (FER). With the progress of assisted reproduction techniques, the chances to have biological children are increasing.

IVF and ICSI belong to the most effective treatment methods of infertility related to testicular cancer, these approaches may also be used in patients with severe oligozoospermia. In case of ejaculation problems, such as anejaculation or retrograde ejaculation, after treatment, spermatozoa could be extracted by testicular sperm extraction (TESE) or transrectal electroejaculation (TE) for realization of IVF or ICSI. The ICSI method is successful in all types of severe male infertility, and its advantage based on much lower need of the number of motile spermatozoa compared to IVF. A frequently used treatment method of male subfertility has been the IUI, but the results indicate that IVF or ICSI have higher rate of success and, in addition to this, they could be used in cases of very low semen quality (Rosenlund et al., 1998). A study of 67 couples with male factor infertility due to cancer (34 testicular cancer patients) underwent an assisted reproduction treatment and 151 cycles of assisted reproduction treatment (ART) was executed in total. The most common technique was ICSI in 82 cases, then IUI in 55 cases and ICSI-FER in 14 cases. This cohort of patients was not treated with IVF. In result, 37 babies were born from the 52 pregnancies in 40 couples. Cryopreserved semen was used in 58 % of pregnancies because of 65 % men with their cryopreserved semen were indicated as azoospermic after treatment. The major technique for fertilisation using cryopreserved semen was ICSI (almost 87 %) which resulted in 22 babies. The rest of pregnancies originated from freshly ejaculated semen (Schmidt et al., 2004).

Azoospermic patients treated with ICSI have a chance to reach clinical pregnancy but the female factors, such as age or the number of mature oocytes, which were retrieved and injected, have an influence on the success rate of pregnancy following ICSI (Friedler et al.,

2002). It could also happen that patients are treated before their semen is cryopreserved and that they become azoospermic after their gonadotoxic treatment. To the treatment preceding cryopreservation goes in various situations, such as the patient is a pre-pubertal boy or is already azoospermic or it is necessary to begin chemotherapy urgently. With the use of TESE, it is possible to retrieve sperm from cancer survivors suffering from post-chemotherapy azoospermia and then use it for ICSI. The success of TESE is not limited by age, serum FSH, testicular volume or time after chemotherapy. A high sperm retrieval rate has been found in seminoma patients (Dar et al., 2018). In case when a patient underwent orchiectomy and when cancer arose after some time in the second testis, there is a chance for obtaining sperm using oncological testicular sperm extraction (onco-TESE), which is based on retrieving of a sample from non-cancerous tissue separated from cancerous tissue (Hamano et al., 2018). Also, the tumour size negatively correlates with spermatogenesis. A possibility of preservation of spermatogenesis is higher when the distance of seminiferous tubules from the tumour is greater (Suzuki et al., 2015).

6 Conclusion

The focus of this thesis is on testicular cancer and its connection with male infertility, changes of fertility during the cause of malignancy and treatment. The testicular cancer has an evident negative impact on spermatogenesis and semen quality. Low semen concentration, poor motility, morphologically abnormal spermatozoa and changing hormone levels are issue in cancer patients. A significant role for poor sperm motility is due to mitochondria stress as their energy production in a form of ATP by oxidative phosphorylation or glycolysis, is also changed during malignant testicular cancer. Defects of mitochondria, such as multiple deletions and mutations or increase mtDNA copy number, can result in serious male fertility problems. It is relevant, that an increased level of mtDNA is relatively newly considered as one of many tumour markers which could help to improve cancer management in the future.

Morover, a treatment of testicular cancer, including orchiectomy and radiation therapy or chemotherapy, has a great negative impact on male fertility. It is important to consider that the dose of radiation influences a range of cells damage. A small dose has already a negative influence on cells but the higher it is, the more serious decrease of spermatozoa number and longer recovery time of spermatogenesis it means.

For the above reasons, semen cryopreservation is a key solution for patients with almost all cases of testicular cancer. Although during this process sperm quality decreases due freeze-thawing, it is a crucial method to preserve a patient's fertility options as with the use of suitable cryopreservation protocol, it is possible to minimalize damage that sperm go through. The assisted reproduction may still be the only way how to become a parent after being diagnosed and treated for testicular cancer. Nowadays, the assisted reproduction is very progressive discipline and constantly more cancer patients can became fathers.

The important message of my thesis is also to remember a general prevention and not only in cases with an increased risk of testicular cancer. There are various organizations in the Czech Republic which point out an importance of prevention and deliver professional lecturers on a self-examination. One of the well-known organisations is non-profit institution Loono with the campaign "I Touch Them Every Month" which goal is to show to the public how easy but effective a self-examination can be. Another project with the original name "STK pro chlapy" ("MOT for men") appeals to men to undergo preventative checkups.

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a symbol * marks out a review

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