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LUND UNIVERSITY
Faculty of Medicine

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Abbreviations

ACT	Adjuvant chemotherapy
AFP	Alpha-fetoprotein
AR	Androgen receptor
β-HCG	beta-humanochoriongonadotrophin
BEP	Bleomycin, etoposide, cisplatin
BEP-if	Bleomycin, etoposide, cisplatin, ifosfamide
BMI	Body mass index
CI	Confidence interval
CIS	Carcinoma in situ
CT	Chemotherapy
DHT	5-alpha-dihydrotestosterone
DNA	Deoxyribonucleic acid
ED	Erectile dysfunction
EMD	Emotional disorders
EP	Etoposide, cisplatin
FISH	Fluorescence in situ hybridization
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
Gy	Gray
HADS	Hospital Anxiety and Depression Scale
hCG	Human chorionic gonadotropin
HDCT	> 4 cycles of chemotherapy
HL	Hodgkin's lymphoma
LDH	lactate-dehydrogenase
LH	Luteinizing hormone
Mk+	Elevation of markers (AFP and/or β-HCG)
NSGCT	Non-seminomatous germ cell tumour
OR	Odds ratio
PEI	Cisplatin, etoposide, ifosfamide
RPLND	Retroperitoneal lymph node dissection
RT	Radiotherapy
SCT	3-4 cycles of chemotherapy
SGCT	Seminomatous germ cell tumour
SO	Surgery only
STI	Sexually transmitted infections
SWENOTECA	Swedish-Norwegian testicular cancer project
TC	Testicular Cancer
TDS	Testicular dysgenesis syndrome
TGCC	Testicular germ cell cancer
TM	Testicular microlithiasis
WHO	World health organization

List of Original Papers

I **Eberhard J**, Ståhl O, Giwercman Y, Cwikiel M, Cavallin-Ståhl E, Lundin KB, Flodgren P and Giwercman A. Impact of therapy and androgen receptor polymorphism on sperm concentration in men treated for testicular germ cell cancer: a longitudinal study. *Human Reproduction 2004 Jun; 19(6):1418-25.*

II **Eberhard J**, Ståhl O, Cwikiel M, Cavallin-Ståhl E, Giwercman Y, Salmonson EC and Giwercman A. Risk factors for developing hypogonadism in testicular cancer patients. *European Journal of Endocrinology 2008 Apr; 158(4):561-570.*

III **Eberhard J**, Ståhl O, Cohn-Cedermark G, Cavallin-Ståhl E, Giwercman Y, Rylander L, Eberhard-Gran M, Kvist U, Fugl-Meyer K S, Giwercman A. Sexual function in men treated for testicular cancer. *Accepted for publication (Journal of Sexual Medicine).*

IV **Eberhard J,** Ståhl O, Cohn-Cedermark G, Cavallin-Ståhl E, Giwercman Y, Rastkhani H, Rylander L, Eberhard-Gran M, Kvist U, Giwercman A. Emotional disorders in testicular cancer patients in relation to hypogonadism, androgen receptor polymorphisms and treatment modality. *Submitted.*

Popular scientific summary

The treatment of testicular cancer has become one of the greatest successes in the field of oncology. Since the introduction of cisplatin, in the seventies, the survival rates have dramatically increased and today more than 95 % of patients can expect to be cured.

In view of the fact that the majority of patients are young, between 20 and 40 years of age, more and more attention has been paid to possible side-effects of the disease and its treatment.

The treatment of testicular cancer may vary in intensity, from surgery, through short chemotherapy or radiotherapy, to intensive cytotoxic treatment. Therefore, the side-effects of the therapy may vary. Contributing to this variation is not only the difference in the treatment given but probably also genetically-determined differences between individuals in their sensitivity to the adverse effects of cancer therapy.

Reproductive function of cancer survivors, including preservation of fertility and normal sexual function, is an important issue. Furthermore, production of sex hormones may influence mental health as well as metabolic and cardiovascular function. In order to improve the counselling of patients regarding their future life quality, research on cancer treatment side-effects in relation to the function of patients' reproductive organs is needed.

The first article focuses on the impact of testicular cancer treatment on sperm concentration in testicular cancer patients. This paper showed that short chemotherapy did not significantly affect sperm production, but men treated by irradiation of lymph nodes in the abdomen, or by chemotherapy for metastatic disease, were at high risk of being sterile six to twelve months after treatment. It seemed, however, that the pre-treatment sperm

production recovered after two to five years, although the size of the study was not sufficient to predict whether the recovery took place in all patients. It means that cryopreservation of sperm should still be offered to all patients before testicular cancer treatment. Interestingly, we found that variation in the androgen receptor gene also influenced the rapidity of post-treatment sperm production recovery. This is, to our knowledge, the first study showing that genetic variation influences the post-treatment recovery of sperm production in cancer patients.

Hypogonadism is a condition related to low levels of the male sex hormone, testosterone. In adult men the symptoms of hypogonadism include low levels of resolution, ambivalence, impaired concentration, fatigue and tiredness as well as osteoporosis, muscle atrophy and loss of sexual desire. The second article has mainly focused on finding predictive factors for hypogonadism, since testicular cancer patients are known to be at risk of developing testosterone deficiency. The article concludes that hypogonadism detected before treatment and certain patterns seen in the remaining testicle by ultrasound are strong predictors of hypogonadism for at least up to five years after treatment. Radiotherapy to abdominal lymph nodes and more than two cycles of chemotherapy were also found to increase the risk of developing hypogonadism, at least transiently, whereas neither age, testicular volume, extension of the disease nor genetic variations in the androgen receptor had any such effect. The findings of this paper will help us in identifying testicular cancer patients at particular risk of developing androgen deficiency.

The third article focuses on sexual dysfunctions in testicular cancer survivors. It concludes that three to five years after treatment, the patients do have significantly increased problems with erectile dysfunction as well as decreased sexual desire. Around 12 % of the patients reported erectile dysfunction compared with 3 % in the general population. Neither different

treatments nor hypogonadism were associated with the risk of having dysfunctions. It means that physicians should focus more on sexual problems in men treated for testicular cancer, although testosterone replacement is not necessarily the treatment of choice.
In the fourth article a possible relation between emotional disorders such as depression or anxiety and hypogonadism, treatment intensity and variation in the androgen receptor was evaluated. Neither hypogonadism nor variations of the androgen receptor were associated with the risk of emotional disorders. Patients receiving more than four cycles of chemotherapy owing to refractory or relapsing disease did have a very high (62 %) risk of suffering from anxiety.

The information obtained as a part of this thesis can therefore be applied in the clinical management of testicular cancer survivors by improving the counselling given to these young men and by pointing out some risk factors of serious, life-quality threatening side-effects of cancer treatment.

Background

Testicular cancer

Epidemiology

Testicular cancer (TC) accounts for roughly 1-2 % of all male neoplasms and hence is the commonest cancer disease among males aged between 20 and 40 years (Huyghe *et al*, 2003). For yet unknown reasons, there is a large variation in the incidence between different regions, the highest incidence in the world being reported in Denmark and Norway (Adami *et al*, 1994). The incidence of this cancer has increased four- or fivefold over the last five decades among Caucasian populations for a still unknown reason (Huyghe *et al*, 2003).

Approximately 95 % of the tumours are of germ cell origin (Campbell & Walsh, 1997), i.e. Testicular Germ Cell Cancer (TGCC). Two types of TGCC are distinguished; seminomatous germ cell tumours (SGCT) or non-seminomatous (NSGCT). The distribution between these two histological forms is approximately equal, but the NSGCT patients are slightly younger than those with SGCT with an approximate median age of 27 vs. 34, respectively (Cooper *et al*, 2008).

Aetiology

Aetiological risk factors are still not fully understood even though a history of cryptorchidism as well as atrophic testes and infertility are overrepresented among patients (Raman *et al*, 2005). This has raised the hypothesis of a testicular dysgenesis syndrome (TDS). Since there is rising incidence of testicular cancer (Adami *et al*, 1994; Moller, 1998) and a trend

towards higher incidence of hypospadia (Paulozzi *et al*, 1997) and cryptorchidism (Boisen *et al*, 2004), concomitantly with a decline in semen quality (Moller, 1998; Swan *et al*, 1997), it has also been suggested that all these disturbances in the male reproductive function could be a part of the same syndrome, with a common aetiology and being of foetal origin (Skakkebaek *et al*, 2001).

Diagnosis and staging

TGCC should be suspected in patients who present with a unilateral, intrascrotal, painless mass which can also be detected by ultrasonography. Complementary measuring of tumour markers such as alpha-fetoprotein (AFP), beta-humanochoriongonadotrophin (β-HCG) and lactate-dehydrogenase (LDH) is mandatory for disease staging. As the primary treatment, an orchiectomy is performed. Carcinoma-in-situ (CIS) in the contralateral testicle is detected by means of a surgical biopsy. The incidence of contralateral CIS in Denmark and in Germany has been reported to be approximately 5 % (Dieckmann & Loy, 1996; Giwercman *et al*, 1987), but data for other countries are lacking. The orchidectomy is followed by pathological examination of the tumour and a radiological examination for the staging procedure.

For staging, there are different classification systems. In Scandinavia, the Royal Marsden Hospital staging system is most commonly used (Dearnaley *et al*, 2001). At the time of diagnosis, approximately 80 % of SGCT are in stage I (no evidence of metastases) or IIA (metastases to abdominal nodes with a transverse diameter < 2 cm), 20 % presenting with more advanced disease. In SGCT, stage I disease, two risk factors for relapse have been identified, tumour size and invasion of rete testis. For NSGCT about

50 % have detectable metastases at the time of diagnosis (Klepp *et al*, 1990a), but those in stage I have a 30 % risk of relapse if no treatment is given after orchiectomy, which points to a high frequency of subclinical metastases (Klepp *et al*, 1990b). Vascular invasion in the primary tumour is the main risk factor in relapse.

Treatment

According to the recently updated guidelines of the European Consensus Group on testicular cancer, the treatment is dependent on the histology, presence of risk factors and stage of the disease (Krege *et al*, 2008a; Krege *et al*, 2008b). In Sweden and Norway uniform guidelines for treatment have been developed by the Swedish Norwegian Testicular Cancer Project (SWENOTECA) (www.ocsyd.se) and these are principally in accordance with the European guidelines.

In the SWENOTECA protocols, a risk-adapted strategy for stage I disease is applied. NSGCT patients without vascular invasion can choose between one cycle of adjuvant chemotherapy or surveillance.

In case of vascular invasion the recommendation is one cycle of adjuvant chemotherapy. The chemotherapy regimen commonly used is the BEP regimen (bleomycin 30 000 IU days 1, 5 and 15; etoposide 100 mg/m^2 days 1-5 and cisplatinum 20 mg/m^2 days 1-5, given every third week).

The guidelines for stage I SGCT have recently been changed. Previously, at the time of inclusion of the patients who form part of this thesis, no difference was made between low- and high-risk subjects. All stage I SGCT patients could choose between surveillance and radiotherapy (RT) administered to para-aortic and ipsi-lateral iliacal lymph nodes to a target dose of 25.2 Gy, given in fourteen fractions. The RT is given with lead shield of the remaining testicle. The radiotherapy decreases the recurrence

rate from approximately 10% to 2 % (www.ocsyd.se). On the basis of a large randomised trial, the recommendation has now been changed, since one single dose of carboplatin was found to be as effective as adjuvant radiotherapy (Oliver *et al*, 2005). Today we distinguish between stage I SGCT with no or one risk factor (low risk) and those having two risk factors (high risk), tumour size > 4 cm or invasion to rete testis. Low-risk patients are recommended surveillance and high-risk patients one cycle of CT (carboplatin), RT is still an option, but is considered more toxic than single dose carboplatin and consequently not recommended. In SGCT clinical stage IIA (CSIIA) radiotherapy is recommended, 27.0 Gy given in fifteen fractions against para-aortal and ipsi-lateral iliacal lymph nodes.
Disseminated disease is treated with cisplatin-based chemotherapy. The basic strategy of the SWENOTECA protocol for NSGCT is to individualise treatment according to the decline of the tumour markers AFP and β-HCG during the initial treatment. Initial treatment for NSGCT patients is two courses of BEP. Patients with satisfactory response receive one or two additional courses of BEP while patients with unsatisfactory half-time for decline in marker levels receive intensified treatment in two steps with the addition of ifosfamide (BEP-if/PEI) in step I. If there is still unsatisfactory response, the treatment is intensified according to step II, involving high-dose chemotherapy with stem cell rescue. Post-chemotherapy surgery, retroperitoneal lymph node dissection (RPLND), is recommended in all patients with abdominal lymph node metastases >= 2 cm at staging and also resection of rest tumours in other locations if possible (Fossa *et al*, 1989). Standard treatment of SGCT > CSIIA is four courses of EP (=BEP minus B), but treatment intensity is governed by tumour response to the treatment given.
The risk of developing a contralateral cancer is approximately 5 %, which is in accordance with the risk of having cancer in situ (CIS) in a contralateral

testicle (Osterlind *et al*, 1991) and CIS is the precursor for TGCC (Hoei-Hansen *et al*, 2005). Patients with confirmed CIS are recommended radiotherapy, 16 Gy given in eight fractions to the contralateral testicle. With these treatment strategies the prognosis of TGCC patients is excellent with a survival rate exceeding 95 % (Verdecchia *et al*, 2007). Owing to this exceptionally good prognosis the question of long-term toxicity of the treatment and the patient's quality of life has become increasingly important.

Spermatogenesis and male endocrinology

Testicular histology

The testicles contain two functionally different parts: the seminiferous tubules, which contain germ cells and Sertoli cells responsible for sperm production, and the interstitial space, with Leydig cells that are responsible for androgen production.

The hypothalamic pituitary testicular axis

Gonadal function is controlled by the gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus. This hormone stimulates production of the gonadotropins, luteinising hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland. LH and FSH are secreted in peaks. The stimulation and inhibition of GnRH release seems to be very complex and is not yet fully understood, involving several neurotransmitters and neurohormones, but also with a feedback system from sex steroids. The release is pulsatile and gives a corresponding pulse in LH and FSH release from the anterior pituitary gland (Veldhuis *et al*, 1983). We also know that

the pulsatile release of GnRH is crucial for the production of the gonadotropins whereas continuous release actually inhibits the release, a phenomenon which is utilised in pharmacologically-induced castration of e.g. prostate cancer patients (Belchetz *et al*, 1978).

LH targets its receptor on the cell membrane of the Leydig cells and stimulates testosterone production. FSH targets its specific receptor on the Sertoli cell membrane, but the more specific mechanism of initiation of spermatogenesis is still poorly understood.

The secretion of LH is mainly regulated by negative feedback from testosterone, but oestradiol is also involved (Morishima *et al*, 1995).

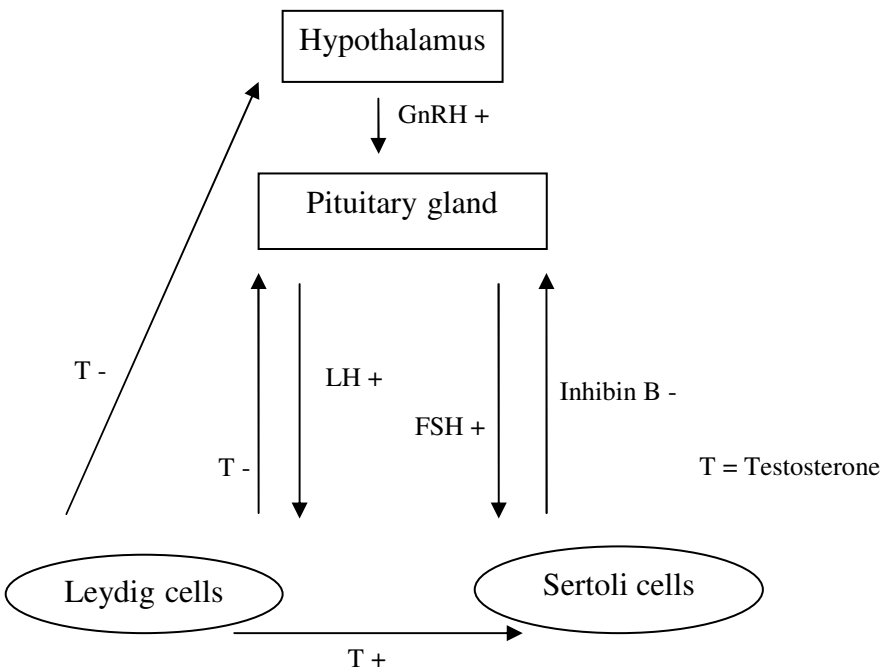

Figure 1: *The hypothalamic pituitary testicular axis. GnRH stimulates secretion of LH and FSH. FSH stimulates spermatogenesis, with a negative feedback from inhibin B. LH stimulates testosterone production. Testosterone inhibits both GnRH and LH release. Testosterone is converted to oestradiol which exerts a negative feedback on the pituitary gland.*

Testosterone decreases the frequency and the intensity of the GnRH pulses from the hypothalamus (Matsumoto & Bremner, 1984) and through a direct effect on the pituitary gland (Sheckter *et al*, 1989).
Inhibin B is a peptide produced by Sertoli cells and its production is dependent on the presence of primary spermatocytes and is under the control of FSH (McLachlan *et al*, 1988). Inhibin B is also involved by a negative feedback mechanism in the regulation of FSH secretion. Oestradiol also appears to have a negative feedback on FSH secretion (Hayes *et al*, 2001) (Figure 1).

Androgen production and function

As a precursor steroid, cholesterol, through several enzymatic steps, converts to testosterone in the testes and the adrenal gland (Figure 2). In a post-pubertal male Leydig cells are responsible for 95 % of all testosterone production, the remaining 5 % secreted from the cortex of the adrenal gland. Testosterone, under the control of LH, is not only secreted in a pulsatile fashion (Winters & Troen, 1986), but there is also a significant diurnal variation with peak levels early in the morning, probably sleep induced (Axelsson *et al*, 2005), and lowest concentration in the evening. In the circulation, most of the testosterone is bound to sex hormone-binding globulin (SHBG) and albumin. The high affinity binding to SHBG accounts for 60-70% of all protein bound testosterone, whereas the low affinity binding to albumin accounts for the remaining 30-40%. Only approximately 2 % of plasma testosterone is in a free form (Dunn *et al*, 1981). The albumin-bound testosterone is usually bioavailable and therefore the biologically active concentration approximately reflects the summary of albumin-bound and free testosterone. The concentration of the SHBG, which is secreted from the liver, is under the influence of a number of

hormonal factors. The concentration of this protein is decreased by androgen replacement therapy and increased by oestrogen administration. Other factors influencing SHBG levels include thyroid hormone increasing and glucocorticoids, insulin and obesity decreasing the levels of this protein (Gascon *et al*, 2000; Tchernof & Despres, 2000; Wallace *et al*, 2003). In healthy, normal men, an increase of SHBG leads primarily to a decrease of free testosterone, but subsequently a reduced negative feedback and increased LH secretion imply a higher level of total testosterone and, thereby, unchanged concentration of the biologically active hormone. In hypogonadal men, the SHBG concentration is usually increased, if the low testosterone levels are not associated with overweight (Selby, 1990).
Testosterone is metabolised to 5α-dihydrotestosterone (DHT) and to oestrogens (Figure 2). DHT is the main androgen acting on epididymis, seminal vesicles and prostate, originating from testosterone through 5α–reductase. DHT is crucial for normal male sex differentiation in foetal life and also plays a role at puberty in developing the adult male phenotype. The conversion to DHT is organ-dependent and occurs in the target organs. Oestrogens act both synergistically and conversely to androgens and also exert a negative feedback on gonadotropin secretion. In males, oestrogens are mainly produced in extragonadal tissue, in particular, fat tissue. This is one of the explanations for the association between the sex hormone levels and body mass index.
Apart from the role of the androgens in sex differentiation and in initiation of puberty, their main effect is maintenance of spermatogenesis and sexual maturation. These hormones also play a crucial role in the metabolism, muscle and cognitive function and hair growth. Androgens are also important for sufficient mineralisation of the bones, although this effect seems to be mediated through the oestrogens (Kenny & Raisz, 2002; Vanderschueren *et al*, 2000).

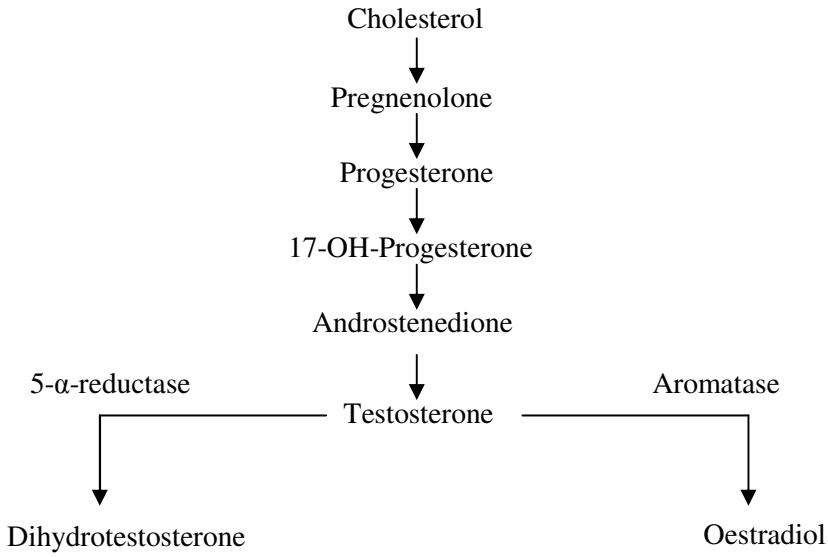

Figure 2: *Testosterone production and metabolism.*

Both testosterone and DHT target the same receptor with high affinity, the androgen receptor (AR). Activation of the AR leads to formation of an active transcriptional regulatory complex that binds with high affinity to androgen response elements in DNA, leading to an up or down regulation of androgen-dependent genes (Lee & Chang, 2003).

The AR is encoded by a gene on the X-chromosome and males, therefore, have only one copy, which they inherit from their mother. The N-terminal domain of the first exon contains a repetitive sequence of CAG triplets encoding a polyglutamine stretch of variable length (Figure 3). In the normal population, the number of CAG repeats varies from approximately ten to thirty (Giwercman *et al*, 1998). There are some ethnic differences, with Africans having shorter and Asians slightly longer repeat lengths than Caucasians (Kittles *et al*, 2001). Among Swedish males, a median number of 22 CAG repeats was reported (Giwercman *et al*, 1998). The repeat length

plays an important role in the transcriptional activity of the AR (Davis-Dao *et al*, 2007; Tut *et al*, 1997; von Eckardstein *et al*, 2001a).

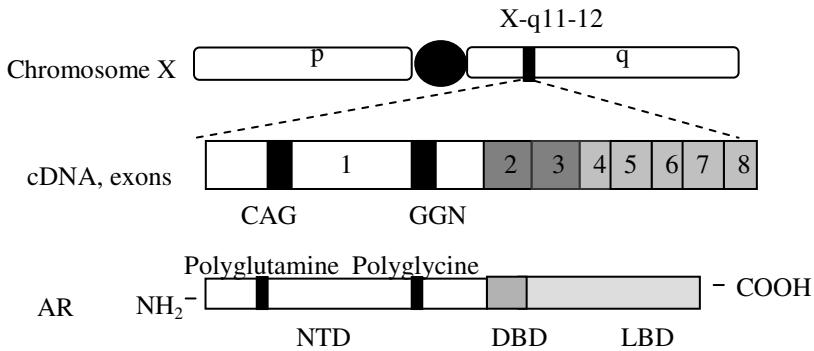

Figure 3: *Human AR gene; structural organisation and protein. NTD= Amino-terminal domain. DBD=DNA-binding domain. LBD=Ligand-binding domain*

There are reports indicating an association between long CAG repeats and decreased sperm concentrations or infertility (Mifsud *et al*, 2001; Tut *et al*, 1997; von Eckardstein *et al*, 2001a), but these findings have not been confirmed by all studies (Giwercman *et al*, 1998; Rajpert-De Meyts *et al*, 2002).

CAG repeat length has also been found to relate to the risk of obesity and metabolic syndrome although the association is not yet fully clarified (Stanworth *et al*, 2008). It seems that the lower androgen sensitivity in subjects with long CAG is compensated, at least partly, by increased testosterone concentration, probably because of higher LH levels owing to reduced negative feedback through a less sensitive receptor (Stanworth *et al*, 2008). In ageing men, the AR CAG repeat length correlates significantly with testosterone and oestradiol. Weaker transcriptional activity of the AR with longer CAG repeats appears to be totally or nearly totally compensated

for by higher testosterone levels. Consequently, oestrogen levels rise and phenotypic correlations may rather reflect oestrogen than testosterone action (Huhtaniemi *et al*, 2009).

Major expansion of the repeat length up to a length of 40 to 75 repeats causes spinal and bulbar muscular atrophy, known as Kennedy's disease (La Spada *et al*, 1991); the aetiology is not yet fully understood but seems not be related to lower androgen sensitivity but rather to intracellular protein aggregation (McEwan, 2001). Nevertheless, androgen receptors are located throughout the brain (Beyenburg *et al*, 2000; Yaffe *et al*, 2003) and the impact of AR CAG repeat length on neurological and psychiatric disorders has been further investigated. In this context, a possible association between CAG repeat length and affective disorders was postulated, where the severity of depression and anxiety was shown to be negatively correlated with the number of CAG repeats in adolescent patients (Su *et al*, 2007). Depressive symptoms have also been associated with long CAG repeats in ageing males (Harkonen *et al*, 2003).

Another polymorphic sequence in the AR gene is the GGN repeat, encoding a polyglycine stretch. The GGN repeat length varies between 10 and 27, 85 % of the population having 23 or 24 GGN (Lundin *et al*, 2003). A GGN repeat length of 23 is the most common and in vitro (Lundin *et al*, 2007) and in vivo (Lundin *et al*, 2006) studies indicate that this is the length that is associated with optimal AR function.

Spermatogenesis

Spermatogenesis includes a number of steps of cell division and differentiation leading to the development of elongated spermatids from spermatogonia, which are the stem cells of spermatogenesis. Spermatogonia A represent the stem cell pool and the differentiation to spermatogonia B

initiates DNA synthesis resulting in tetraploidic primary spermatocytes. Each primary spermatocyte undergoes a first meiotic division, giving rise to two secondary spermatocytes, which subsequently enter second meiotic division resulting in a total of four haploid spermatids. The next steps include reorganisation of the nucleus and the cytoplasm, development of a flagellum and the head being covered by an acrosomal cap, forming the mature sperm. These last steps are named spermiogenesis. In humans the total duration of spermatogenesis is approximately 70 days. Additionally fourteen days are required for transport of the mature sperm through the epididymis to the ejaculatory ducts. As mentioned above, the hormonal control involves FSH and testosterone acting through their receptors on the Sertoli cells. In the testis, the concentration of testosterone is approximately 100 times higher than in peripheral circulation (Maddocks *et al*, 1993) and a Sertoli cell selective androgen receptor knockout in mice caused spermatogenic arrest at primary spermatocyte level (De Gendt *et al*, 2004), showing that AR in Sertoli cells is an absolute requirement for androgen maintenance of complete spermatogenesis, and that spermatocyte/spermatid development/survival critically depends on androgens. Patients with complete FSH receptor mutation were shown, however, to have some sperm production, but the concentration of sperms was very low (Tapanainen *et al*, 1998).

Exogenous administration of testosterone, in particular when given as injections, leads to inhibition of gonadotropins secretion leading to lack of testosterone production in Leydig cells and thereby azoo- or oligozoospermia (Nieschlag *et al*, 2001).

Germ cells are among the most rapidly dividing cells in the body, which makes them quite sensitive to the effects of CT and RT. Total eradication of spermatogonia B, the first differentiation of the stem cells of spermatogenesis, will lead to permanent azoospermia. This effect has been

seen following more than 4 Gy of irradiation (Rowley *et al*, 1974) and can also be caused by cytotoxic drugs, mainly those with alkylating effect (Howell & Shalet, 2005). If the stem cells are not eradicated, regeneration of spermatogenesis should be expected. In mice, this process usually takes three to six months (Meistrich, 1993), corresponding well to the duration of spermatogenesis. In humans, however, for unknown reasons this process is rather prolonged in some patients, and re-appearance of sperms in the ejaculate has been reported more than five years after completion of cytotoxic treatment (Anserini *et al*, 2002).

Infertility

The definition of infertility is failure to conceive during twelve months of frequent unprotected intercourse (WorldHealthOrganization, 2000). The prevalence of infertility among couples in the western world is approximately
15 %. Around 20 % is reported to be because of a male factor, 38 % to a female factor, 27 % to both male and female factors and 15% is so-called unexplained infertility (WHO Task Force on the Diagnosis and Treatment of Infertility. *et al*, 1987). Thus, in roughly 50 % of all infertility cases some pathology may be found in the male.
The investigation of male infertility includes clinical examination including measurement of testicular volume, semen analysis and hormonal evaluation. Standard semen analysis includes parameters such as ejaculate volume, sperm concentration, motility and morphology assessed according to the WHO criteria (World Health Organization., 1999). Optionally, an evaluation of sperm chromatin can be performed since a high proportion of sperms with chromatin strand breaks is associated with risk of infertility

(Spano *et al*, 2000). Hormonal analysis should include assessment of FSH, LH, testosterone, SHBG, inhibin B and oestrogen.
The causes of male infertility can be divided into hypothalamo-pituitary disease, testicular or post-testicular defects or idiopathic cause. Despite careful assessment, causes of abnormal sperm number, morphology or function cannot be clarified in 40 to 50 % of infertile men (de Kretser, 1997). Testicular disease or primary hypogonadism has been suggested to be responsible for 30 to 40 % of male infertility (de Kretser, 1997).

Testicular cancer, side-effects of disease and its treatment

The high survival rate in TGCC patients during the last few years has implied an increasing attention given to the side-effects of both the disease per se and the treatment given. Thus, the life quality of the survivors has come into focus. As already indicated, the severity of the disease and the treatment modalities differ substantially between patients. In addition, some level of inter-individual variation in the susceptibility to the side-effects of the therapy can be expected (Fry *et al*, 2008). Consequently, frequency, modality and severity of side-effects may vary between patients receiving the same treatment.
When discussing side-effects, we discriminate between acute, transient and/or late side-effects. The most frequent acute side-effects of chemotherapy are nausea, neutropenia and alopecia. Nephropathy, neuropathy and ototoxicity may occur, mainly as a side-effect of cisplatinum and, if these symptoms appear during therapy, there is a risk of chronicity. These side-effects are well investigated and strategies to reduce the risk of developing them are integrated into the routine management of the patients. Another serious late side-effect is the risk of inducing a second

malignancy. This risk has been reported to be two or threefold higher than in the general population (Robinson *et al*, 2007; Travis *et al*, 2005). The risk of developing haematological malignancies is mainly related to etoposide (Nichols *et al*, 1993) and is dependent on the cumulative dose given (Pedersen-Bjergaard *et al*, 1991). Concerning solid tumours, there is an increased risk mainly after RT within the radiation field (Travis *et al*, 2005).

Testicular cancer and infertility

Patients diagnosed with TGCC have an increased risk of being sub- or infertile (Brydoy *et al*, 2005; Moller & Skakkebaek, 1999). The spermatogenesis might be defective already before orchiectomy and deteriorates further after orchiectomy (Petersen *et al*, 1999a; Petersen *et al*, 1999b). The orchiectomy is reported to cause azoospermia in up to 10 % of patients (Petersen *et al*, 1999a).

Furthermore, infertile men have a high risk of developing TGCC. In men presenting with abnormal semen quality, a twenty fold increased risk of developing TGCC was reported (Raman *et al*, 2005). TGCC is also related to cryptorchidism, failure of the testicles to descend to the scrotum during foetal life. Cryptorchidism is associated with poor semen quality and the relative risk of developing TGCC in men with a history of this congenital malformation was reported to be two to ten times higher than in the background population (Dieckmann & Pichlmeier, 2004; Swerdlow *et al*, 1997; Wood & Elder, 2009). Infertility in TGCC patients can also be acquired or accentuated owing to side-effects of TGCC treatment surgery, chemotherapy (CT) and/or radiotherapy (RT).

Overall, approximately 20 % of all treated patients have long-lasting sequelae with infertility or sexual dysfunction (Hartmann *et al*, 1999; Kuczyk *et al*, 2000). Chemotherapy was shown to cause impaired sperm

production and decreased fertility (Bokemeyer *et al*, 1996; Fossa *et al*, 1985b; Hendry *et al*, 1983; Lampe *et al*, 1997; Pont & Albrecht, 1997; Stephenson *et al*, 1995) and semen quality was found to be significantly more impaired in CT-treated than only orchidectomised patients (Hansen *et al*, 1990). Following CT, as many as one-third of the men were found to develop at least transient azoo-oligozoospermia (Stephenson *et al*, 1995). The risk of being permanently azoospermic, however, has been reported to be only between zero and 5 %, but these studies did not include patients receiving more than four cycles of BEP (Gandini *et al*, 2006; Hansen *et al*, 1989). The ability of sperm regeneration is dose-dependent. Cumulative doses of cisplatinum exceeding 400 mg/m^2, equivalent to four courses of BEP regimen, gave long-lasting impairment of gonadal function (Pont & Albrecht, 1997). Nevertheless, after ten years, 38 % of TGCC patients attempting to fertilise, that had received a total dose > 850 mg cisplatin, had achieved paternity without the use of cryopreserved sperm. These figures increased to 48 % after fifteen years (Brydoy *et al*, 2005).

Adjuvant RT to abdominal lymph nodes might also impair sperm production owing to scattered radiation to the remaining testicle. But if scattered doses can be limited <0.2 Gy permanent radiation-induced effects on the remaining testicle are very unlikely (Sedlmayer *et al*, 1999). In the adjuvant setting and RT given by modern technique, the risk of permanent azoospermia is very small, current reports mainly pointing towards a transient effect (Centola *et al*, 1994; Sedlmayer *et al*, 1999). Radiation doses exceeding 4 Gy have been reported to induce permanent azoospermia (Rowley *et al*, 1974). Direct radiotherapy to the remaining testicle with doses of 14-20 Gy given for eradication of CIS implies permanent azoospermia (Giwercman *et al*, 1991).

Retroperitoneal lymph node dissection (RPLND) (Fossa *et al*, 1985a) is a well-known cause of retrograde ejaculation. With a unilateral nerve-sparing

procedure, introduced during the 1980s, the risk of retrograde ejaculation varies from a few percent to almost 30 % (Donohue, 2003; Jacobsen *et al*, 1999; Krege *et al*, 2008a; Krege *et al*, 2008b), while with more extensive bilateral surgery the risk is very high.

Since it is not completely predictable which men will develop long-standing or permanent azoospermia, sperm cryopreservation is routinely recommended to TGCC patients. In a large study on TGCC survivors, approximately 50 % were interested in pre-treatment semen cryopreservation (Magelssen *et al*, 2005). A considerable number of these achieved fatherhood without the use of frozen semen, but the psychological impact of pre-treatment cryopreservation was undeniable. For 7 % of the survivors, however, assisted reproductive techniques with cryopreserved sperm offered the only chance of post-treatment paternity (Magelssen *et al*, 2005).

Several studies have addressed the issue of recovery of spermatogenesis following TGCC treatment and whether it could be predicted or not (Aass *et al*, 1991; Fossa *et al*, 1990; Lampe *et al*, 1997). Pre-treatment decreased gonadal function (low sperm count and increased FSH) is a risk factor of persistent testicular dysfunction (Fossa *et al*, 1990; Lampe *et al*, 1997). Other pre-treatment risk factors include defective sperm chromatin structure as well as age (Aass *et al*, 1991; Fossa *et al*, 1997).

Since androgens play an important role in spermatogenesis and the physiological effect of testosterone in humans is modified by the length of CAG and GGN repeats of the AR gene, it could be speculated that these polymorphisms might also play a role in the regeneration of sperm production following cancer therapy.

Although several studies have addressed the issue of semen quality and fertility in TC survivors, the current literature has several limitations. Many

studies do not discriminate between the effects of different treatment modes including CT, RT, orchiectomy and RPLND (Fossa *et al*, 1985b; Hansen *et al*, 1990; Hendry *et al*, 1983; Joos *et al*, 1997; Lampe *et al*, 1997; Pont & Albrecht, 1997; Stephenson *et al*, 1995) and do not take the intensity of treatment into account (Bokemeyer *et al*, 1996; Hansen *et al*, 1990; Stephenson *et al*, 1995). Also, knowledge about the time course is still scarce. Furthermore, the issue of genetically-determined inter-individual variation in post-treatment recovery of spermatogenesis has not yet been investigated.

To be able to give adequate information to TGCC patients about future fertility, there is a need for longitudinal studies taking into consideration treatment modality, length of follow-up period and also identification of possible genetic markers of restoration of sperm production.

Testicular cancer and hypogonadism

The diagnosis of hypogonadism is based on a combination of biochemical and clinical features.

In men with symptoms of androgen deficiency, the combination of high gonadotropin levels and low testosterone indicates testicular origin of hypogonadism or primary hypogonadism, which is the predominant type in TGCC patients.

The clinical symptoms of hypogonadism include: loss of libido, erectile dysfunction, impairment of memory, depression, lethargy, osteoporosis, loss of muscle mass and strength and some regression of secondary sexual characteristics, commonest in post-pubertal men (Carnegie, 2004). Furthermore, it has been shown that hypogonadism is a risk factor for development of metabolic syndrome and cardiovascular disease (Kupelian *et al*, 2006) and TGCC patients have an increased risk of both

cardiovascular risk profile and manifest disease (Huddart *et al*, 2003; Nuver *et al*, 2005b; Vaughn *et al*, 2008; Wethal *et al*, 2007). The pathophysiology behind this association is still not fully understood and different mechanisms have been postulated. It has been speculated that this effect is related to a direct negative effect of chemotherapy on blood vessels (Nuver *et al*, 2005a) and may also be a consequence of impaired renal function (Bosl *et al*, 1986). A link between hypogonadism and cardiovascular disease should also, however, be considered.

TGCC patients are at risk of developing hypogonadism (Nijman *et al*, 1987; Willemse *et al*, 1983). The disease per se seems to be associated with Leydig cell insufficiency, since orchidectomised men with TGCC have lower levels of testosterone compared with orchidectomised non-TGCC patients (Willemse *et al*, 1983). Orchidectomy is followed by a decrease in testosterone levels (Fossa *et al*, 1984) but a time-related compensatory improvement may occur, which is why hesitation in initiating replacement therapy shortly after surgery is warranted (Petersen *et al*, 1999a). Chemotherapy and radiotherapy might affect Leydig-cell function negatively. A total of 60 % of TGCC patients receiving cisplatinum-based CT owing to metastatic disease had elevated LH levels compared with 11 % of those not given CT several years after treatment (Hansen *et al*, 1990). The negative effect of cisplatin-based chemotherapy with persistent LH elevation and low testosterone is further confirmed in other investigations (Fossa *et al*, 1995; Hansen & Hansen, 1993; Strumberg *et al*, 2002). There are, however, some studies where no association between cisplatin-based CT and Leydig cell dysfunction could be found (Fossa *et al*, 1985b; Petersen *et al*, 1994). These dissimilarities might be because of differences in chemotherapy used, doses and length of follow-up period.

The impact of adjuvant sub-diaphragmal RT is less studied but a possible transient increase of LH, following this treatment, has been reported (Joos *et*

al, 1997). Leydig cells are, however, generally considered to be less sensitive to irradiation than the germinal epithelium.

RT to the contralateral testis is given when cancer in situ is diagnosed. Endocrine function seems to be impaired already before treatment with further impairment after testicular irradiation with 14-20 Gy (2 Gy x 7-10) but only with minor dose dependency in the range of 14 to 20 Gy (Petersen *et al*, 2003; Petersen *et al*, 2002). An unresolved issue is whether the long-term age-dependent decrease in testosterone levels is accentuated by this Leydig cell damage.

Despite the fact that many studies have focused on testosterone and LH levels in TGCC-treated men, little is known about risk factors of developing hypogonadism. In a ten-year follow-up study, men treated for TGCC had a three- to fourfold risk of developing hypogonadism and the risk increased with age and treatment intensity (Nord *et al*, 2003). The predictive value of treatment intensity was further confirmed where both radiotherapy and chemotherapy resulted in additional Leydig cell impairment compared with surveillance only (Huddart *et al*, 2005).

Apart from the treatment-related factors, however, other characteristics might also be associated with the risk of testicular dysfunction and hypogonadism.

Testicular microlithiasis (TM), detected by ultrasonography, is over-represented among TGCC patients, found in men during infertility investigation (Costabile, 2007). Thus, one could speculate whether men presenting with TM also have an increased risk of developing post-treatment hypogonadism. Another factor of potential interest is the genetically-determined sensitivity to androgens, related to the polymorphisms in the AR gene (Lundin *et al*, 2006; Tut *et al*, 1997).

Bearing in mind that the symptoms of hypogonadism are rather uncharacteristic and therefore may be overlooked during the follow-up, it

would be of help to define men at high risk. Characteristics such as age, pre-treatment hormone levels, treatment modality, stage of disease, presence of TM, contralateral testicular volume and genetic markers should, therefore, be further investigated to identify risk factors for androgen deficiency in TGCC patients.

Testicular cancer and sexual dysfunction

The commonest male sexual dysfunctions are erectile dysfunction (ED), ejaculatory dysfunction and decreased sexual desire or interest (Halvorsen & Metz, 1992a). The prevalence in the general population has been reported as 4 to 9 % for ED, 4 to 10 % for absent or delayed ejaculation and 36 to 38 % for premature ejaculation (Spector & Carey, 1990). There are studies suggesting an increase in the frequency of absent or delayed ejaculation as well as erectile dysfunction and a decrease in premature ejaculation. Desire disorders have increased in prevalence among patients referred for sexological treatment (Spector & Carey, 1990). The aetiology of different categories of sexual dysfunction differs substantially and includes neurological, vascular, endocrinological, psychological, traumatic or pharmaceutical causes. The anamnesis could give a hint about the underlying cause; loss of nocturnal erection indicates a neurological or vascular cause, sudden onset a traumatic or psychological reason and an unsustained erection might indicate a vascular or psychological cause (Halvorsen & Metz, 1992b).

Metabolic syndrome is a risk factor for cardiovascular disease, including reduced penile blood flow with subsequent ED (Fung *et al*, 2004), and since TGCC patients have an increased risk of developing metabolic syndrome (Wethal *et al*, 2007) it could be hypothesised that these patients also have an

increased risk of ED. There are even reports indicating that ED could predict later coronary heart disease (Min *et al*, 2006; Thompson *et al*, 2005). TGCC patients are at risk of sexual dysfunctions, the most established being retrograde ejaculation owing to RPLND surgery (Fossa *et al*, 1985a). Many patients consider this side-effect as a more important problem in relation to infertility then its impact on sexual life. Today, there are different options to assist these patients in achieving fatherhood, including use of α adrenerg receptor stimulators (Ochsenkuhn *et al*, 1999), penile vibration, trans-rectal electroejaculation (Ohl *et al*, 1991) or use of testicular or epididymal sperm extraction followed by in vitro fertilisation or intracytoplasmic sperm injection (Rosenlund *et al*, 1998). Another option is using cryopreserved sperms.

Among TGCC patients, however, several other sexual dysfunctions have been reported and, apart from ejaculatory dysfunction, erectile dysfunction (Jonker-Pool *et al*, 2001) and absent or reduced orgasm are overrepresented (Nazareth *et al*, 2001), which is also true for decreased sexual enjoyment and desire (Joly *et al*, 2002).

Since the aetiology of sexual dysfunctions differs substantially, a number of risk factors have been evaluated. High age at treatment (Aass *et al*, 1993; Caffo & Amichetti, 1999) as well as treatment modality, chemo- or radiotherapy (Jonker-Pool *et al*, 1997; Tinkler *et al*, 1992), has been reported as having a negative impact on sexuality. Recovery over time has been seen, more patients reporting dissatisfaction with sexual life six months after therapy than before treatment, but with some recovery after three years (Aass *et al*, 1993).

Another aspect of importance in TGCC patients with sexual dysfunctions is the issue of psychological stress caused by the threat from a malignant disease (Jonker-Pool *et al*, 2001). An additional factor to be taken into consideration is hypogonadism, since both TGCC and sexual dysfunction

have associations with androgen deficiency. There is, however, a lack of studies focusing on this association. Although Wiechno and colleagues (Wiechno *et al*, 2007) found significant association between increased LH levels and symptoms of sexual dysfunction as assessed by use of the Sexual Functioning Questionnaire (SFQ), low testosterone levels were not found to be a risk factor for abnormal SFQ or erectile dysfunction assessed by the International Index of Erectile Function (IIEF) (Wiechno *et al*, 2007).
In most reports, as a methodological tool global or domain-specific questionnaires were used to assess sexual function, giving composite scores. Item by item comparison with a control group has not been done. Longitudinal reports are also scarce. Owing to these methodological shortcomings, full knowledge about risk factors and frequency of sexual dysfunctions in TGCC patients is still lacking. Consequently, since sexual function is an issue of great importance to many patients, further investigations are warranted in order to improve knowledge about the cause of this problem. Such information is important for prevention, early detection and also treatment of sexual dysfunction in TGCC survivors. Thus, androgen deficiency can easily be treated by replacement therapy, whereas sexual problems not related to lack of testosterone require other management strategies.

Testicular cancer and emotional disorders

In oncological care the main objective concerning psychiatric co-morbidity is to identify the major general diagnoses.
According to the standardised psychiatric diagnose system, diagnostic and statistical manual of mental disorders (DSM IV) (American Psychiatric Association, 1995) there are several emotional disorders (EMD), however,

in current thesis only anxiety disorders and depression are discussed as EMD.

The lifetime risk of having a mood disorder or depression is approximately 20 % (Kessler *et al*, 1994) and the risk within a twelve-month period of having an anxiety disorder is 18 % whereas it is approximately 9 to 10 % for depression (Kessler *et al*, 2005; Kroenke *et al*, 2007). Also, in developed countries world-wide mental illness is reported to count for about 15 % of total disability (Murray & Lopez, 1997).

A recent report observes that the non-psychiatrist physician's accuracy in recognising depression is low and that it is necessary to develop methods to improve the physician's ability to recognise depression (Cepoiu *et al*, 2008).

The diagnostic criteria for major depression according to DSM IV are presentation of at least five of the following symptoms most of the day, nearly every day, for at least two repeated weeks:

- depressed mood;
- loss of interest/pleasure;
- change in sleep;
- change in appetite or weight;
- change in psychomotor activity;
- trouble concentrating;
- loss of energy;
- thoughts of worthlessness or guilt;
- thoughts about death or suicide.

Sometimes patients have fewer than five of these symptoms or the symptoms are lighter, which could then be characterised as minor depression or dysthymic disorder.

Anxiety disorders can be divided into several different diagnoses, such as panic disorder, different phobias including social phobia, generalised

anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder, according to DSM IV (American Psychiatric Association, 1995). Accordingly, different anxiety diagnoses have their specific diagnostic criteria, but some of them are common to all anxiety disorders such as excessive, irrational fear and dread. Other common symptoms for many of the disorders are unfounded worry, irritability, sleeping disorders, feeling nervous, psychosomatic symptoms such as palpitations, difficult in breathing, dizziness and chest pain.

Several reports indicate that these psychiatric disorders are underdiagnosed (Kroenke *et al*, 2007; Wittchen *et al*, 2001; Wittchen *et al*, 2002) as well as being very common in society (Kessler *et al*, 2005; Kessler *et al*, 1994; Kroenke *et al*, 2007). To reveal a correct diagnosis the best way is still structured face-to-face questioning based on the DSM IV criteria, but in the hope of facilitating the diagnostic procedure and also to screen for the disorders, several questionnaires have been developed. A number of these tools are well-validated and applicable in different clinical situations. One of the most extensively validated and commonly used is the Hospital Anxiety Depression Scale (HADS). The HADS questionnaire consists of fourteen questions, seven concerning depression (HADS-D) and seven concerning anxiety (HADS-A). Each question is scored from 0 to 3 and the score on each subscale (HADS-A and HADS-D) ranges from 0 to 21. HADS-A≥8 indicates anxiety, HADS-D≥8 indicates depression (Zigmond & Snaith, 1983). A review based on 747 papers showed sensitivity and a specificity of approximately 0.80 for both anxiety and the depression-related part of HADS (Bjelland *et al*, 2002). Furthermore, a comparative validation between HADS and Structured Clinical Interview for DSM IV (SCID) showed that by use of the recommended cut-off points for HADS a sensitivity of 85 % and a specificity of 76 % for diagnosing EMD were achievable (Lowe *et al*, 2004).

Even though TGCC patients report good general satisfaction with life (Fleer *et al*, 2004; Joly *et al*, 2002), these men have an increased risk of anxiety (Dahl *et al*, 2005b; Fossa *et al*, 2003) and also, if presenting with chronic fatigue, there is an association between both depression and anxiety following successful cancer treatment (Dahl *et al*, 2005a). Little is known, however, about possible predictive causes. Treatment modality is one possible cause but no association has been found between the different treatment modalities and risk of reduced quality of life (Mykletun *et al*, 2005). Hypogonadism and depression have several symptoms in common such as low level of resolution, ambivalence, impaired concentration, fatigue and low energy serving an obvious problem in making the right diagnose. A possible association between depression and biochemical hypogonadism has been reported, where high LH levels were associated with increased risk of being depressed (Wiechno *et al*, 2007).
In this context, another interesting observation was a study on ageing males that pointed to a higher risk of depression in men with long CAG repeats (Harkonen *et al*, 2003), while in another investigation on adolescent men, short CAG repeats pointed to not a higher incidence, but more severe depressive symptoms (Su *et al*, 2007). Additionally, greater CAG repeat length was associated with lower scores on cognitive tests, probably also implying an association with EMD (Yaffe *et al*, 2003).

In general, although several studies have addressed the issue of long-term sequelae of TGCC and its treatment, there is still a significant lack of information regarding the predictive factors for such complications and also the strategies for their prevention. These obstacles represent a serious hindrance in optimal management of TGCC survivors.

Aims of the Thesis

The overall aim of this thesis was to increase the current level of knowledge regarding impairment of reproductive function and risks of emotional disorders related to TGCC and its treatment and thereby improve the management and counselling of this group of men.

The specific aims of the individual studies were, in TGCC patients, to:

- assess the effect of CT and RT on semen quality with special attention to the dose response effect and the time course of recovery;
- investigate the impact of different AR polymorphisms on pre-treatment sperm characteristics and as a predictor of sperm regeneration after treatment;
- identify risk factors for developing androgen deficiency following cancer treatment;
- estimate the prevalence and characterise the type of sexual dysfunction, three to five years after therapy;
- evaluate the impact of hypogonadism, genetically determined androgen sensitivity and treatment intensity in relation to the risk of post-treatment sexual dysfunction and emotional disorders.

Material and Methods

Patient inclusion

All TGCC patients referred to the Department of Oncology, Lund University Hospital, Lund between the ages of 18 and 50 and diagnosed within a period of five years prior, were asked to participate in a study of fertility.

The study was initiated in March 2001. In November 2003 additional inclusion started at the Department of Oncology, Radiumhemmet and Södersjukhuset, Karolinska University Hospital, Stockholm.

The inclusion was discontinued in June 2006, and as the patients are followed for longitudinal investigation for five years the study will go on until 2011.

Of 461 eligible patients, 334 were included (72 %). Seventy-five patients declined participation (16 %) and 52 patients were exclude due to mental co-morbidity, linguistic difficulties, bilateral testicular cancer or physically disabled (Figure 4).

Article I and II only included patients from Lund, article III and IV from both Lund and Stockholm.

All patients participated with a written informed consent according to protocols approved by the ethical review boards of Lund University.

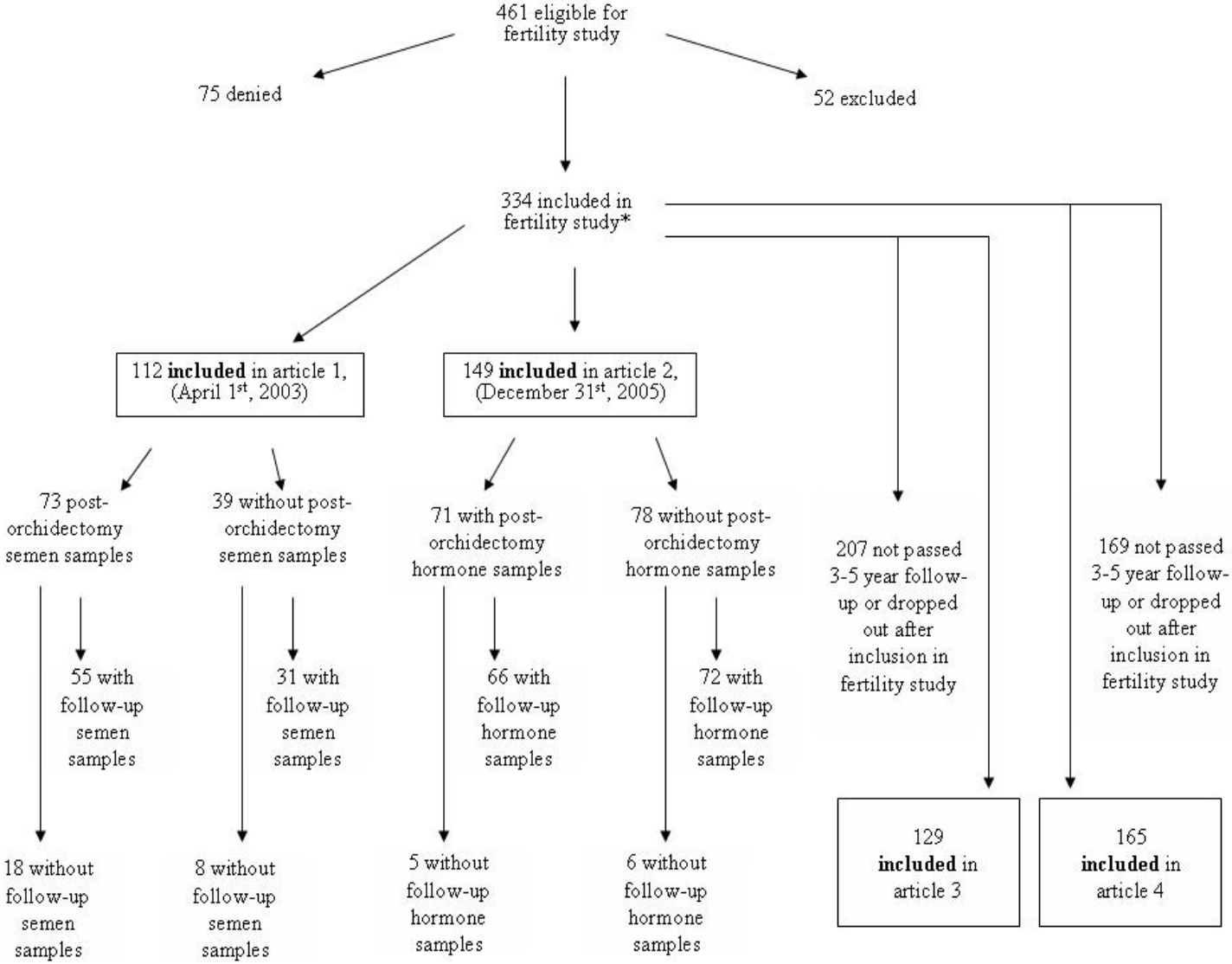
461 eligible for fertility study
75 denied
52 excluded
334 included in fertility study*
112 **included** in article 1, (April 1st, 2003)
149 **included** in article 2, (December 31st, 2005)
73 post-orchidectomy semen samples
39 without post-orchidectomy semen samples
71 with post-orchidectomy hormone samples
78 without post-orchidectomy hormone samples
207 not passed 3-5 year follow-up or dropped out after inclusion in fertility study
169 not passed 3-5 year follow-up or dropped out after inclusion in fertility study
55 with follow-up semen samples
31 with follow-up semen samples
66 with follow-up hormone samples
72 with follow-up hormone samples
18 without follow-up semen samples
8 without follow-up semen samples
5 without follow-up hormone samples
6 without follow-up hormone samples
129 **included** in article 3
165 **included** in article 4

Figure 4: *Flow-chart describing the patient material. Article I and II, being longitudinal and III and IV cross-sectional.*

* Additionally two patients eligible for study III (n=336).

Methods

From included patients were obtained:

- clinical information regarding histological diagnose, stage of disease and cancer treatment;
- semen samples;
- blood samples for hormone analyses;
- blood sample for DNA analysis;
- ultrasound of contra-lateral testicle;
- questionnaire concerning sexual function, socio-demographics, quality of life and emotional disorders.

Cancer treatment (articles I-IV)

All patients were treated according to the SWENOTECA protocols (Albers *et al*, 2005; Klepp *et al*, 1997), the details described on pages 15-17. For staging, the Royal Marsden Hospital (RMH) staging system was used (Dearnaley *et al*, 2001; Horwich *et al*, 1989).

Sperm (article I) and hormone analyses (articles II-IV)

Six time-points for delivery of both ejaculates and blood samples for hormones were defined (Figure 5):

- after orchiectomy but before further treatment;
- 6, 12, 24, 36 and 60 months after completion of treatment.

The ejaculates were analysed according to the WHO (1999) manual and sperm motility, morphology, and concentration as well as ejaculate volume were determined (World Health Organization., 1999). For those recruited in Lund, all sperm analyses were performed at the Reproductive Medicine Centre (RMC) (former Fertility Centre), Malmö University Hospital, Malmö. Patients from Stockholm were not included in this part of the study (article I). A few cryopreserved samples, collected prior to inclusion, were analysed in the fertility laboratory, Lund University Hospital, Lund.

Hormone analyses included luteinizing hormone (LH) and testosterone. Blood sampling was performed between 8 am and 3 pm. All analyses were performed in one of three laboratories, the Departments of Clinical Chemistry in Malmö, Lund and Stockholm. During the study period, the methods for LH and testosterone analyses were changed in Lund and Malmö and conversion factors were obtained, both for the intra- and inter-laboratory variation. In a pooled dataset, no difference in hormone levels measured by the different laboratories was found.
The reference levels for testosterone and LH were identical between Malmö/Lund and Stockholm. Patients were categorized as being hypogonadal if serum testosterone was below 10 nmol/L and/or serum LH was 10 IU/L or more (Nieschlag *et al*, 2004). Since the blood samples were taken between 9 am and 3 pm and the levels of testosterone, but not LH, decrease during the day, we also used LH>10 IU/L as the only indicator of hypogonadism.

Depending on time from diagnosis and inclusion in the study, a patient could contribute with one to six samples during the study period (Figure 5).

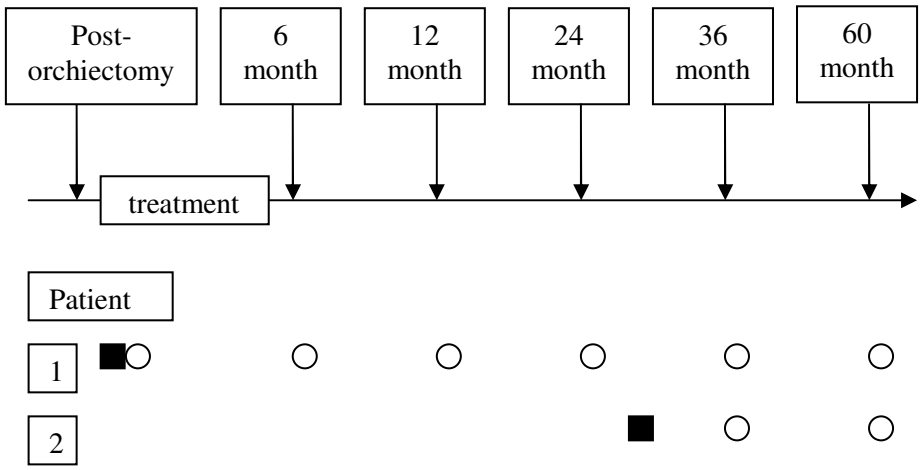

Figure 5: *Study Design. The circle represents semen/blood sampling, the black square time of inclusion.*

DNA analysis (articles I, II and IV)

Androgen receptor CAG and GGN repeat lengths were analysed in DNA extracted from peripheral leukocytes and amplified by polymerase chain reaction. Subsequently, direct sequencing was done using Beckman Coulter CEQ 2000XL (Beckman Coulter, Bromma, Sweden) sequencing gear (Lundin *et al*, 2003).

Evaluation of sexuality and socio-demographics (article III)

In 1996 a nation wide Swedish study on sexual life in Swedes age 18 to 74 years was performed by the Swedish National Institute of Public Health (Fugl-Meyer *et al*, 2000). The questionnaire used in this national study was the source of the questionnaire in the preset study.

Questions relevant for our study population were selected by a multidisciplinary group, involving one psychiatrist/sexologist, one andrologist and two oncologists. From the national questionnaire, nineteen questions were chosen reflecting:

- socio-demographic status including information regarding having/not having a partner, paternity status, sexually transmitted infections (STI), alcohol, smoking and snuffing habits, weight and height;
- sexual functions/dysfunctions with propensity on sexual desire, sexual interest, erectile dysfunction (ED), personal problem due to ED, premature and delayed ejaculation, time since last intercourse, sexual satisfaction and need for sexual advice;
- general satisfaction.

At two time-points, at time of the inclusion and after 60 months, all men filled out the questionnaire. As reference population, 916 age-matched men, who had participated in the national study, were used.

Measures of emotional disorders (article IV)

At the time of inclusion and after 60 months, the patients filled in the hospital anxiety depression scale (HADS), which is a well established and validated self-rating scale developed to screen for depression and anxiety (Zigmond & Snaith, 1983). HADS contains fourteen items; seven depression items (HADS-D) and seven anxiety items (HADS-A). Each item is scored from 0-3 and the score on each subscale (HADS-D and HADS-A) ranges from 0 to 21. A HADS-D score ≥ 8 was used as the cut-off score for

depression and HADS-A ≥8 was used for anxiety. These cut-off levels correspond to those used in prior studies (Bjelland *et al*, 2002).

Testicular characteristics (article II)

The remaining testicle was evaluated with ultrasound concerning volume and presence of microlithiasis (TM) between six and twelve months after inclusion of the patient. For determination of testicular volume, the formula for volume of ellipsoid: 1/3 x π x length x wide x thickness x ½ was used (Lenz *et al*, 1993). TM was defined as at least five uniform, non-shadowing echogenic foci of 1 to 3 mm, scattered throughout the testicular parenchyma (Lenz *et al*, 1987; von Eckardstein *et al*, 2001b). All examinations were made by the same radiologist. The consistency was investigated by palpation and assessed as being normal or soft.

Statistical analysis

All statistical analysis was performed using the SPSS 11.0 software (SPSS Inc., Chicago, USA). For all statistical tests $p<0,05$ was considered statistically significant.

Details of material and methods are presented in the original articles of the thesis. For each article, a summary is presented below.

Article I

Aims

-To evaluate the impact of different TGCC treatment modalities on semen quality, focusing on dose response effect and time course of recovery.
-To evaluate the impact of different AR polymorphisms on pre-treatment sperm concentration and as a predictor of sperm regeneration after treatment.

Patient inclusion and treatment

Until April 1st 2003, 112 of 144 eligible patients (78 %) were recruited (Figure 4).
Details on treatment are given in pages 15-17 and table 1. Eleven patients developed retrograde ejaculation due to retroperitoneal lymph node dissection (RPLND). For details on number of semen samples delivered postorchidectomy and at follow-up, see figure 4. Eight of the 112 included patients had not yet delivered a semen sample at the time of study evaluation.

Methods

Sperm and DNA analyses are described on page 43.
When the analyses were initiated, blood samples for DNA analysis were available only from the first 81 men. The number providing semen samples were 56, 23, 42 and 31 at pre-treatment, six months, one to two years and three to five years, respectively. This subgroup of men for whom the CAG and GGN repeat lengths could be determined, did not differ from the

remaining 31 with respect to age, disease, histological type, stage, treatment or sperm concentration post-orchidectomy.

Statistical analysis

For each treatment modality, a longitudinal analysis of data was performed. In order to obtain sufficient numbers, samples collected at 24, 36 and 60 months were pooled. If a patient delivered more than one sample during this time interval, the one with the highest sperm concentration was included in the analysis.
Additionally, comparison of semen parameters between groups, a cross-sectional analysis was performed.
For longitudinal comparisons of more than two time points Friedman's test was used. For intra-individual comparison of values at two time points only, Wilcoxon test for paired data was applied. In the cross-sectional analyses, Kruskal-Wallis test and Mann-Whithey test for unpaired data were used. Spearman's rho was calculated in order to find the correlation between the CAG or GGN repeat length and the sperm concentration at any of the following time points:

- after orchidectomy but before further treatment;
- six months, one to two years and three to five years.

DNA data were caculated for the whole group as well as separately for the therapy groups ACT, SCT and RT.
Subsequently, in order to calculate the predictive value on the pre-treatment sperm concentration, multivariate linear regression analysis was used with the type of tumour, as discrete variable, and age and CAG repeat length as continuous variables,. Similar type of analysis was done for sperm

concentration at six months, one to two years and three to five years, with type of therapy as discrete variable and CAG repeat lengths and age as well as sperm concentration pre-treatment as continuous independent variables. Sperm concentrations were log transformed (after adding 0.1 to all sperm concentrations in order to be able to transform 0 values) prior to the analysis. All statistical tests were two-sided.

Article II

Aims

-To identify patient, disease and treatment related risk factors associated to and predicting post-treatment hypogonadism.

Patient inclusion and treatment

Until December 31st, 2005, 149 of 200 eligible patients (74 %) were included and 143 were evaluable (six patients had not delivered any blood samples) (Figure 4).
Details on treatment are given in pages 15-17 and table 1. For details on number of samples for hormone analyses delivered post-orchidectomy and at follow-up, see figure 4.

Methods

Hormone and DNA analyses as well as ultrasound of the testicle are described on pages 41-43 and 45.
Blood samples for DNA analysis were, as in study I, available from 81 men. These 81 subjects did not differ from the remaining 62 regarding age,

disease, histological type, stage, treatment or sperm concentration post-orchidectomy. Of the 143 patients, 107 underwent an ultrasound of the remaining testicle.

Table 1: Treatment of patient in article I and II.

	Article I						***Article II***					
Treatment modality	**SO**	**ACT**	**SCT**	**HDCT**	**RT**	**All**	***SO***	***ACT***	***SCT***	***HDCT***	***RT***	***All***
No of patients	7	32	37	5	31	**112**	*13*	*43*	*42*	*9*	*36*	***143***
Age (median)	24	28	28	28	35	**29**	*29*	*29*	*27*	*29*	*36*	***30***
SGCT	0	0	7	2	31	**40**	*5*	*2*	*8*	*3*	*36*	***54***
NSGCT	7	32	30	3	0	**72**	*8*	*41*	*34*	*6*	*0*	***89***
Stage I	7	32	7	0	31	**77**	*13*	*43*	*9*	*2*	*36*	***103***
Stage II	0	0	20	1	0	**21**	*0*	*0*	*21*	*2*	*0*	***23***
Stage III	0	0	4	1	0	**5**	*0*	*0*	*4*	*2*	*0*	***6***
Stage IV	0	0	6	3	0	**9**	*0*	*0*	*8*	*3*	*0*	***11***

Royal Marsden Hospital (RMH) staging system has been used (Horwich *et al*, 1989)

SO	No further therapy after orchidectomy;
ACT	1-2 cycles of adjuvant chemotherapy;
SCT	3-4 cycles of chemotherapy;
HDCT	More intense treatment, >4 cycles of chemotherapy;
RT	Adjuvant radiotherapy;

Statistical analysis

Patients with an increased level of human choriogonadotropin (HCG) were excluded from the analysis of post-orchidectomy hypogonadism. Seven men being on androgen replacement therapy were considered hypogonadal from the time the replacement was given. Two of them were in the ACT group, two in the SCT group, two in the RT group and one was in the HDCT group.

Primarily, using binary logistic regression analysis, OR for hypogonadism was calculated for TGCC patients using the ACT group as a reference group. A separate comparison was done for each treatment modality (SCT/RT) and post-treatment time point. Furthermore, the risk for the whole group of TGCC men for developing hypogonadism was tested at different post-treatment time points, with respect to the following potential predictive factors:

- biochemical hypogonadism before treatment (yes/no);
- androgen receptor CAG (<21, 21-22, >22) and GGN (<23, 23, >23) repeat number;
- stage of disease (I-IV);
- testicular consistency (normal/soft);
- ultrasound (+/- microlithiasis);
- volume of the contralateral testis (<15ml vs.≥15ml);
- age (<30 vs.≥30 years).

Patients presenting with TM, were complementary analysed after excluding those being hypogonadal postorchidectomy, but before further treatment. The method of hormone analysis was used as potential confounder.
All analyses were performed separately for each time point.

Article III

Aims

-To study the prevalence and type of sexual dysfunctions 3 to 5 years after treatment for TGCC and compare these figures to those in an age-matched group of men from the general population.
-To relate our findings to type of oncological treatment and presence of hypogonadism among TGCC patients.

Patient inclusion and treatment

Until 30th of June 2006, 334 patients were included in the fertility study. Additional two patients, not eligible for the fertility study due to bilateral TGCC were considered eligible in study III (n=336). Of these patients 129 had past the three year control and were thus included in study III (Figure 4). Details on treatment are given in page 15-17 and table 2. Eight patients had retrograde ejaculation after RPLND.

Methods

Evaluation of sexuality and socio-demographic as well as hormone analyses are described on pages 41-44. For the sexual functions/ dysfunctions a six-graded answering alternative scale was used:

1. never;
2. hardly ever;
3. rather rarely;
4. rather often;
5. nearly all the time;
6. all the time.

A patient who stated that the dysfunction occurred rather often/nearly all the time/all the time was considered to suffer from manifest dysfunction per se (Fugl-Meyer & Fugl-Meyer, 2002). If the dysfunction led to personal erectile distress the same scale was used.

Times since last intercourse was dichotomised in two different ways, primarily with an approximate 50 % distribution (less than 5 days vs. 5 days or more), secondly with an approximate 10 % distribution (3 months or less vs. more than 3 months)

Sexual satisfaction was assessed by the question ”How satisfying is your sexual life?” derived from the well-validated generic instrument LiSat-11 checklist (Fugl-Meyer *et al*, 2002). Six answering alternatives ranging from very dissatisfied to very satisfied were offered. The scale is test-retest reliable and it is valid to dichotomize the scale into “satisfied” (very satisfying or satisfying) and “not satisfied” (rather satisfying/rather dissatisfying/dissatisfying/very dissatisfying).

Statistical analysis

To evaluate the likelihood probability for co-occurrence of TGCC and sexual dysfunctions, OR with 95 % CI were calculated, using logistic regression. All analyses were adjusted for age. In addition, potential confounders such as occupation, paternity status, failing to become biological father, smoking, snuffing, sexually transmitted infections and BMI, were included in the models, one at a time. These items were kept in the model if they changed the age-adjusted effect estimate with more than 15%. Patients reporting absence of antegrade ejaculation was excluded from the analyses concerning premature and delayed ejaculation need for sexual advice as well as for the question regarding consulting an expert.

The impact of different therapeutic modalities in relation to indices of sexual dysfunction was assessed by comparing them to each other. We used SCT as reference since it was the largest group and as we, a priori, expected the SCT treated men to be the most seriously affected.
For all statistically significant associations between disease/treatment and sexual function outcomes, biochemical hypogonadism as predictor of sexual dysfunction was then tested, using binary logistic regression. For the variables analysed by binary logistic regression, the outcomes were dichotomised. Patients on testosterone replacement (n=9) were excluded from this analysis.

Article IV

Aims

-To evaluate whether biochemical signs of hypogonadism and/or AR polymorphisms are predictors of emotional disorders (EMD) in TGCC survivors.
-To assess the association between treatment intensity and EMD.

Patient inclusion and treatment

Until 30th of June 2006, 334 patients were included in the fertility study. One-hundred and sixty-five patients who went through their three-year check-ups were included in the study (Figure 4). Details on treatment are given in pages 15-17 and table 2.

Table 2: Treatment of patients in article III and IV.

	Article III						Article IV					
Treatment modality	**SO**	**ACT**	**SCT**	**HDCT**	**RT**	**All**	***SO***	***ACT***	***SCT***	***HDCT***	***RT***	***All***
No of patients	11	33	47	2	36	**129**	*13*	*41*	*54*	*8*	*49*	***165***
Age (median)	32	33	33	40	37	**35**	*32*	*35*	*35*	*34*	*40*	***36***
SGCT	3	0	15	1	36	**55**	*4*	*0*	*16*	*3*	*49*	***72***
NSGCT	8	33	32	1	0	**74**	*9*	*41*	*38*	*5*	*0*	***93***
Stage I	11	33	10	1	36	**91**	*13*	*41*	*12*	*1*	49	***116***
Stage II	0	0	25	0	0	**25**	*0*	*0*	*28*	*2*	*0*	***30***
Stage III	0	0	4	0	0	**4**	*0*	*0*	*4*	*1*	*0*	***5***
Stage IV	0	0	8	1	0	**9**	*0*	*0*	*10*	*4*	*0*	***14***

Royal Marsden Hospital (RMH) staging system has been used (Horwich *et al*, 1989)

SO	No further therapy after orchidectomy;
ACT	1-2 cycles of adjuvant chemotherapy;
SCT	3-4 cycles of chemotherapy;
HDCT	More intense treatment, >4 cycles of chemotherapy;
RT	Adjuvant radiotherapy;

Methods

Evaluation of emotional disorders as well as hormone and DNA analyses are described on pages 43-45. Blood samples for DNA analysis were available from 140 men for CAG repeat length and from 135 on GGN repeats. These subjects did not differ from the remaining 25/30 regarding age, disease, stage, histological type, biological hypogonadism or prevalence of EMD.

Statistical analysis

Using binary logistic regression, OR with 95 % CI were calculated to evaluate the association between biochemical signs of hypogonadism, length of CAG repeat, and length of GGN repeat as potential predictors for EMD. The analyses concerning the impact of hypogonadism were performed both with and without including men on testosterone replacement. Furthermore, these two groups – those treated with testosterone and those not treated – were compared to each other.

The CAG length was evaluated as a continuous variable as well as divided into four categories, (<20, 20-21, 22-23 and >23) using the shortest CAG length interval as reference. The GGN repeat length was categorized into three groups, (<23, 23 and >23) using the most common length of 23 as the reference. When assessing the impact of hypogonadism, potential confounders such as: age, smoking, body mass index (BMI) and laboratory (Lund vs. Stockholm) were included in the models, one at a time. These factors were kept within the model if they changed the risk estimate more than 15 %.

The association between the different treatment modalities and EMD was evaluated using Fisher's exact test. One model was applied comparing all treatment groups to each other and one comparing HDCT to all the others. The reason for not using logistic regressions when comparing treatment groups was the low numbers of individuals in some of the treatment modality groups.

Results

Impact of therapy and AR polymorphism on sperm concentration (article I)

Azoospermia

In total 73 men delivered post-orchidectomy samples before other treatment. Among those four (5.5 %, 95 % CI: 1.5-13 %) were azoospermic. One was still azoospermic at six months and one patient did have few sperms in the ejaculate at twelve months. For two patients no post-treatment samples were yet available. Of the 69 men having spermatozoa in the ejaculate after orchidectomy, 16 have not yet delivered any post-treatment samples. Among 53 patients who delivered one or more post-treatment samples, five became azoospermic after CT or RT treatment (9.4 %, 95 % CI: 3.1 -21 %). None of the 26 men in the ACT became azoospermic (95 % CI: 0-13 %). Among seventeen patients in the SCT group, two were azoospermic after six and one at sixty (18 %, 95 % CI: 3.8-43 %) months. From these three patients only one post-treatment sample was available. Two of ten men in the RT group (20 %, 95 % CI: 2.5-56 %) were azoospermic six months after treatment, one regained sperm production after one year, whereas the other did not yet have further follow-up.

Sperm concentration

No significant decrease in sperm concentration was seen, at any time point, in men who received 1 to 2 cycles of adjuvant chemotherapy, ACT group (Figure 6).

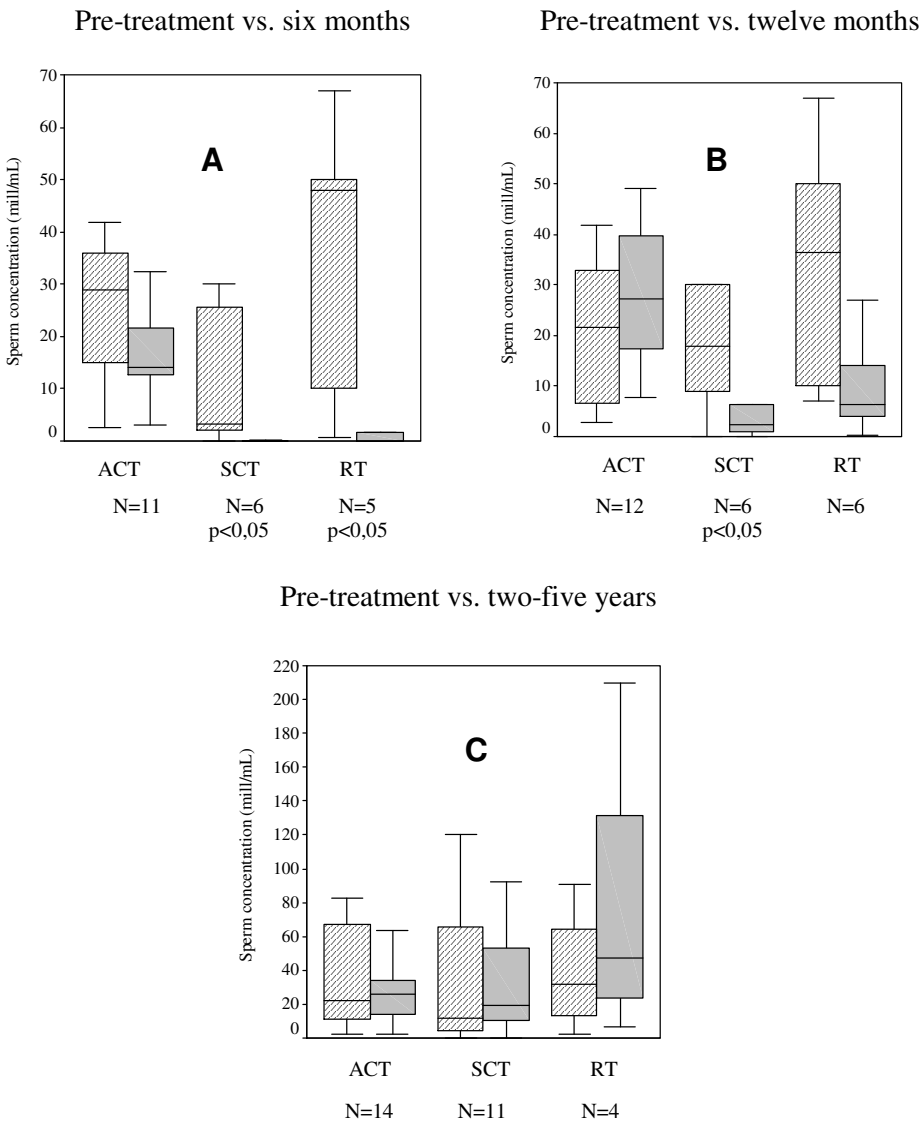

Figure 6: *Sperm concentration, longitudinal data: ACT, SCT, RT as defined in the text. Bars correspond to median value, boxes to the interquartile interval and whiskers to 95 % CI. (A) Pre-treatment vs. six months; (B) Pre-treatment vs. twelve months; (C) Pre-treatment vs. two to five years. Black boxes represent pre-treatment values. Adapted from Eberhard et al, Human Reproduction 2004.*

In the SCT group, the median sperm concentrations after six and twelve months were significantly lower than before treatment, 0.05 vs. 3.4 x 10^6/mL (p=0.043) after six months and 2.4 vs. 18 x 10^6/mL (p=0.046) after twelve months corresponding to a decease of 99 % and 87 % respectively. After two to five years the sperm concentration had increased to 19 x 10^6/mL, not statistically significantly different from the pretreatment value of 12 x 10^6/mL (p=0.8) (Figure 6).

In patients treated with RT, the medium sperm concentration decreased from 48 x 10^6/mL pre-treatment to 0.1 x 10^6/mL (p=0.04) at six months corresponding to a decrease of almost 100 %. After twelve months there was a decrease in concentration, 6.3 vs. 36 x 10^6/mL pre-treatment, however not statistically significant (p=0.075). After two to five years the sperm concentration had increased to 47 x 10^6/mL, not statistically significantly different from the pretreatment value of 32 x 10^6/mL (p=0.27) (Figure 6). At twelve month the concentration had increased significantly to 6.8 x 10^6/mL vs. 0.9 x 10^6/mL at six months (p=0.03).

There was no significant difference in median sperm concentration before treatment, neither between the NSGCT and SGCT patients, nor between the treatment groups. After six and twelve months there was a statistically significant difference between the groups (p=0.0001), the ACT group having significantly higher sperm concentration than both the SCT (p=0.0001) and the RT group (p=0.001). Concentrations after two to five years did not differ between the therapy groups.

Factors predicting sperm concentration

Type of treatment, but not the pre-treatment sperm concentration or CAG/GGN repeat lengths, independently predicted concentration after six months (p<0.0005) and after one to two years (p=0.004). No statistically

significant association with the treatment modality was found after three to five years.

When analyzing the group of men receiving SCT separately, only the CAG length (p=0.02) but neither GGN number, sperm concentration postorchidectomy nor age did significantly predict the concentration after one to two years.

Correlation between sperm recovery and AR polymorphisms

There was a significant correlation between CAG repeat and sperm concentration after one to two years in men treated with 3 or 4 cycles of CT, SCT, (rho= -0.72; p=0.03) (Figure 7). The repeat number did not correlate with sperm concentration in the other therapy groups or at other time points – including postorchidectomy. No correlation was found for GGN length.

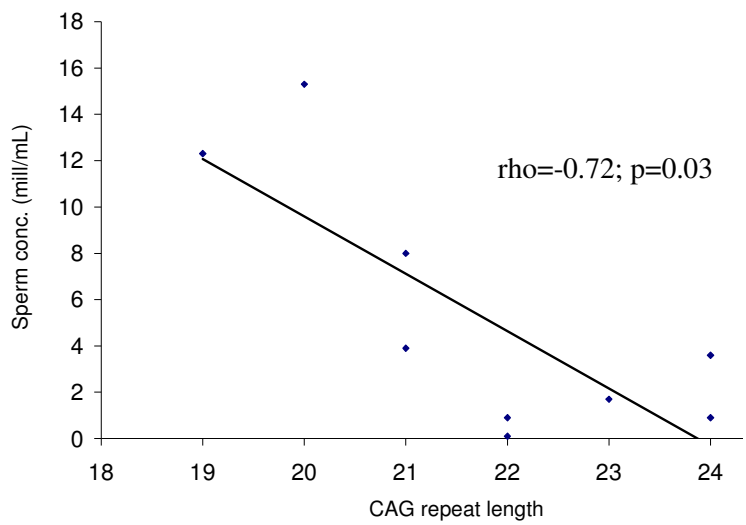

Figure 7: *Correlation between CAG repeat length and sperm concentration in patients treated with three to four cycles of chemotherapy one to two years after treatment. Adapted from Eberhard et al, Human Reproduction 2004.*

Risk factors for developing hypogonadism (article II)

Post-treatment hypogonadism, relation to hypogonadism before treatment

Hypogonadism postorchidectomy, but before further treatment, strongly predicted hypogonadism after six (OR 53, 95 % CI: 19-145), twelve (OR 125, 95 % CI: 37-430), twenty-four (OR 88, 95 % CI: 26-300) and thirty-six (OR 121, 95 % CI: 32-460) months. Postorchidectomy, 22 of 58 men (38 %) were hypogonadal. After one year, twelve out of twenty (60 %) and after two years six out of eleven (55 %) had normal hormone levels (Figure 8).

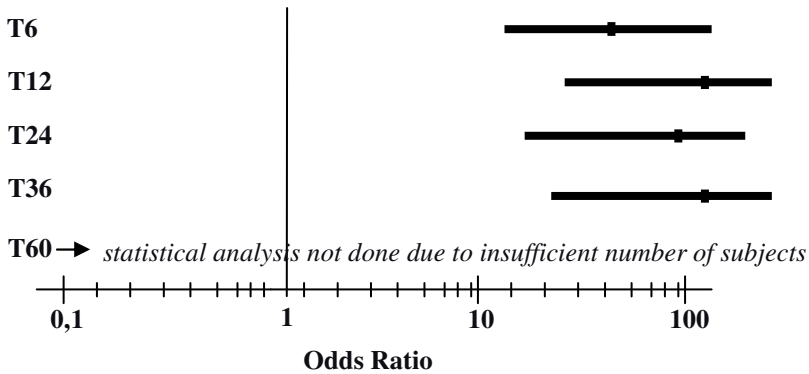

Figure 8: *Odds ratios for hypogonadism for TGCC patients in relation to hypogonadism at T0. T0=postorchidectomy samples. T6, T12, T24, T36 and T60=six, twelve, twenty-four and thirty-six months after treatment. Adapted from Eberhard et al, Eur. J. Endocrin., 2008.*

Hypogonadism in relation to treatment

For the SCT group, a significantly increased OR for hypogonadism, as compared to the ACT group, was found after six (OR 22, 95 % CI: 4.4-118) and twelve (OR 5.8, 95 % CI: 1.5-22) months post therapy (Figure 9).

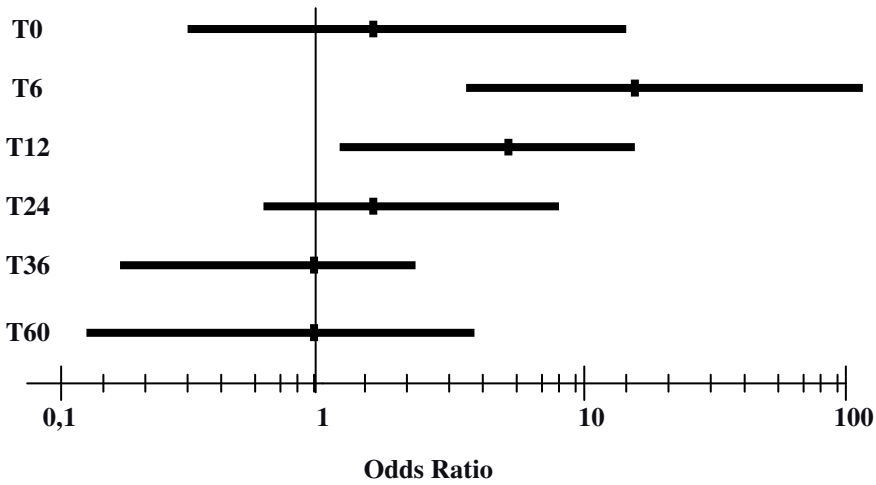

Figure 9: *Therapy dependent odds ratios of developing hypogonadism with the group given adjuvant chemotherapy as reference in patients treated with three to four cycles of chemotherapy. T0=postorchidectomy samples. T6, T12, T24, T36 and T60=six, twelve, twenty-four, thirty-six and sixty months after treatment. Adapted from Eberhard et al, Eur. J. Endocrin., 2008.*

Also in the RT group, as compared to the ACT treated men, a significantly increased OR was observed after six (OR 10, 95 % CI: 2.1-47) and after twelve (OR 3.9, 95 % CI: 1.1-14) months post therapy (Figure 10).

Hypogonadism in relation to age, stage and androgen receptor polymorphisms

No statistically significant relation to the risk of hypogonadism was observed for age, stage of disease or androgen receptor CAG and GGN repeat lengths.

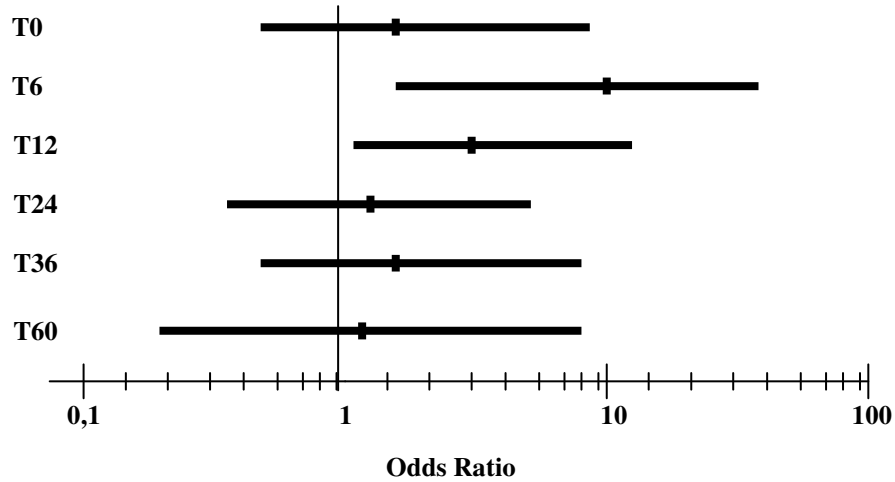

Figure 10: *Therapy dependent odds ratios of developing hypogonadism with the group given adjuvant chemotherapy as reference in radiotherapy treated patients. T0=postorchidectomy samples. T6, T12, T24, T36 and T60=six, twelve, twenty-four, thirty-six and sixty months after treatment. Adapted from Eberhard et al, Eur. J. Endocrin., 2008.*

Hypogonadism in relation to testicular characteristics

Testicular microlithiasis was predictive for hypogonadism postorchidectomy (OR 11, 95 % CI: 1.2-112) and twelve (OR 3.9, 95 % CI: 1.1-13), twenty-four (OR 3.0, 95 % CI: 1.0-8.8), thirty-six (OR 5.4, 95 % CI: 1.7-17) and sixty (OR 4.4, 95 % CI: 1.2-16) months post-treatment (Figure 11). When excluding patients being hypogonadal postorchidectomy a similar trend was observed, reaching statistical significance after thirty-six (OR 5.2, 95 % CI: 1.5-19) and sixty (OR 4.7, 95 % CI: 1.0-21) months. The contralateral testicles volume and consistency were not associated with any increased risk for hypogonadism.

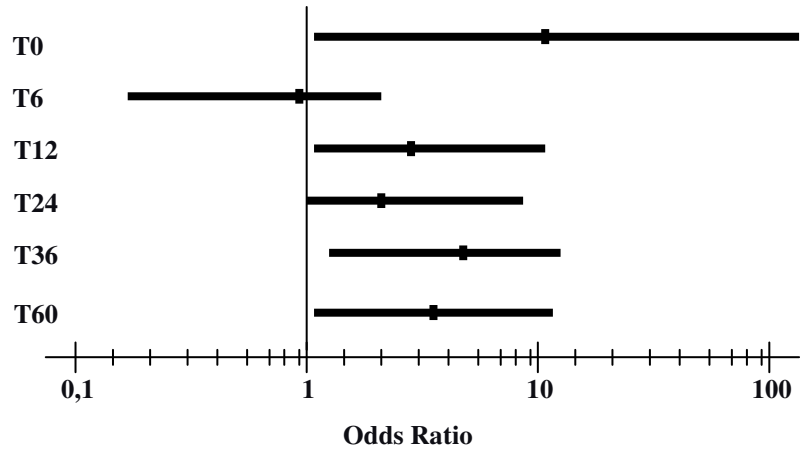

Figure 11: *Odds ratios for hypogonadism in relation to microlithiasis of the remaining testicle. T0=postorchidectomy samples. T6, T12, T24, T36 and T60=six, twelve, twenty-four, thirty-six and sixty months after treatment. Adapted from Eberhard et al, Eur. J. Endocrin., 2008.*

Sexual function and relation to hypogonadism and treatment (article III)

Sexual function

Patients reported more often manifest low sexual desire (adjusted OR 6.7, 95 % CI: 2.1-21.0), and manifest erectile dysfunction (adjusted OR 3.8, 95 % CI: 1.4-10.0) than the reference group. Twelve percent of TGCC patients reported erectile dysfunction compared to 3 % for controls, whereas the corresponding figures for low sexual desire were 4 % and 2 % respectively. Low sexual desire and manifest erectile dysfunction per se as well as manifest erectile distress concurred for 25 % of the patients and 40 % of the reference group, respectively. No other significant differences between

patients and comparators were found when adjustment for potential confounders was made (Table 3).

Sexual function in relation to hypogonadism

Twenty-nine percent of the men were biochemically hypogonadal. No significant association between biochemical hypogonadism and low sexual desire (OR 1.2, 95 % CI: 0.11-14) or manifest erectile dysfunction (OR 1.1, 95 % CI: 0.26-4.5) was found. Inclusion of patients treated with testosterone did not change the OR estimates given above. Using LH above 10 IU/L as the only indicator of hypogonadism did not change the results.

Sexual function in relation to treatment modality

With a few exceptions, the three therapeutic groups SO, RT and ACT did not differ significantly from the SCT-group regarding the age-adjusted OR's for sexual dysfunctions. The only statistically significant difference between the therapy groups was more prevalent erectile dysfunction (OR 8.8, 95 % CI: 1.2-62) and perception of their sexual life as not satisfying (OR 9.9, 95 % CI: 1.7-58) in SO patients, as compared to the SCT group. Furthermore, a higher proportion of patients in the SCT group than in the SO group reported having intercourse during the past five days (OR 9.9, 95 % CI: 1.7-58).

Table 3: OR for TGCC patients compared to Swedish nationally representative comparators for variables assessing sexual function, frequency of intercourse, sexual satisfaction and treatment seeking.

	Age-adjusted OR (95 % CI)	**Adjusted OR* (95 % CI)**
Sexual dysfunctions:		
Low sexual desire	2.4 (0.85-6.8)	**6.7[acd] (2.1-21)**
Less sexual desire than 5 years ago	0.83 (0.56-1.2)	0.83 (0.56-1.2)
Decrease in sexual interest	0.91 (0.53-1.7)	0.91 (0.53-1.7)
Erectile dysfunction [f]	**6.6 (3.1-14)**	**3.8[bd] (1.4-10)**
Erectile dysfunctional distress [f]	**6.5 (2.6-16)**	2.5[bd] (0.65-9.8)
Premature ejaculation [ef]	0.90 (0.40-2.0)	0.68[abd](0.29-1.6)
Delayed ejaculation [ef]	3.9 (0.94-15.9)	2.4[bd](0.45-12.9)
Frequency of intercourse:		
Less than 5 days since last sexual intercourse	0.71 (0.50-1.1)	0.71 (0.50-1.1)
More than 3 months since last sexual intercourse	1.1 (0.57-2.0)	1.3[b] (0.67-2.7)
Satisfaction:		
Dissatisfying sex life	0.84 (0.50-1.4)	0.84 (0.50-1.4)
Treatment seeking:		
Need for sexual advice or help [e]	0.87 (0.50-1.5)	0.87 (0.50-1.5)
Consulted an expert for sexual advice or help [e]	0.99 (0.84-1.2)	0.99 (0.84-1.2)

*Adjusted for age and one or more of the variables a-e described below.
a=occupation
b=children/have or want to
c=smoking/snuffing
d=Body Mass index
e= eight patients with absence of antegrade ejaculation are excluded
f=nine patients and 69 men from general population not having had intercourse the last twelve months excluded

EMD in relation to hypogonadism, AR polymorphism and treatment (article IV)

Frequencies of emotional disorders

Among the 165 patients, 19 % scored ≥ 8 on HADS-A and 5 % on the HADS-D. Among the 20 patients on testosterone replacement therapy, 30 % scored ≥ 8 on HADS-A and 10 % on the HADS-D.

Hypogonadism in relation to EMD

Of the 165 included patients, three refused to deliver a blood sample and twenty were on testosterone replacement therapy. Among the remaining 142, 36% were biochemically hypogonadal. If using only LH ≥ 10 as the criterion, 20% were hypogonadal.

The risks of anxiety (OR 1.0, 95 % CI: 0.40-2.4) and depression (OR 1.1, 95 % CI: 0.20-6.4) were not increased in biochemically hypogonadal TGCC patients. When men on testosterone replacement therapy (n=20) were compared to those not on replacement (n=142), no difference was found.

AR polymorphisms in relation to EMD

Twenty-six percent had CAG repeat length <20, 28 % had 20-21, 22 % had 22-23 and 24 % had >23. Sixteen percent had GGN repeat length <23, 50 % had 23 and 34 had >23.

There was no significant correlation between AR polymorphisms and EMD. The results were similar irrespective of whether we included or excluded the patients on testosterone replacement in the analyses.

EMD in relation to treatment modality

In the SO group, none suffered from EMD. In the ACT group, 10 % suffered from anxiety and 2 % from depression. In the SCT group, 15 % had anxiety and 2 % depression and the corresponding figures in the RT group were 29 % and 10 %, respectively. Finally, in the HDCT group, 62 % had anxiety and 12 % depression (Table 4). When comparing all treatment groups to each other, there was a significant difference regarding frequency of anxiety (p=0.002), but not regarding depression (p=0.19). When the HDCT group, was compared to the other groups, there was a significant difference in the prevalence of anxiety (p=0.006), but not in the prevalence of depression (p=0.38).

Table 4. EMD in relation to treatment in 165 TGCC patients 3-5 years after treatment.

	HADS-A ≥ 8 Anxiety (n) (%)	HADS-D ≥ 8 Depression (n) (%)
All patients	31 (19)	8 (4.8)
Treatment received		
SO n=13	0	0
ACT n=41	4 (9.8)	1 (2.4)
SCT n=54	8 (15)	1 (1.9)
RT n=49	14 (29)	5 (10)
HDCT n=8	5 (62)	1 (12)

Discussion

Major findings and clinical implications

This thesis provided information which may be valuable in the counselling and management of patients treated for TGCC:

(1) Patients receiving adjuvant RT or SCT are at high risk of developing severe oligozoospermia, with high chance of recovery 24 months after completion of the therapy. On the other hand, ACT has only a minor influence on sperm concentration, but the number of patients was too limited to exclude development of azoospermia in some susceptible subjects. These findings do not, however, change the common practice of offering sperm cryopreservation prior to cancer treatment. In the RT and SCT treated men, the azoospermia may be permanent or prolonged, which may seriously reduce the chance of fathering a child.

(2) The association we found between the AR CAG repeat length and regain of sperm concentration one to two years after SCT is to our knowledge the first report of the impact of genetic factors on the recovery of spermatogenesis after cancer therapy. If this finding can be confirmed in a larger material, the CAG length could serve as a potential instrument in determining inter-individual differences in the rapidity of sperm regeneration after cancer therapy. Polymorphisms in other genes related to spermatogenesis may also prove to be valuable in prediction of the susceptibility to the gonadotoxic effect of cancer therapy.

(3) TGCC patients are at increased risk of androgen deficiency, which to a certain degree has not received enough attention in the follow-up of these

men. A significant increase in the OR for hypogonadism was observed six to twelve months post-treatment in patients receiving three to four cycles of chemotherapy and in the RT group, with a decreased risk after two to five years. Previous studies on the risk of hypogonadism in men treated for TGCC (Brennemann *et al*, 1997; Hansen *et al*, 1990; Palmieri *et al*, 1996) did not clearly discriminate between treatment modalities and/or the post-treatment investigations were not performed at specific time-points, which is why a more precise mapping of the process of Leydig cell recovery could not be done. An important observation was not only the increased OR of hypogonadism but also the possible recovery of the Leydig cell function, warranting some hesitation in initiation of androgen replacement. Microlithiasis in the remaining testicle was a risk factor for hypogonadism both pre- and post-treatment. In addition, hypogonadism prior to chemo-or radiotherapy was another predictor of post-treatment hypogonadism, whereas AR polymorphisms and age as well as testicular volume did not have any predictive value. Defining risk factors of hypogonadism enables the identification of patients to be recommended for future screening for androgen deficiency, including testosterone and LH measurements as well as andrological counselling.

(4) Three to five years after cancer treatment, low sexual desire and manifest erectile dysfunction were commoner among TGCC patients than in the age-matched general male population. Interestingly, these outcomes were associated with neither biochemical signs of hypogonadism nor with treatment intensity. From a clinical point of view, erection seemed to be the most important issue, since as many as 12 % of the patients reported frequent erectile dysfunction during the last year compared with only 3 % in the general population. This impairment of sexual function in TGCC survivors should also be given more attention and looking for signs of

sexual dysfunction should not be restricted to hypogonadal and/or heavily treated men.

(5) Even emotional disorders (EMD) seem to be rather frequent in TGCC survivors, with anxiety found in 19 % and depression in 5 %, three to five years after treatment. The risk of these EMD was not associated with markers of androgen action as biochemical signs of hypogonadism or AR polymorphisms. The intensity of treatment influenced the risk of anxiety, being present in 62 % of patients treated with ≥ 5 cycles of chemotherapy. Our findings indicate, however, that hypogonadism and emotional disorders, although sharing some symptoms, seem to be two different entities.

Spermatogenesis and Leydig cell function

In agreement with earlier reports, we found the negative impact of CT on spermatogenesis to be dose-dependent (Petersen *et al*, 1994) and ACT did not have any significant influence on sperm production (Cullen *et al*, 1996). The time course of the recovery of sperm production was similar in men treated with RT and SCT, with pre-treatment levels of sperm concentration reached after 24 to 60 months (Figure 6). Also, in agreement with earlier reports, the type of therapy predicted sperm concentration (Brydoy *et al*, 2005; Pont & Albrecht, 1997). We found treatment modality to be a predictor of sperm concentrations after six months, but also after one to two years.

We found no patients developing azoospermia after ACT, but the number of men included in the study was too low to exclude that this side-effect may occur in some of them. Almost 40 % of SCT or RT treated men developed azoospermia six months after treatment. Since follow-up samples were

lacking in most of these men, however, no firm conclusions regarding the subsequent period could be drawn.

With regard to postorchidectomy, before further treatment, 43 % was hypogonadal among patients later treated with SCT or RT. In these patients, analogously with the effects of the treatment on sperm concentration, we found decreased testosterone levels and/or increased LH in 64 %, six to twelve months post-treatment. Corresponding figures after two years were 46 % and after five years 24 % (Figures 9 & 10). The time-related post-treatment recovery of Leydig cell function is in discordance with the data published by Nord and colleagues, who found an increased risk of hypogonadism ten years after completion of TGCC treatment (Nord *et al*, 2003). An age-dependent deterioration of Leydig cell function, related to a longer follow-up period cannot, however, be excluded.

Although stages of disease and treatment intensity are closely related, the latter but not the former was associated with the risk of hypogonadism. Among stage 1 patients, however, some were treated with ACT and some with RT. These two treatment modalities differ in their impact on Leydig cell function, which might explain why disease stage was not a predictor of post-treatment Leydig cell insufficiency.

Scrotal ultrasound is routinely used in the diagnosis of TGCC. Among 107 patients evaluated with ultrasonography, 46 % had microlithiasis in the contralateral testis, which is in concordance with earlier reports (Costabile, 2007). The finding was associated with post-treatment risk of hypogonadism with an OR of 3-11. We found an increased risk of post-treatment hypogonadism after six months and at subsequent time-points in men with microlithiasis. Since microlithiasis has been reported in patients with testicular dysgenesis syndrome (TDS) (26) and is believed to be associated with confirmed testicular cancer (27), pre-treatment Leydig cell

dysfunction might be the cause of post-therapy hypogonadism. Even after exclusion of the subgroup of men who were hypogonadal postorchidectomy, before further therapy, however, microlithiasis remained a predictor of hypogonadism. To my knowledge there are no studies indicating that cancer therapy can induce microlithiasis and therefore I assume that the pathological ultrasonic pattern was also present prior to treatment.

Sexual and psychological function

Impairment of sexual function among TGCC patients, including decrease in sexual desire, ejaculation, orgasm, sexual satisfaction, sexual activity, libido, arousal and erection, have previously been reported by others (Fegg *et al*, 2003; Jonker-Pool *et al*, 1997). These studies did not, however, include control groups and patients with varying post-treatment observation time were included, thus not considering possible dynamic changes in these conditions related to post-treatment stress and/or possible recovery over time. Our study also lacks the longitudinal aspect, but it is valid for the specific three- to five-year follow-up period.

Aas and colleagues (Aass *et al*, 1993) followed 76 patients longitudinally with a questionnaire before and up to 36 months after treatment. They found that cancer treatment had a negative impact on the patient's satisfaction with his sexual life initially after cancer therapy, but this problem partly resolved later in the follow-up. Lackner and colleagues found no increased risk of erectile dysfunction in TGCC survivors, possibly because of the low statistical power of the study (Lackner *et al*, 2005). Also in a case-control study on stage 1 and 2 radiotherapy-treated patients, no difference in frequency of sexual dysfunction between patients and healthy controls was observed (Incrocci *et al*, 2002). The follow-up period, however, varied from

one month to ten years, which, at least partly, might invalidate the conclusions of this study.

Our findings in TGCC patients of a decreased erectile function and low sexual desire might be considered as different ways of measuring the same outcome, as it is known that low sexual desire may cause erectile dysfunction and vice versa (Fugl-Meyer & Fugl-Meyer, 2002). In our study 40 % of the reference group, but only 25 % of the patients, who stated that they had erectile dysfunction, also had low sexual desire. This indicates that in TGCC patients these dysfunctions should be considered, at least partly, as separate conditions.

A low level of sexual satisfaction generally accompanies all sexual dysfunctions and in particular erectile distress (Lewis *et al*, 2004). The finding that the reference group and the patient group had similar prevalence of not being sexually satisfied, despite the patients' higher prevalence of erectile and desire dysfunctions, suggests that the patients were reasonably psychologically adjusted to their situation. In line with this are the findings that there was no difference in reported erectile distress or in the need for sexual advice between the patients and the reference group. Interestingly, although sexual desire was low among the TGCC subjects, they did not differ in regard to incidence of decrease of sexual desire during the last five years, compared with the general population. These two parameters differ from each other, the first reflecting the present situation, while the latter refers to change over time. This may indicate that the relatively low sexual desire in TGCC patients is not related to the cancer diagnosis or the therapy, a suggestion supported by the fact that no significant difference was seen in sexual desire between the therapy groups. It is, therefore, tempting to hypothesise that low sexual desire in the TGCC men may rather be associated with the disease per se than with its treatment.

Male hypogonadism and EMD share several symptoms including low levels of resolution, ambivalence, impaired concentration, fatigue and lethargy (American Psychiatric Association, 1995; Carnegie, 2004). It could, therefore, be anticipated that disease- and treatment-induced hypogonadism is the underlying cause of EMD in TGCC patients. This could also be hypothesised from a biological point of view, since AR receptors are found in several parts of the brain (Beyenburg *et al*, 2000) and possibly several neurological, cognitive and psychiatric conditions are influenced by androgen action (Almeida *et al*, 2004; Cherrier *et al*, 2005; Colangelo *et al*, 2007). Our results indicate, however, that the EMD symptoms in TGCC patients are not related to hypogonadism. The two conditions seem to be two separate entities which, from a clinical point of view, may represent a problem in making the right diagnosis.

Our results are in accord with those presented by Wiechno and colleagues (Wiechno *et al*, 2007), who also reported no association between the results of the HADS and elevated LH or low testosterone. When using another depression scale, however, the Beck Depression Inventory (BDI), they noted an increased risk of depression when LH levels were increased. Both scales are well validated and the sensitivity and specificity in detecting major depression is very high for both. In the case of minor depression, however, there are indications that in cancer patients following curative treatment (Katz *et al*, 2004), the HADS demonstrates greater specificity, sensitivity and also a higher positive predictive value, although the difference between the scales was found not to be statistically significant. Although the study was based on a limited number of patients and therefore needs confirmation from a larger sample, the finding of a 62 % risk of having anxiety three to five years after treatment in the HDCT group raises both clinical concerns and aetiological speculations. In the clinical follow-up, these patients should be evaluated for anxiety disorders. The aetiology

could be because of the psychological stress, these young patients experiencing a serious threat to their lives. This EMD could also be related, however, to the high doses of CT. In a recent report, no cognitive impairment was seen in TGCC patients receiving CT compared with orchidectomised +\- RT patients. In this study, however, the CT patients received standard treatment doses (Pedersen *et al*, 2009). There are recent data indicating a dose-dependent cognitive dysfunction following cisplatin-based CT (Skoogh, 2008), which is also reported for chemotherapy-treated patients with other diagnosis (Nelson *et al*, 2007). Current research indicates that the cognitive domains that may be most affected by chemotherapeutic agents are visual and verbal memory, language, attention, and psychomotor functioning and subsequent anxiety. The potential mechanisms that cause such disruption remain largely unknown, although contributing factors could be vascular injury and oxidative damage, inflammation, direct injury to neurons or autoimmune responses (Nelson *et al*, 2007; Skoogh, 2008). Another study reported negative impact of CT in breast cancer patients on selected domains of cognitive function. These changes remained significant even after controlling for anxiety, depression, fatigue and haemoglobin level, which might support a psychological mechanism for anxiety among HDCT patients (Jansen *et al*, 2008).

Impact of androgen receptor polymorphism

We found a negative correlation between the length of CAG repeat in the AR gene and sperm concentration one to two years post three to four cycles of BEP. Furthermore, in a multivariate analysis, together with sperm concentration postorchidectomy, the CAG length was shown to be a significant, independent, predictor of sperm concentration in SCT-treated patients after one to two years. Previous in vitro and in vivo studies have

shown that the length of the CAG repeat is inversely correlated to the transcriptional activity of the AR and thereby to the sensitivity to the androgens (Tut *et al*, 1997). Some studies have demonstrated longer CAG repeats in infertile men (Dowsing *et al*, 1999) and an inverse correlation between sperm concentration and CAG length (von Eckardstein *et al*, 2001a). We did not find any correlation between CAG lengths and pre-treatment sperm concentration. Furthermore, the results of the multivariate analysis indicated that the effect of this AR polymorphism is not exerted through regulation of the pre-treatment state of spermatogenesis, but is rather implicated in the process of recovery. We did not find any association between sperm number and the length of the other repetitive sequence of the AR gene – the GGN repeat.

The finding of an association between the CAG segment and recovery of spermatogenesis is intriguing. Since androgens are mostly involved in the regulation of post-meiotic stages of spermatogenesis (Sofikitis *et al*, 2008), our finding indicates that after SCT recovery of late stages of spermatogenesis (Zhang *et al*, 2003) plays an important role in reaching pre-treatment levels of sperm concentration. Our study indicates that decreased androgen action after BEP treatment delays the recovery of sperm production. Inclusion of larger groups of men is necessary, however, to draw any firm conclusions regarding this issue.

Lower androgen sensitivity, with higher LH levels, was found in men with long CAG tracts (28). It could be anticipated that the risk of hypogonadism is modified by the length of this repeat. We found no association, however, between any of the AR polymorphisms and biochemically-defined hypogonadism.

In elderly Finnish men, long CAG repeats were found to be associated with the risk of depression (Harkonen *et al*, 2003) indicating a more important role of androgens in the pathogenesis of EMD in this category of subjects,

while in an investigation on adolescent men, short CAG repeats pointed towards not a higher incidence but more severe depressive symptoms (Su *et al*, 2007). Additionally, higher CAG number was associated with lower scores on cognitive tests, maybe also implying an association to EMD (Yaffe *et al*, 2003). Our study did not give any support for an impact of AR polymorphisms on EMD among TGCC patients.

Strengths and weaknesses of thesis

Different analytic strategies were applied in the studies which formed part of this thesis, with a longitudinal approach in articles I and II and cross-section analyses in articles III and IV. The longitudinal approach implies a better option for studying causality and also gives a mapping of time-related changes. The disadvantage, however, is a relatively low number of subjects on whom such analyses could be based, and additional studies are needed to prove or disprove our findings. For the two latter articles, longitudinal analyses will be possible when the vast majority of the patients have passed the five-year follow-up, which will take another one or two years. Both articles have, however, been based on well-validated instruments including a considerable number of subjects. In article III the questions used to evaluate sexual function and satisfaction were validated in a large population study, which also provided data for an age-matched reference group.

The participation rate in the two first studies was quite high, 82 %, reducing the risk of selection bias. In articles III and IV there were two inclusion criteria, primarily to be included in the fertility study and secondarily to have passed the three- to five-year control. The participation rate in these articles was 79 %.

In the third study, the 59 % response rate for the controls may appear somewhat low, but post hoc analyses (Fugl-Meyer *et al*, 2000) have shown that the studied male population is adequately representative of Swedish men aged eighteen to 74. Seventy-five of the 409 (461 eligible minus 52 excluded) men (18 %) who were asked to participate in the fertility study declined to take part. In comparison with those 129 who were finally included in study III (Figure 5), we found no difference regarding their age, but the men not willing to participate had received significantly less advanced treatment. Since we did not find any obvious impact of the treatment intensity on the risk of sexual dysfunction, however, we do not think that this would influence the results.

In study I information about abstinence time was only available for 91 of 177 samples (51 %). It could be anticipated that semen samples collected postorchidectomy, but before further treatment, compared with other time-points, were preceded by a longer abstinence time owing to the disease as well as surgery-related stress. No significant difference, however, in abstinence time was found between samples collected at different time-points.

The signs of hypogonadism are non-characteristic and good biochemical markers of androgen deficiency are lacking. The clinical diagnosis is usually based on the combination of symptoms and serum levels of testosterone and LH. In our studies we have only used biochemical parameters in defining hypogonadism. The levels of 10 nmol/L for total testosterone and 10 IU/L for LH are generally accepted as useful markers of hypogonadism in younger males (22). A shortcoming in articles II, III and IV is the change in the methods for testosterone and LH measurements. In the material from Lund, however, conversion of the results from the method used in the first part of these studies to the one applied during the second part was based on more than 30 subjects tested with both methods.

Furthermore, no statistically significant difference between measurements performed with the different methods was found at any time-point. In the material from Stockholm, no methodological changes were made during the interval from study initiation to the time of data analysis. The reference intervals used in the Lund material after conversions were identical to those used in Stockholm. Finally, the type of method applied for hormone measurement was included as a confounding factor in the statistical analysis in studies II to IV. We therefore believe that our results are reliable despite this methodological drawback.

For logistic reasons, we obtained blood samples at different time-points between 9 a.m. and 3 p.m. This might have blurred the difference between truly hypogonadal and eugonadal men, owing to the diurnal variation of testosterone. It has been reported, however, that the diurnal variation in testosterone levels is less pronounced in hypogonadal men (Winters, 1991). Furthermore, the association between hypogonadism and the outcomes in papers II to IV was unchanged when LH above 10 IU/L was used as the only indicator of androgen deficiency. Unlike testosterone, LH does not decrease during the day. Studies regarding the association between testosterone levels and metabolic signs of hypogonadism reported no difference in the risk estimates regardless of whether the time of blood sampling was taken into consideration or not (Agledahl *et al*, 2008). In study II, men on androgen replacement were considered hypogonadal from the time of initiation of therapy. In studies III and IV, men already on androgen replacement therapy were excluded, which could lead to an underestimation of the prevalence of patients with problems related to hypogonadism and TGCC. Inclusion of patients on testosterone replacement, however, did not influence the magnitude of the risk estimates in either of these two studies.

General conclusions

- Adjuvant chemotherapy did not induce a significant decline in sperm concentration. After three to four cycles of chemotherapy and adjuvant radiotherapy against abdominal lymph nodes a reduction of sperm concentration was observed, recovering to pre-treatment levels two to five years post-treatment.

- In patients treated with three to four cycles of chemotherapy, androgen receptor CAG number was associated with the recovery of spermatogenesis.

- Hypogonadism postorchidectomy, but before further treatment and testicular microlithiasis, and type of treatment were predictive factors for the risk of post-treatment hypogonadism.

- Compared with the general age-matched population, TGCC patients three to five years after completion of therapy were at significantly higher risk of having low sexual desire and erectile dysfunction. These sexual dysfunctions, however, were not significantly associated with treatment intensity or hypogonadism.

- Biochemical hypogonadism and androgen receptor polymorphism seem not to be risk factors for anxiety or depression in TGCC patients.

- Patients with refractory or relapsed disease receiving five or more cycles of cisplatinum-based chemotherapy may, to a higher degree than patients receiving less intense therapy, suffer from anxiety.

Future Perspectives

The material from the study provides an excellent source to answer several future questions of interest and importance, mainly from a clinical, but also from a biological, perspective. It can provide further data for subgroups of TGCC patients, but also improve our knowledge on inter-individual differences among these patients. Such knowledge can be of value for more individualised counselling concerning several treatment-related side-effects, such as need for cryopreservation, susceptibility to developing infertility, hypogonadism and possibly also sexual and affective disorders. The possibility of evaluating the material with a longitudinal design constantly increases, as the collection of data is ongoing until definite study closure June 2011.

Concerning sperm parameters, a conformational longitudinal study is warranted, including more semen samples with parallel analyses of FSH and inhibin B. The aim would be to evaluate further the risk of developing azoospermia and to investigate the predictive value of the hormone values in relation to assessment of spermatogenesis and its recovery.
The finding that in patients treated with three to four cycles of chemotherapy the androgen receptor CAG number was associated with the recovery of spermatogenesis is intriguing, but since it is based on nine subjects only, it definitely needs confirmation in a larger sample. Other polymorphisms in strategic genes should also be studied.

More information regarding the Leydig cell function can be obtained by using the hCG test data, with serum levels of testosterone measured before and 96 hours after hCG administration. In the present study, the hCG test has been performed one and five years after completion of therapy, which

may reveal some more discrete impairments of Leydig cell function, not apparent when measuring testosterone levels without any stimulation (data not yet analysed).

In parallel with current projects, another project has been focusing on sperm DNA integrity in the same patient material (Stahl *et al*, 2004; Stahl *et al*, 2006). Possible associations between defect sperm DNA and genetic polymorphisms and Leydig cell dysfunction, as well as testicular microlithiasis, could also be investigated

The questionnaire includes a well-validated instrument for measuring general satisfaction, the LiSat-11 checklist (Fugl-Meyer *et al*, 2002) also included in the national survey. These parameters should be evaluated longitudinally to reveal causality and association with hypogonadism, sexual dysfunctions and treatment intensity as well as socio-demographics, in order to find potential agents related to low general satisfaction.

Both sexual dysfunctions and emotional disorders should be further evaluated with a longitudinal approach. Some findings need confirmation in a larger sample, in particular the increased risk of anxiety in HDCT patients. Furthermore, the risk of developing depression depending on treatment modality was not possible to evaluate in the current thesis owing to the insufficient number of depressed patients in the subgroups.

Metabolic syndrome is overrepresented in hypogonadal men and also among TGCC patients and the causes are still largely unknown. A direct link has been hypothesised and in that context the study could be expanded with data concerning metabolic syndrome and outcomes tested for

correlations to treatment intensity, hypogonadism and AR polymorphisms. The aim would be to find risk factors for developing metabolic syndrome.

The finding of a predictive value of microlithiasis in relation to the risk of hypogonadism both pre- and post-treatment should also be expanded to an investigation of possible association between this ultrasound pattern and semen quality in TGCC patients. A positive finding would be a support for the TDS hypothesis and also indicate the possibility of using ultrasonographic investigation as a tool in prediction of future fertility.

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