Reproduction and pregnancy outcome in cancer survivors

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Contents

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Oslo, January 2008

Henriette Magelssen

2. Abbreviations used

ABVD: Adriamycin, bleomycin, vinblastine, darcabazine

ART: Assisted reproductive techniques

BOT: Borderline tumours of the ovary

CHOP: Cyclophosphamide, adriamycin, vincristine, prednisolone

CI: Confidence interval

CMF: Cyclophosphamide, methotrexate, 5-fluoruracil

CNS: Central Nervous System

CRN: Cancer Registry of Norway

ED: Erectile dysfunction

FEC: 5-fluoruracil, epirubicin, cyclophosphamide

FSH: Follicle stimulating hormone

GnRH: Gonadotropin releasing hormone

HL: Hodgkin´s lymphoma

ICSI: Intra-cytoplasmic sperm injection

IUI: Intra-uterine insemination

IVF: In vitro fertilisation

LBW: Low birth weight

LH: Luteinising hormone

MBRN: Medical Birth Registry of Norway

MESA: Microsurgical epididymal aspiration

ML: Malignant lymphoma

MOPP: Mechlorethamine, vincristine, procarbazine, prednisolone

NRH: The Norwegian Radium hospital

OR: Odds ratio

ORadj: Adjusted odds ratio

POF: Premature ovarian failure

RPLND: Retroperitoneal lymph node dissection

RRMC: Rikshospitalet-Radiumhospitalet Medical Center

SCP: Semen cryopreservation

TC: Testicular cancer

TDS: Testicular Dysgenesis Syndrome

TESE: Testicular sperm extraction

3. List of papers

This thesis is based on the following papers:

Paper I:

Magelssen H, Brydoy M, and Fossa SD (2006) The effects of cancer and cancer treatments on male reproductive function. Nat Clin Pract Urol. 2006 Jun; 3(6): 312-322. Review.

Paper II:

Magelssen H, Haugen TB, von During V, Melve KK, Sandstad B, and Fossa SD (2005) Twenty years experience with semen cryopreservation in testicular cancer patients: who needs it? Eur Urol. 2005 Nov; 48(5): 779-785.

Paper III:

Fossa SD, Magelssen H, Melve K, Jacobsen AB, Langmark F, and Skjaerven R (2005) Parenthood in survivors after adulthood cancer and perinatal health in their offspring: a preliminary report. J Natl Cancer Inst Monogr. 2005; (34): 77-82.

Paper IV:

Magelssen H, Melve KK, Skjaerven R, Fossa SD (2007) Parenthood probability and pregnancy outcome in patients with a cancer diagnosis during adolescence and young adulthood. Hum Reprod. 2008 Jan; 23(1): 178-186. Epub 2007 Nov 16.

4. Background

4.1 Epidemiological aspects of cancer survivorship

In 2005, almost 168 000 individuals were alive in Norway with a prior cancer diagnosis, which represents three to four percent of the Norwegian population (1). In the US more than 10 million people are cancer survivors (= individuals with at least one cancer diagnosis, independent of the time since diagnosis). The proportion of cancer survivors is expected to increase due to changes in age distribution, increasing size of the population in the coming years, due to increasing incidence of cancer and improved curative treatment.

In 2001-2005 almost 12% of new cancer diagnoses in men living in Norway were made in the age group 30-54 years and almost 2% were in the age group 15-29 years, the comparable figures for females being 20% and 1.5% (1). Testicular cancer (TC), malignant melanoma and malignant lymphoma (ML) were the most frequent cancer types diagnosed among males aged 15-44 years in the years 1971-1997 (Cancer Registry of Norway (CRN) 2007, personal communication) (Figure 1a). Breast cancer, gynaecological cancers and malignant melanoma were the most frequent new cancer diagnoses among females aged 15-45 years old in this time period (Cancer Registry of Norway (CRN) 2007, personal communication) (Figure 1a).

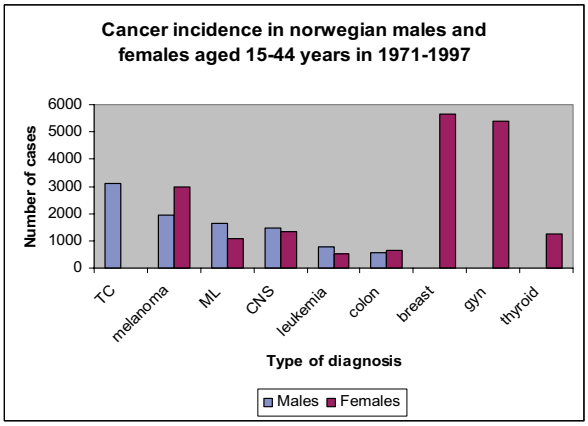


Figure 1a)
Cancer incidence in Norwegian males and females aged 15-44 years at time of diagnosis in 1971-1997 (Cancer Registry of Norway (CRN) 2007, personal communication). TC: Testicular cancer, ML: Malignant lymphoma, CNS: Cancer in the Central Nervous System, gyn: Gynaecological cancer.

More than half of the male patients in Norway aged 15-44 years when diagnosed with TC or ML in the years 1971-1997 were treated at the Norwegian Radium Hospital (NRH) (Figure 1b), with an even higher percentage for female patients diagnosed with ML or cervical cancer (Figure 1c).

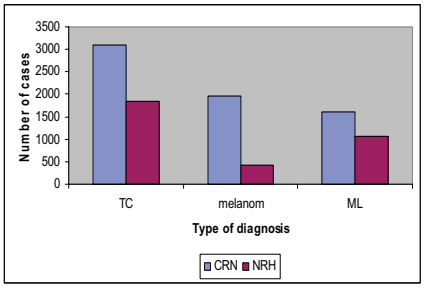


Figure 1b) Testicular cancer (TC), malignant melanoma and malignant lymphoma (ML) diagnosed in Norwegian males aged 15-44 years at time of diagnosis in 1971-1997, the total number diagnosed in Norway (numbers from the Cancer Registry of Norway (CRN)) and the numbers diagnosed at the Norwegian Radium Hospital (NRH).

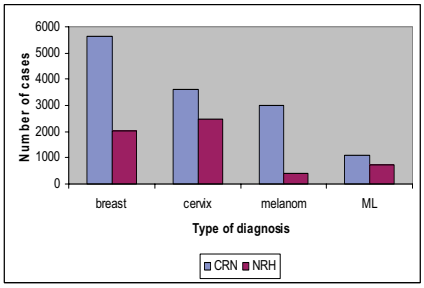


Figure 1c) Breast cancer, cervical cancer, malignant melanoma and malignant lymphoma (ML) diagnosed in Norwegian females aged 15-44 years at time of diagnosis in 1971-1997, the total number diagnosed in Norway (numbers from the Cancer Registry of Norway (CRN)) and the numbers diagnosed at the Norwegian Radium Hospital (NRH).

Due to improved treatment and high survival rates, in particular among males aged 15-45 years (TC, ML), late effects after cancer as well as life after cancer have thus become an important issue for cancer specialists and primary health care officers, and represent a challenge for clinical, epidemiological and translational research. Most information on long-term effects after cancer and its treatment is based on studies of childhood cancer (2-12). Less is known about survivors after cancer in adolescence and young adulthood (age group 15-45 years).

The above cancer demographics imply that increasing numbers of patients have their parenthood probabilities affected by their cancer experience. The gradual successes of cancer treatment, particularly for malignancies that affect young people have yielded a large population of cancer survivors who may wish to have children (13). At the same time, population-based studies have shown that an increasing proportion of individuals delay first-time parenthood to their 4th or even 5th decade of life (14) (See MBRN statistics at: *http://mfr-nesstar.uib.no/mfr/*) . When cancer is diagnosed in young individuals, clinicians are thus faced with multiple questions from the patient and/or his/her partner, such as; Does my cancer or its treatment affect my chance to experience parenthood in my life time? Will possible children have increased risk of birth defects or genetic aberrations? Will a possible pregnancy imply an increased risk of obstetric problems? What tasks can be performed aimed to prevent infertility or at least restore my reproductive ability? (15)

Gonadal toxicity or other somatic barriers are, however, not the only impediments to parenthood after cancer (13;15). Some young survivors may have concerns whether they after their cancer treatment will remain sufficiently attractive to find a partner. Others may ask whether treatment may lead to loss of vitality and energy, decreasing the desire and/or the ability to have children after cancer treatment (13). Financial concerns related to the cancer experience may also have impact on post-treatment reproduction plans (13).

Certain aspects of the health care system and the population structure in the Nordic countries provide good conditions for long-term outcome cancer research: Each individual living in the Nordic countries has a unique national identification number. Well-functioning population-based registries exist, and emigration has so far been limited. During the last 20 years, Norwegian researchers have therefore taken advantage of these conditions and have become involved in the investigation of long-term effects after cancer, utilising information from hospital-based and population-based registries. The present study deals with some of the above issues of post-diagnosis reproduction, based on a large cancer hospital's experience during the previous century´s last three decades. It does not, however, consider psychosocial aspects of post-diagnosis reproduction.

4.2 General fertility issues

4.2.1 Gonadal function

Males: The origin of primordial germ cells is in the fetal life. The testicular dysgenesis syndrome (TDS) is a result of disruption of the physiological regression of primordial germ cells and their development to gonads during fetal life, manifested as one or more of the disorders low sperm counts, undescended testis, hypospadias or TC (16).
Normal male fertility requires undisturbed spermatogenesis starting at puberty and undisturbed transport of the mature sperm cells followed by antegrade ejaculation. The testes have both an endocrine (hormone producing) and an exocrine (sperm producing) function, which is controlled by the hypothalamus through the pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Gonadotropin releasing hormone (GnRH) is produced in the hypothalamus and promotes the production of LH and FSH in the hypophysis and their release to the blood stream.

The duration of the spermatogenic cycle takes approximately 70 days (17), starting from the stem cells of the germinative epithelium (spermatogonia). Simplified, at the testicular level, LH regulates testosterone production by the Leydig cells (17). FSH and testosterone stimulate the Sertoli cells, which provide both endocrinological and nutritional support for the spermatogenesis (17;18). Sertoli cells secrete Inhibin B which controls FSH secretion through a negative feedback mechanism (17). New cycles are initiated at regular time intervals (every 2-3 weeks) before the previous ones are completed. Any cytotoxic injury to the germinative epithelium is followed by increased FSH levels, and Leydig cell hypofunction leads to LH increase (primary gonadal failure). Furthermore, any reduction of the pituitary gland function, as cranial radiation, leads to decreased LH and FSH, followed by both endocrine and exocrine (secondary) gonadal failure.

From the testis, the sperm cells pass through the epididymis for further maturing to become fertile and motile. Erection and ejaculation are dependent on neural stimuli and reflexes mediated by the parasympathic and sympathic pelvic nerves.The sperm cells are transported by the seminal fluid produced by the prostate and the seminal vesicle. Disruption of any of these mechanisms may have consequences for fertility.

Females: At birth, women have a fixed number of primordial follicles in their ovaries, which are progressively lost during life from about 400 000 at puberty to about 1000 at the age of 50

when menopause normally occurs. Combined stimulation by LH and FSH matures the follicles: FSH is primarily involved in stimulating the growth of ovarian follicles, while LH controls ovulation and regulates steroidogenesis (mainly production of estradiol and progesterone). The secretory pattern of LH and FSH in the female is more complex than in the male. The release of LH varies with the stage of the menstrual cycle, and the highly increased release on about day 14 of the cycle leads to ovulation. The first half of the cycle is dominated by growing follicles that secrete estradiol. After ovulation, the follicle is transformed into the corpus luteum which releases progesterone to the blood stream. For almost all of the effects of progesterone (growth of the myometrium, preparing for implantation), a preciding or simultaneous action of estradiol is essential (maturation of follicle and egg, proliferation of the endometrium, facilitating migration of sperm). Any insult that reduces the number of follicles leads to an increased risk of premature ovarian failure (POF), defined as menopause before the age of 41 years. A decreased oocyte reserve may also result in a lower chance of subsequent conception, despite maintenance of menstrual cycles (19;20).

4.2.2 The effect of cancer treatment on fertility

Fertility in cancer survivors is affected by treatment, which may lead to both primary and secondary gonadal failure. In addition, cancer treatment may reduce or abolish the function of genital organs in women or the sperm cell transport in males.

Surgery

Males: After unilateral orchiectomy in TC patients, sperm cell concentrations are reduced compared to concentrations prior to the surgical procedure (21), in part due to the reduction of the germinative epithelium, but also due to reduced function of the contralateral testicle. Former used bilateral radical retroperitoneal lymph node dissection (RPLND) leads to dry ejaculation in almost 100% of the patients, as a result of the resection of post-ganglionic nerves of the hypogastric plexus which innervate the pelvic structures necessary for ejaculation (22). After the introduction of unilateral RPLND early in the 80'ies (23) and of the nerve-sparing techniques, "dry" ejaculation remains an adverse effect in not more than 10 % of the patients, even in case of post-chemotherapy RPLND. After surgery for cancer of the rectum, 20-40% of male patients develop complete erectile dysfunction (ED), and 16% of patients suffer from postoperative ejaculatory dysfunction (24).

Females: Uterine cervical cancer and ovarian cancer are the most common gynaecological cancers during reproductive age, and infertility is in most cases inevitable as the genital organs are removed. However, in women with early-stage cervical cancers who want to preserve fertility, other treatment alternatives exist: laser conization or trachelectomy (20;25;26). The clinical guideline for borderline tumours of the ovary (BOT) was until recently still similar to that of epithelial ovarian cancer: total hysterectomy and bilateral salpingoophorectomy. Today, fertility-sparing treatment with unilateral salpingoophorectomy or only the removal of the BOT (cystectomy), is considered a safe procedure with a good future possibility to obtain spontaneous pregnancy (27), provided that the patient is willing to undergo careful and prolonged follow-up (28).

Radiotherapy

Males: The germinative epithelium represents one of the most radiosensitive tissues, and even low doses of radiation can cause impairment of spermatogenesis, at least transiently. Damage may be caused by direct radiation, for example in case of cancer in situ of the testicle, or more commonly from scattered irradiation during treatment directed at adjacent tissues (29). The immature spermatogonium is more radiosensitive than the mature sperm cells, with doses as low as 0.1 Gy causing morphological and quantitative changes. Recovery of spermatogenesis takes place from surviving stem cells and is dependent on the dose of testicular radiation, with complete recovery within 9-18 months following single-dose radiation of ≤ 1 Gy, within 30 months following radiation of 2-3 Gy and –if at all- after 5 years or more after radiation doses of 4 Gy (29). Single radiation doses of more than 6 Gy most often result in permanent azoospermia (29). However, fractionated irradiation, as commonly used in the clinical setting, increases the gonadal toxicity, with threshold for permanent testicular damage around 1.2 Gy (29;30), and permanent infertility after fractioned doses of more than 2 Gy. Fractionated testicular doses of less than 0.2 Gy give no significant effect on sperm counts, fractionated doses between 0.2-0.7 Gy result in transient reduction in sperm concentration mirrored by elevated FSH, with return to normal values within 1-2 years (29;31). In young men, testicular shielding should always be considered during pelvic radiotherapy, to reduce the scattered irradiation dose to the testicles (32).

Females: Radiotherapy causes DNA damage and induces apoptosis in the primordial follicles. However, in females only few cells during each ovarian cycle are in meiotic activity, and the ovaries are hence less sensitive to radiation than the testes. Due to their location within the

pelvis, however, the ovaries more often receive relatively high doses of radiation. Ovarian impairment is related to the dose of radiotherapy and the woman´s age at treatment (or number of remaining follicles). Radiation doses of about 4 Gy to the ovaries lower the follicle reservoir by 50%(33-35). In females below 40 years of age an estimated radiation dose of 20 Gy will destroy all the follicles, with comparable figures for females above 40 years being only 6 Gy (14;33;35;36). Limitation of radiation exposure by shielding of the ovaries should be practiced whenever possible. Oophoropexy, surgical transpositioning the ovaries outside the radiation field, may also reduce the radiation dose(33), but due to altered ovarian blood flow, scattered irradiation and also subsequent remigration of the ovaries, the success rate is only about 50% (19;20). The uterus is also extremely vulnerable to high dose radiation and decreases in volume by 40% (14;37). Even if pregnancy is achieved, these patients may have an increased risk of obstetric complications, including early pregnancy loss, premature labour and low birth weight children due to impaired uterine growth and blood flow (7;14;20;37).

Chemotheraphy

Dependent on the choice of the cytostatic agent and the combinations of these, the cumulative doses and the age of the patient, there is a risk of persistent post-chemotherapy infertility (Table I).

Males: The cytostatic agents disrupt spermatogenesis by targeting various cell types (Leydig cells, Sertoli cells, germ cells) (38). The most gonadotoxic cytostatic agents are procarbazin and alkylating drugs, in particular cyclophosphamide. Procarbazin was previously often used in the treatment of Hodgkin's lymphoma (HL) by the MOPP-regime (mechlorethamine, vincristine, procarbazine, prednisolone), resulting in persisting azoospermia in a high proportion of patients (39). The currently used ABVD-combination (adriamycin, bleomycin, vinblastine, dacarbazine) is less gondotoxic with recovery of spermatogenesis seen in the majority of the patients (39). The cytostatic agents used in the treatment of Non-Hodgkins lymphoma (CHOP: cyclophosphamide, adriamycin, vincristine, prednisolone) is less gonadotoxic than chemotherapy used for HL, probably related to the absence of procarbazin (and less alkylating agents) (40). This is also presumably the cause of less gonadotoxic effects of ABVD used for HL. Cisplatin-based chemotherapy for TC results in temporary azoospermia in most men, with post-chemotherapy recovery of spermatogenesis in about 50% after two years, and in 80% by five years (41). The cumulative dose of the cytotoxic agent

used is, however, important. Additive effects must be considered if cytostatics are combined with low-dose testicular irradiation.

Table I

Cytotoxic drugs and gonadotoxicity

High risk -**Alkylating agents** • Cyclophosphamide • Mechlorethamine • Ifosfamide • Busulfan • Chlorambucil -**Miscellaneous** • Procarbazine	M**edium risk** -**Platinum analogues** • Cisplatin • Carboplatin -**Antibiotics** • Doxorubicin
Low risk -**Plant derivatives** • Vincristine • Vinblastine -**Antibiotics** • Dactinomycin (Actinomycin D) • Bleomycin -**Antimetabolites** • Methotrexate • Mercaptopurine	**Combinations** **High risk** -MOPP(mechlorethamine, vincristine, procarbazine, predn) -ChlVPP(chlorambucil, vincristine, procarbazine, prednisolone) -COPP(cyclophosphamide,vincristine, procarbazine, predn) **Medium risk** -ABVD(adriamycin, bleomycin, vinblastine, dacarbazine) -BEP(cisplatin, etoposid, bleomycin)

Females: Also in females, alkylating agents are considered the most damaging cytostatics, causing DNA damage and inducing apoptosis of the follicles. Permanent amenorrhea and elevated FSH levels post-treatment indicate that the follicle-reserve is lost. The MOPP regimen previously commonly used in patients with HL leads to POF and thus infertility in females (19). After ABVD the risk of permanent amenorrhea has been reported to be below 20%(20). The gonadal effects of adjuvant treatment with 6 cycles of FEC (5-fluoruracil, epirubicin and cyclophosphamide) for breast cancer are dependent on age, with a high (above 80%) risk of permanent amenorrhea in women above 40 years, a medium risk for women in their 30`s, and low (<20%) for women in their 20`s (19;20). Breast cancer survivors treated with adjuvant CMF regime (cyclophosphamide, methotrexate and 5-fluoruracil) (in Norway used up to 1999) have developed permanent amenorrhea in as much as 45-68% (14;42).The possibility of preserving fertility in females exposed to chemotherapy by administration of a GnRH-agonist is still controversal. In the lack of randomized studies, there are uncertainties

regarding application in humans, and the benefit of ovarian protection by GnRH analogues is unproven (20;33;43).

4.2.3 Cryopreservation/IVF

Even if many cancer patients restore fertility after treatment, it is not possible to predict the recovery in the individual patient. For some patients, assisted reproductive techniques (ART) offer the only chance of post-treatment parenthood of a biological child. In addition, the psychological impact of cryopreservation is undeniable (20;44).

*Males: C*ancer patients at risk of permanent or long-lasting post-treatment infertility, who do not exclude post-treatment fatherhood at the time when the fertility threatening treatment is started, should be offered semen cryopreservation (SCP)(45). SCP and subsequent sperm cell thawing result in a 25% to 75% postthaw decrease in sperm motility relative prefreeze values, with similar percentage decline in semen quality in patients with TC or HL as in healthy men (46;47). Improvement in cryopreservation techniques and cryopreservation media will further increase sperm quality after cryopreservation even in oligospermic men (46;48-50) .

Though SCP obtained by masturbation has become the "standard" for fertility-saving in post-pubertal males with normal or almost normal spermatogenesis, new but still experimental techniques can be offered to pre-pubertal boys and to men with disturbed semen transport, but with normal spermatogenesis. In the adult men mature sperm cells for ART may be obtained by microsurgical epididymal aspiration (MESA) or testicular sperm extraction (TESE)(51;52). Ectopic xeno- or auto- grafting of testicular tissue represents another and even more experimental approach to obtain mature sperm cells, whereas in vitro maturation of testicular tissue has so far been unsuccessful for the achievement of mature sperm cells (53). Finally, it seems possible that pluri-potent embryonic stem cells may form germ cells in vitro. This latter approach, if successful in humans, would also circumvent the threat of transmission of cancer cells to the recipient if the pre-treatment testicular tissue is contaminated by cancer cells (54).

Females: There are several methods for preservation of fertility in females, with embryo cryopreservation as the most established method. This procedure, however, requires a partner, and is also limited by the need of hormonal stimulation in vivo which has to start at the beginning of the menstrual cycle. Consequently, cancer treatment must be postponed for 2-6

weeks. For women with hormone responsive tumours, there are medical concerns as to the stimulation by estrogens. Tamoxifen or aromatase inhibitors in combination with gonadotropin treatment have been studied as alternatives with reduced oestrogen exposure for stimulation in these patients (14;20;55).
Cryopreservation of unfertilised oocytes or of ovarian cortical strips/biopsies is still regarded experimental, as these cells are more vulnerable for cryopreservation than embryos. It is however, the only available method for pre-pubertal women or women without a partner. The first child conceived by this method world-over was born in Belgium in 2004. Freezing of ovarian tissue does not require hormone stimulation and does not delay cancer treatment more than the time required for the procedure. The ovarian tissue can later be transplanted, theoretically by three strategies: autotransplantation (orthotopically (transplanted back to the original site (ovaries)) or heterotopically (transplanted to a different part of the body (abdomen or forearm))), xenotransplantation (human ovarian tissue transplanted to mice) and in vitro maturation (isolation of mature follicles)) (14). Orthotopic transplantation allows the possibility of spontaneous conception, heterotopic transplantation and xenotransplantation requires oocyte harvesting and *in vitro* fertilisation. Both procedures have resulted in mature ova (56-58), whereas in vitro maturation has so far not given any results (59).
Xenotransplantation has been used purely for experimental purposes, and will not be an object for clinical applications unless the safety and ethical issues are solved (14;60).

4.2.4 The situation in Norway regarding cryopreservation and IVF

The first sperm bank in Norway was established in 1980 at St.Olav's Hospital in Trondheim, a travelling distance of more then 500 km from Oslo. During the first eight to ten years a principal condition for SCP was a sperm concentration of $\geq 5 \times 10^6$ /ml. From about 1987, and along with increasing experience with ART, any semen sample with living sperm cells was frozen. In 1994, a second sperm bank was opened at Rikshospitalet University Hospital in Oslo. Up to 1994 SCP was offered to patients if they were less than 40 years at diagnosis. Along with the establishment of the second sperm bank, this age limit was increased to 55 years in 1995.

Semen specimens are obtained either at home, at the hospital, or at a Andrology Laboratory by masturbation into a container. The recommendation is 2 to 7 days of sexual abstinence before the day of cryopreservation. The volume of semen is estimated by weighing. The

sperm concentration is determined by Hamilton-Thorne Sperm Analyzer (HTM-IVOS). The semen samples are diluted with a glycerol-containing phosphate buffer, pH 7.4, to a final concentration of 7% glycerol (v/v).The semen is frozen in 0.5 ml straws in three decreasing temperature steps; 15 minutes in -30° C nitrogen vapour, 15 minutes in -70° C nitrogen vapour, and then in liquid nitrogen for storage. If additional delay of treatment is justified, up to two more samples are frozen, preferably with 2 days interval or more.

Embryo cryopreservation has in Norway been performed since the 1980ies. Cryopreservation of unfertilised oocytes or of ovarian cortical strips/biopsies has been performed since 2004. Because of age-related follicle loss, the age-limit for the latter procedure is 35 years.
In Norway, ovarian tissues from 22 women aged 14-35 years have been cryopreserved by January 2007.

During the early eighties, ART was performed as intra-uterine insemination (IUI). In vitro fertilization (IVF) has been used since 1989, and intra-cytoplasmic sperm injection (ICSI) has been performed since 1995.
Until 2002, ART has been paid for by the health care system with minor expenses for the patient. Today, the patient has to pay up to kr 15000 to the government for the medications needed for ART (the rest is refunded), and kr 1500 to the hospital for each attempt of ART (leading to embryo transfer). The rest is refunded for until three attempts.

4.3 Pregnancy outcome

Cancer survivors are concerned about potential health problems for their children. One concern is that the survivor´s past cancer and/or its treatment could lead to a child with a birth defect or genetic abnormality. This concern is justified in cancer patients with a known genetic defect; retinoblastoma, Wilms tumour or BRCA positive breast cancers (61-64).
It has been suggested that prior flank irradiation in females with Wilms tumour during childhood may increase the risk of congenital anomalies (3). So far, however, there is no epidemiological proof that there is an increased risk of genetically induced congenital anomalies in children born after one parent´s cancer treatment (12;65-71). Cancer and cancer therapies can, however, affect pregnancy outcomes and impact the offspring by direct effects on the female reproductive tract or by neuroendocrine pathways and by developmental disturbance of the growing embryo (72;73), in particular during the first trimester. In female

cancer survivors, increased risk of low birth weight children has been demontstrated (7;8;14). Most studies have analysed infants born to survivors of childhood cancer, and most of these infants were conceived many years after the parent´s treatment. More large studies of survivors treated in adulthood are needed (13). Concern also remains that using ART to treat cancer-related infertility may allow conception with genetically damaged gametes (20;74;75).

Birth weight and gestational age are essential key variables in perinatal epidemiology, and perinatal mortality is a central outcome. Perinatal mortality was introduced as a concept in 1936 by a German paeditrician who claimed that the time period just prior to, during and after birth is characterised by a peak in mortality of the fetus or the new born infant (76). The usual definition of perinatal mortality is the number of stillbirths and early neonatal deaths per 1000 births(live and still) (76). The early neonatal mortality rates have decreased, especially for the preterm births and for infants with low birth weight (LBW), for a larger part attributable to new clinical procedures for the most immature infantsb(77). In the 1960s and 1970s less than 10% of the newborns weighing between 500 and 1000g survived, whereas around 75% of these infants survive today**.** In 1950 the WHO recommended that birth weight below 2500g should be used as a standard for either"prematurity" or"immaturity". Birth weight is now recognised as a product of the intrauterine growth (velocity) of the fetus and the lenght of gestation. There is a continuous rise in the mortality rate as the birth weight decreases, also below the traditional cut-off level at 2500g defining LBW children with high risk (78). The risk of perinatal mortality is also highly dependent on the lenght of gestation, and increases drastically below 29 weeks(79). Above 30 weeks, the survival is more than 90% (79).

The tendency for a mother to deliver similar-sized children has also been studied in relation to the perinatal mortality of the infants (80;81). Babies whose birth weight and weight for gestation was similar to that of their older siblings were in general found to have the lowest risk of perinatal death (80). Perinatal mortality for preterm second births is higher among mothers whose first infant was born at term compared with mothers whose first born child was delivered moderately preterm (82). These results indicate that "women are to some extent "programmed" to produce offspring of a certain fetal age and size" (83). When analyzing risk of adverse pregnancy outcomes, one of the most important predictors to consider is therefore the outcome of the mother's previous pregnancy (-ies). For many outcomes, the situation will be that risk is heterogeneous between women, but relatively constant for the same women in their successive pregnancies (80;82).

In devolped countries, congenital anomalies account for a majority of perinatal and infant deaths, alongside preterm-associated conditions and severe growth restriction. The usual understanding of a major birth defect is a structural abnormality of prenatal origin present at birth, which seriously interferes with viability or physical well-being (84). Depending somewhat on the definition, the prevalence of major birth defects is estimated to be around 3% (84). The prevalence usually found in registries that depend on routine examination at birth is around 2-3% (85). Minor birth defects, i.e. abnormalities that do not interfere with viability or physical well-being, are present in approximately 10% of newborns. Many studies group all major birth defects as one category, for instance in studies of overall prevalence. This is what we have done in our analyses of birth defects in the present work (Paper III and IV). When more detailed studies are designed, it is advisable to group the anomalies either on the basis of their underlying mechanism or on the basis of which organ(s) is/are involved. The latter is used in the ICD-classification, and is what we use in the part describing the congenital anomalies in Paper IV.

Though post-diagnosis fertility had been investigated in several mono-institutional studies and for several cancer types, large scale investigations were lacking when the present study was initiated. In particular, comparisons with observations from the general population were only rarely performed. However, most clinicians anticipated that cancer patients would be subfertile after their treatment. Further, the general view among oncologists was that children born after one parent´s cancer treatment were not at increased risk of having a congenital anomaly compared to children born to the general population.

5. Aims

The principal aim of the present work was to study the impact of cancer and its treatment on reproduction and pregnancy outcome in patients diagnosed during adolescence and young adulthood, with emphasis put on male cancer survivors. We also compared reproduction and pregnancy outcomes with the general population.

Specific objectives were:

- To present an overview on the effects of cancer and cancer treatments on male reproductive function.

- To evaluate the proportion of pre-treatment SCP among newly diagnosed TC patients and to document the post-treatment utilization of thawed semen over a twenty years period.

- To assess demographic and medical pre-treatment variables which are associated with post-treatment parenthood probability.

- To document the 10-year cumulative first-time post-cancer parenthood probability in cancer survivors diagnosed with malignancies typical for adolescence and young adulthood.

- To compare the cumulative first-time parenthood probability between adult-onset cancer patients at the age of 35 years with that found for individuals with similar age and gender from the general population.

- To estimate the risk of adverse pregnancy outcomes, including congenital anomalies, in cancer survivors compared to the figures in individuals with similar age and gender from the general population.

6. Material and Methods

6.1 Paper I

Data for the review-article were obtained using The PubMed database, searching for articles published from 1985 to September 2005. Only articles written in English were reviewed. Reference lists of relevant articles were checked for additional publications of interest. Search terms included “neoplasms” and “male infertility”.

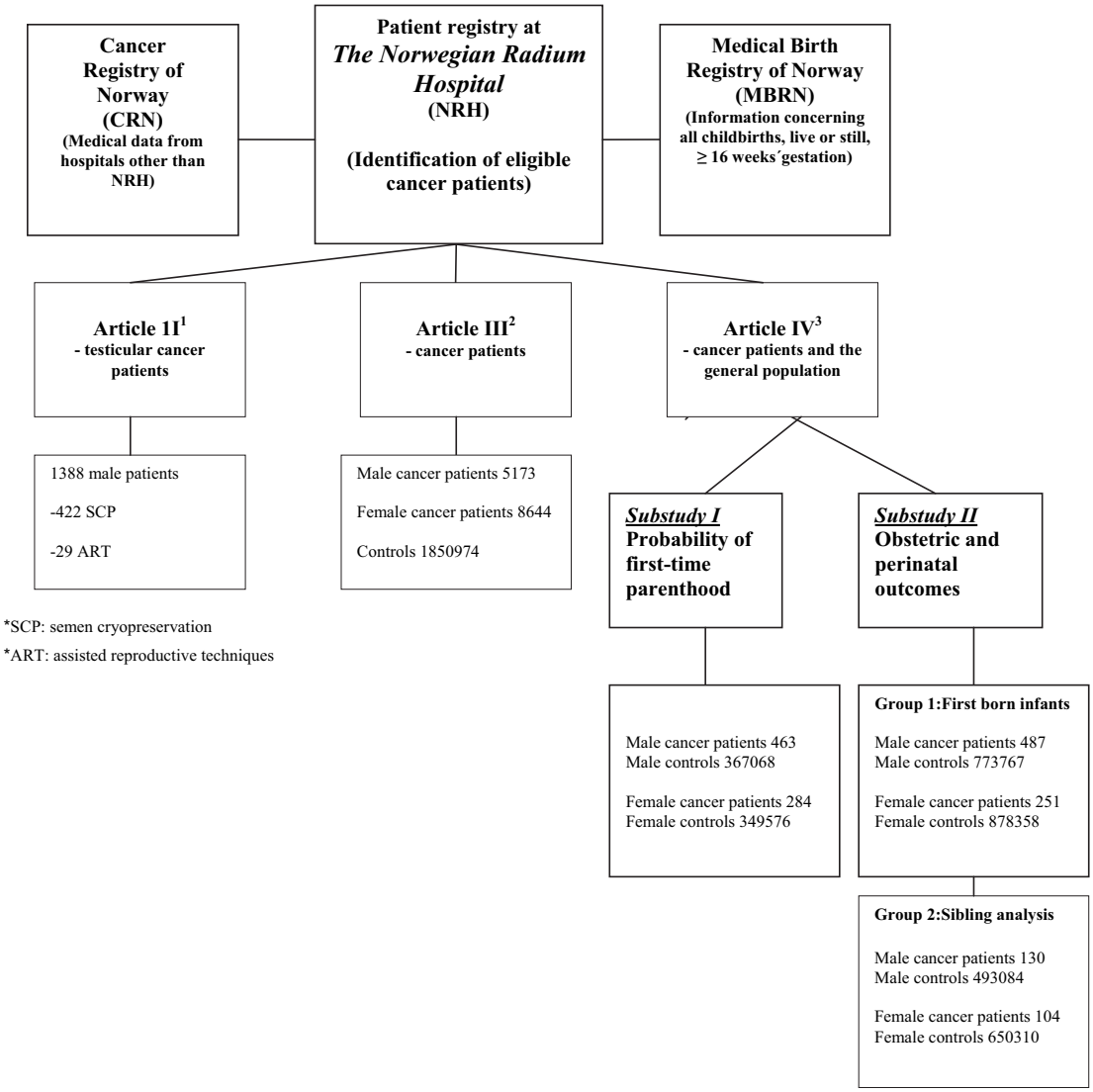
6.2 Principal sources of information as to the original articles (Paper II-IV)

The Cancer Registry of Norway (CRN): Reporting a cancer diagnosis to this registry has been compulsory in Norway since 1953, recording date and type of diagnosis, initial extent of the disease (localised, regional, distant), histology, date of death and initial treatment (except hormone treatment). No information is recorded on recurrence and its treatment. For Paper III-IV we traced the records of the CRN for information on medical data not available in the records from the NRH.

The Medical Birth Registry of Norway (MBRN): Since its start in 1967, this population-based registry collects, with compulsory notification, information on all childbirths in Norway, live or still, of at least 16 weeks’ gestation. Registered information on each childbirth includes demographic data of the parents, their previous reproductive history, use of IVF (registered since 1988), maternal health before and during pregnancy, complications and interventions during delivery and the results of the medical examination of the new-born (including congenital anomalies). Since 1999 the Registry also receives notification from neonatal intensive care units for infants transferred to such units after birth. All records are routinely matched with the Norwegian Population Registry where national identification numbers are provided, and for information on infant deaths. Individuals born before 1967 without parenthood in this year or later are not registered in the MBRN, whereas all individuals born in Norway in 1967 or later, are covered by the Registry. Individuals born in 1967 or later, thus registered in the MBRN, can be followed for their own reproduction.

Patient registry of the NRH: The NRH functions as a referral hospital for patients needing multidisciplinary oncological treatment. An electronic patient registry contains information on each patient’s cancer diagnosis if hospitalised since 1971, including treatment given during the hospitalisation(s) at the NRH. In addition, treatment given prior to the first referral is recorded in broad terms; surgery, radiotherapy, cytostatics and hormone treatment.

Figure 2: Flow chart; Patient selection for Paper II-IV



*SCP: semen cryopreservation

*ART: assisted reproductive techniques

[1] Article II: Twenty Years Experience with Semen Cryopreservation in Testicular Cancer Patients: Who needs It?
Eligibility criteria for cases, all registered in the hospital's database of testicular cancer patients: invasive testicular cancer, referred to NRH 1983-2002, 15-50 years at time of diagnosis and referral.

[2] Article III: Parenthood in Survivors after Adulthood Cancer and Perinatal Health in Their Offspring: A preliminary Report.
Eligibility criteria for cases, all registered in the hospital's database: invasive cancer, referred to NRH 1971-1997, 15-45 years at time of diagnosis and referral.

[3] Article IV: Parenthood Probability and Pregnancy Outcome in Patients with a Cancer Diagnosis during Adolescence and Young Adulthood.
Eligibility criteria for cases, all registered in the hospital's database: invasive cancer, referred to NRH 1980-1997, 15-35 years at time of diagnosis and referral. Substudy I: born 1967-78, Substudy II: born 1945-82.

6.3 Paper II

From 1983 onwards, the routine at the hospital was to discuss fertility issues and SCP with each TC patient up to the age of 40 years (up to 1994, 55 years thereafter) if the clinician anticipated a treatment –induced infertility problem.This policy implied that SCP was not performed in TC patients with planned treatment of radiotherapy only, or those allocated to the wait & see policy. However, with the improved availability of cryopreservation after 1994, individual wishes in each patient were increasingly taken into consideration, even when planned treatment was considered not to reduce post-treatment fertility.

The medical records and the clinical database of all TC patients referred to the NRH between 1983 and 2002 were screened for information on reproduction (Figure 2). These TC patients represent almost 100% of all new patients with TC within the south-eastern part of Norway, supplemented by a small and, with time, decreasing proportion of TC patients from other geographical regions of the country. The medical records contain information on the patient´s pre-and post-diagnosis fatherhood, possible infertility problems and the use of IVF/ART, provided in part from the TC patient himself and/or in epicrises from andrological or gynaecological units (including sperm banks). Additional data on reproduction were available from two surveys/clinical examinations performed in 1988 and 2000/2002 (86;87). For the purpose of this study we selected the patients with unilateral orchiectomy less than 2 months before referral, and a palpable testicle in the contralateral scrotum. None of the eligible patients had started with radiotherapy or chemotherapy at referral to the NRH. After their post-orchiectomy treatment at the NRH, the TC patients are generally followed for 5-10 years at the NRH´s out-patient clinic. The study period was subdivided into five intervals representing the years of diagnosis: 1983 to 1986, 1987 to1990, 1991 to 1994, 1995 to 1998 and 1999 to 2002.

The patients of Paper III and IV were identified by linking the three registries described above (Figure 2):

By means of the unique national identification numbers, given to each citizen in Norway, the records from the NRH patient registry were merged with data from the CRN for additional information on the cancer diagnosis, and with the MBRN for data on reproduction and pregnancy outcomes.

6.4 Paper III

Cases were selected based on the following eligibility criteria: 1) aged 15-45 years at their first cancer diagnosis treated at the NRH between 1971 and 1997, 2) histologically verified invasive malignancy, based on ICD-7 (140-207, except 189).

In order to estimate the probability of any conception *after* cancer, only childbirths 9 months or more after the cancer diagnosis were counted, without consideration of the cancer survivor´s pre-diagnosis parenthood. We estimated these post-diagnosis parenthood probabilities as to gender, age groups, previous parenthood, year of treatment and different cancer types (ML, malignant melanoma, choriocancinoma, TC, others).When evaluating perinatal outcome, all deliveries after a parent's malignant diagnosis, independent on the length of the post-diagnostic interval were studied and compared with results from deliveries reported for all other individuals in the MBRN. The cut-off date for registered childbirths was Januar 1 st, 1999.

In this paper no comparison with the general population was done for parenthood probability, and sibling-relations in perinatal outcomes were not considered.

6.5 Paper IV

Data from the MBRN were updated and were available for the observation period from January 1, 1967 through June 30, 2004 (cut-off date of the study).

We conducted two independent substudies in cancer patients (Cases) and their controls, identified in the general population. Cases had to fulfill all of the following eligibility criteria: 1) Born from 1945 through 1982, 2) 15-35 years old at the time of diagnosis of their first invasive cancer (ICD-7: 140-206, except 189), 3) Referral to the NRH from 1980 throughout 1997. For each substudy, appropriate control groups were provided by the MBRN.

1. *Substudy I: First-time parenthood probability.*

Among the individuals born from 1945 to 1982, we selected all individuals born in Norway from 1967 to 1978, and followed them for their first childbirth (yes/no) until June 2004. This selection was done since only individuals born after 1967 would be registered in the MBRN even if they did not reproduce during the observation period. Within this population we identified our cases, which had to fullfill criteria 2 and 3.

The term *"first-time parenthood probability"* takes into account the individuals´ first childbirth (≥ 16 weeks gestation) during the observation period, without considering whether the pregnancy was initiated prior or after the cancer diagnosis. For three of the most frequent cancer types (ML, TC and gynaecological cancer) we also calculated the "*post-diagnosis parenthood probability"* defined as first-time parenthood after cancer in patients who were childless when their malignancy was diagnosed. Four categories of treatment (surgery alone, radiotherapy (+/- surgery), chemotherapy (+/- surgery) and radiotherapy & chemotherapy (+/- surgery)) were defined, based on the summarised treatment information available in the hospital registry and the CRN ("overall treatment").

2. Substudy II: Obstetric and perinatal outcomes including congenital anomalies.

By means of the national identification numbers, the births in the MBRN were linked to their mothers or fathers, all born between 1945 and 1982. Sibship files were established with the mother or father as the observation unit. Among cancer patients we constructed two separate groups and performed two separate analyses: Individuals in Group1 were childless at the time of the malignant diagnosis and had at least one post-diagnosis pregnancy, for which obstetric and perinatal outcomes were compared with first births in the general population (Controls1). In these analyses we compared all first childbirths in cancer patients with all first childbirths in the general population, independent of the number of children born later on. Cases in Group2 had one birth prior to and at least one after the cancer diagnosis, and we compared obstetric and perinatal outcomes for first- (pre-diagnosis) and second- (post-diagnosis) born siblings with outcomes for first- and second-born siblings in the general population (Controls2) (sibling analysis).

LBW was birth weight < 2500 g, and delivery before 37 completed weeks of gestation was recorded as preterm delivery. Perinatal death was defined as stillbirth (after 16 weeks gestation) or early neonatal death occurring within the first 7 days of life. All congenital anomalies were grouped together as one entity. The term IVF (excluding births following hormone manipulation only) did not discriminate the different types of ART. Maternal age, maternal education, time period and, when appropriate, paternal age and interbirth interval, were evaluated as possible confounders for the association between a cancer diagnosis and the different perinatal outcomes in the post-diagnosis births.

7. Statistical analyses

In Paper II, using SPSS 11.5, associations between categorical variables were assessed using Chi-square tests, and t-tests were used for testing differences between groups with continuous variables. “Post-orchiectomy fatherhood rates” during the observation period were estimated using the Kaplan-Meier method assessing inter-group differences with log rank tests. Cox regression analyses identified factors associated with post-treatment fatherhood. The cut-off date for all observations in the present report was Aug 31, 2003. The observation period was counted from the date of orchiectomy until the date of first post-orchiectomy fatherhood, the patient’s death or the cut-off date of Aug, 31, 2003, whichever occurred first.

In Paper III, common descriptive measures were used together with the Kaplan Meier procedure to estimate the probability of parenthood. Patients were censored at death or emigration or, for surviving patients, on January, 1st 1999, whichever occurred first. Differences between curves were evaluated by the log-rank test. Pregnancy outcomes were measured with categorical variables; we calculated crude odds ratios (OR) with 95% confidence intervals (CI). We used logistic regression analyses to adjust for confounders (maternal age, parity, year of childbirth).

In Paper IV standard descriptive methods were applied using SPSS for Windows version 12.

Substudy I: The cumulative parenthood probability was assessed by the Kaplan Meier procedure with log rank tests evaluating differences, the event being the first time childbirth recorded in the MBRN. For *first-time parenthood probabilty* the observation time started at the mother’s/father’s date of birth. The observation time for *post-diagnosis parenthood probability* started at the date of the malignant diagnosis. All persons were followed to the date of death or emigration, first childbirth or June 30, 2004, whichever occurred first.

Substudy II: Chi-square and logistic regression analyses were used to compare perinatal outcomes recorded in the interval from 1967 to June 2004 in cancer patients and controls (Odds ratios: OR). Logistic regression was used to adjust for mother’s age, educational level and time period, and when appropriate, for father’s age and time interval between the two recorded births (Adjusted Odds ratios: ORadj). Birth weight and preterm delivery were analysed in singletons only. Maternal (and paternal) age was modelled as a categorical

variable and grouped as <20 years, 20-24, 25-29, 30-34, 35+ years. Data on parents' education were categorized as low (<=10 years), medium (11-13 years), and high (14+ years). Time trends were analysed by grouping year of first birth in five-year categories from 1967 (last category 1997-2004). The interbirth interval was the number of years between the first and the second birth and was modelled as a continuous variable.

The level of significance was set at $p<0.05$ in all studies, and all tests were two-sided. 95% CI were calculated.

8. Main findings

8.1 Paper I

The effects of cancer and cancer treatments on male reproductive function.

Based on the literature studied, we concluded:

1) Cancer and its treatment threaten male fertility and reduce the chances of post-treatment paternity. This should be discussed with the patient prior to initiating treatment for cancer, and preventive measures should be considered by multi-disciplinary co-operation with oncologists, urologists, andrologists and those specializing in reproductive medicine.

2) The application of fertility-saving treatment is the first option to prevent post-treatment infertility in males together with pre-treatment SCP.

3) Pre-treatment SCP should be offered to all young adult males with ongoing spermatogenesis in whom cancer treatment involves a risk of subsequent infertility.

4) Post-treatment male infertility can in some cases be circumvented by IVF using frozen semen, oligospermic fresh semen or sperm cells collected by TESE/MESA.

5) With developing technology in mind, today's experimental techniques of harvesting gonadal tissue may be considered in young males, though unrealistic expectations for future fertility should be avoided.

8.2 Paper II

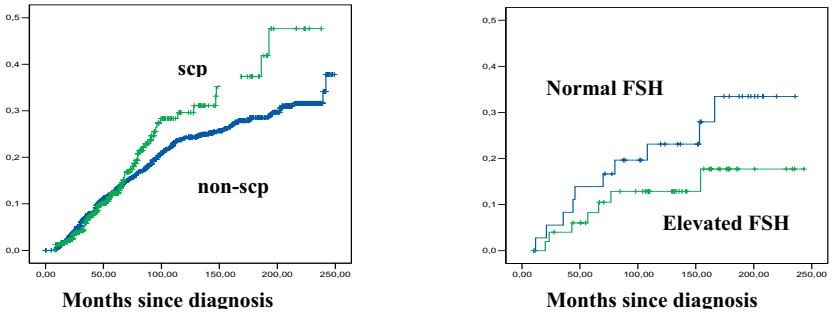
Twenty years experience with semen cryopreservation in testicular cancer patients: who needs it?

Among 1388 eligible, newly diagnosed TC patients, 422 (30%) had pre-treatment SCP. During the study period of 20 years, an increasing percentage of patients had pre-treatment SCP, reaching 43% after 1994. Patients who had SCP were on average four years younger than those without, they more often had non-seminoma and more frequently presented with metastases. Chemotherapy with or without post-orchiectomy surgery was the most common

treatment in the patients with SCP. In 966 men SCP was not done. The reasons for omittance of SCP could be evaluated in 669; 21 men were unable to produce an ejaculate, 94 had azoospermia and the procedure was considered unnecessary or, for few cases, was not done due to immediate onset of treatment in 554 men.

Twenty-nine (7%) of the 422 patients with SCP had used their frozen semen for ART at least once to achieve fatherhood. Pregnancies were achieved in 16 of these patients' partners, but two of these pregnancies ended in abortions. Two of the patients trying ART without succsess have later fathered children without the use of frozen semen. A total of 67(17%) of 393 men with SCP fathered at least one child without use of frozen semen. The comparable figures for those without SCP were 205 out of 966(21%).

Twenty years after orchiectomy, the cumulative incidence of first post-treatment fatherhood was 47% for the 393 patients who had SCP but did not use it for ART, and 34% for the 966 patients without SCP (p=0.12) (Figure 3a). Including all evaluable patients (without using frozen semen), treatment (high dose chemotherapy more detoriating), age (higher age more detoriating) and serum FSH (elevated FSH levels [>12U/l] more detoriating) were the strongest indicators of post-treatment fatherhood. Azoospermia at the time of diagnosis did not exclude the probability of post-treatment fatherhood. After 15 years, the probability of post-treatment fatherhood was 34% in patients with azoospermia and normal FSH at the time of diagnosis, compared to 18% in those with azoospermia and elevated FSH (p=0.18) (Figure 3b).



Figur 3a and 3b

Probability of natural post-treatment parenthood in survivors of testicular cancer

a) with (scp) or without (non-scp) semen cryopreservation

b) with azoospermia and normal (Normal FSH) or elevated serum FSH (Elevated FSH) at time of diagnosis

8.3 Paper III

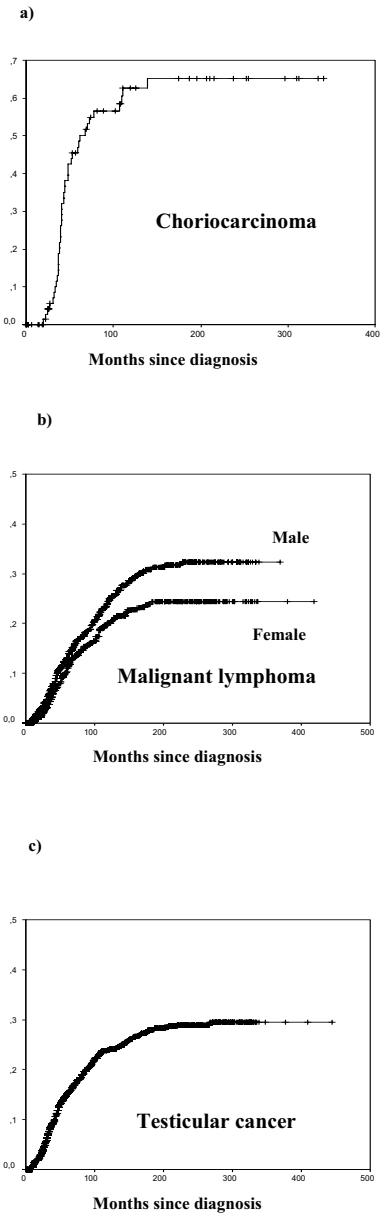
Parenthood in survivors after adulthood cancer and perinatal health in their offspring: a preliminary report.

Among the 13 817 eligible patients, a total of 5183 males (37%) and 8644 females (63%) were identified. TC (36%) and ML (21%) dominated among the male cancer patients and cervical cancer (29%) and breast cancer (23%) among the females. At the time of diagnosis 60% of the male cancer patients were 30 years or above, the comparable figure being 81% in the female cancer patients. Fourty-nine percent of the males and 62% of the females had children prior to their malignant diagnosis.

Post-diagnosis parenthood: Independent of pre-treatment parenthood, a total of 1531 patients had 2307 children after the diagnosis, 972 males had 1479 children, and 559 females had 828. A total of 1217 patients (784 males and 433 females) had a child ≥9 months after the diagnosis (1899 childbirths, 1221 among male cancer patients and 678 among female patients). Almost 90% of these 1217 patients had 1 or 2 children after the diagnosis, and one TC patient had 7 children born after his treatment. Post-diagnosis parenthood was significantly more frequent among males than among females ($p<0.01$).

For all patients, the post-diagnosis parenthood probability was 8% at 5 years and 14% at 10 years without major increase thereafter. Favourable results were seen in patients <30 years at diagnosis, in patients childless at diagnosis and in males compared to females. The overall 10-year post-diagnosis parenthood probability for male cancer patients was 23% compared to 8% in females. However, women with uterine choriocarcinoma displayed the highest 10-year probability of post-diagnosis parenthood (64%), followed by patients with ML (males 28%, females 22%) and TC (27%) (Figures 4 a-c).

Figure 4



Figures 4a, 4b and 4c (Figures from the original article (Paper III)):

Probability of post-diagnosis parenthood in a) Patients diagnosed with choriocarcinoma b) Males and females diagnosed with malignant lymphoma c) Patients diagnosed with testicular cancer

Pregnancy outcome: The mean age at post-diagnosis delivery for female cancer survivors was 29.8 years, whereas mean age for male cancer survivors at the time of post-diagnosis birth was 32.9 years. This was significantly higher than the mean age at childbirth in the control population (mean maternal age 26.8 years and mean paternal age 30.4 years). Among the post-diagnosis pregnancies in our patients, 36% were first pregnancies, 39% were second pregnancies and 25% represented higher parities.

Compared to the general population, after excluding multiple births, female cancer survivors gave birth to post-diagnosis infants with on average 130 grams lower birth weight and 6 days shorter gestation (mean). Infants fathered by male cancer survivors did not differ from the controls with respect to birth weight or gestational age. There was no increase in the prevalence of congenital anomalies in the offspring of cancer survivors as compared to offspring in the general population. Multiple births and deliveries by caesarean sections were increased among cancer patients.

8.4 Paper IV

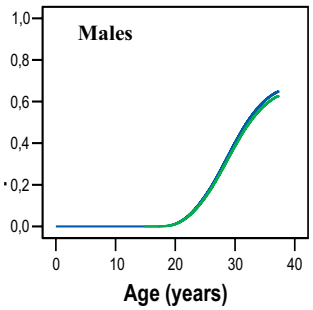
Parenthood probability and pregnancy outcome in patients with a cancer diagnosis during adolescence and young adulthood.

8.4.1 First-time parenthood probability: **(**Substudy I) The substudy population consisted of 463 male and 284 female cancer patients and 367,068 males and 349,576 females from the general population. TC, ML and gynaecological cancer were the most frequent malignant diagnoses.

A total of 142 males and 85 females had their first child after the malignant diagnosis. The male cancer patients had a mean of 1.62 children (both before and after diagnosis) compared to 1.72 among male controls (p = 0.08). The comparable figures for female cancer patients were 1.76 children versus 1.92 among controls (p = 0.02). At the end of the observation time the male cancer patients´ cumulative *first-time parenthood probability* was 63 % (95% CI: 56-70) versus 64% (95% CI: 63.7-64.3) among controls (p = 0.41) (Figure 5a). The *first-time parenthood probability* among female cancer patients was 66% (95% CI: 59-73), compared to 79% (95% CI: 78.8-79.2) among the controls (p = 0.007) (Figure 5b).

There was no statistically significant difference between the most frequent cancer types as to 10- year *first-time post-diagnosis parenthood probability* in patients who were childless at diagnosis (ML (41%) versus TC (42%) in males (p=0.47), ML (44%) versus gynaecological cancer (33%) in females (p=0.30)), however with few events in some of the groups. Patients with localised or regional disease at diagnosis displayed a 44% 10 year post-diagnosis parenthood probability compared to 34% in those with distant metastases (p = 0.047), without differences between overall treatment modalities.

Figure 5
a)



b)

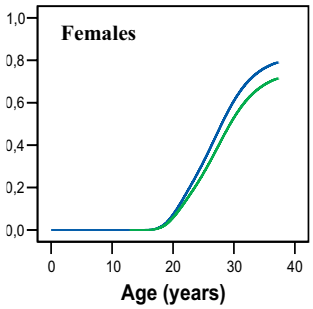


Figure 5a and 5b (Figures from the original article (Paper IV)):
Probability of overall first parenthood in individuals born 1967-1978

a) in male cancer patients (cases; green) and the male general population (controls; blue)

b) in female cancer patients (cases; green) and the female general population (controls; blue)

8.4.2 Perinatal outcome: (Substudy II) A total of 487 male and 251 female cancer patients, childless at diagnosis, achieved at least one post-diagnosis pregnancy (Group1), whereas 130 males and 104 females were identified as having parented one child before and at least one child after the cancer diagnosis (Group2).

Successful IVF was used by 39 of the males from Group1 (8%) compared to 0.8% in the Controls1 (Adjusted odds ratio (ORadj) 5.3; 95% CI: 3.8-7.5). Among the male cancer patients, no significant association was found between the children with congenital anomalies and the use of IVF (2/39 vs 25/448, p = 1.0, Fisher´s exact test). Twenty-seven post-diagnosis first-born infants (5.5%) of previously childless male cancer patients had congenital

anomalies, compared to 3.2% among first births to males in the general population (OR 1.8; 95%CI: 1.2-2.6; ORadj 1.5; 95% CI: 1.1-2.3). These 27 post-cancer infants were fathered by patients with TC (n=12); ML (n=10) and others (n=5). Anomalies in the musculoskeletal system (limbs, hands and feet) were diagnosed in 21 of the infants (skeletal deformities: 12; hip joint dysplasia: 8: anomaly of m.sterno-cleidomastoideus: 1). In four children, three of them fathered by survivors after TC, anomalies of the genito-urinary tract were reported. Overall treatment of the 27 fathers had consisted of surgery only in 4, radiotherapy (+/- surgery) in 9, chemotherapy (+/- surgery) in 6 and radiotherapy & chemotherapy (+/-surgery) in 8. The 27 children were born after a median of 54 months after diagnosis (range: 2-244).

No increased risk of congenital anomalies was observed among post-diagnosis infants to female cancer patients. However, perinatal mortality was significantly increased in first births to females who were childless at diagnosis (7 perinatal deaths [2.8% versus 1.4 % in the general population] [ORadj 2.3; 95% CI: 1.1-5.0]). Further, there was a significantly increased risk of LBW and preterm delivery in these births: 10% of first-born singletons delivered by female cancer patients displayed LBW compared to 5% in the general population (ORadj 2.1; 95% CI: 1.4-3.2), and 15% were delivered preterm compared to 7% in the general population (ORadj 2.2; 95% CI: 1.3-3.6).

The sibling analysis (Group2) further revealed that the risk of LBW was 4-fold increased in the post-diagnosis second birth to females who had one child before diagnosis, whereas the risk of LBW in their first birth did not differ significantly from first-born infants in the general population. Contrary to the well known parity effect in birth weight from first to second births observed in the general population (increasing weight) (88), a reduction in mean birth weight was found for the second relative the first sibling among female cancer patients from Group2 (Figure 6). This reduction in mean birth weight was even more pronounced after adjustment for the time interval between the births. Second siblings (post-diagnosis) in Group2 were also significantly more often than the Controls2 delivered preterm (ORadj: 3.1; 95% CI: 1.8-5.5), whereas this was not so for the first (pre-diagnosis) siblings.

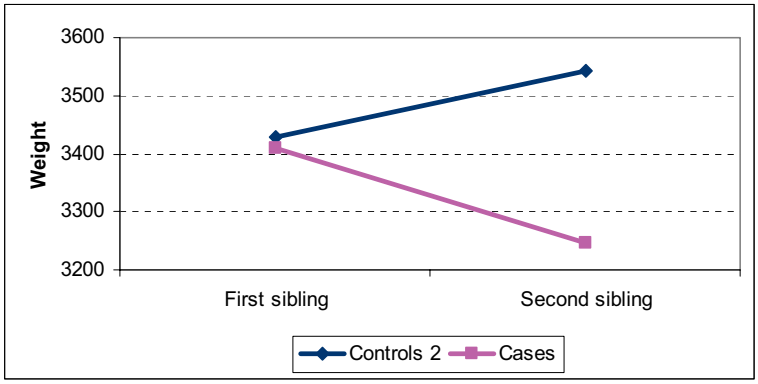


Figure 6:

Mean birth weight (grams) in first and second sibling (Figure from the original article (Paper IV))

Cases: female cancer patients with first sibling born prior to diagnosis and the secons sibling born post-diagnosis

Controls: the female general population

9. Discussion

9.1 Methodological considerations

9.1.1 Reviews

Generally, reviews may be grouped into the following two categories (http://ph.cochrane.org/en/authors.html):

1) Systematic reviews (with or without meta-analysis)

2) Traditional literature reviews/narrative reviews

Systematic reviews are much driven from the methods developed by the Cochrane Collaboration (evidence-based medicine). The scope of systematic reviews is identified in advance (review question/sub-questions) and comprehensive search is used to identify all relevant studies. Systematic reviews use explicit criteria to include/exclude studies, apply established standards for critical appraisal of study quality and are based on explicit methods of extracting and synthesising study findings (http://ph.cochrane.org/en/authors.html). Systematic reviews have many advantages: they reduce bias, are replicable, resolve controversy between conflicting findings and provide reliable basis for decision making.
A meta-analysis is the statistical combination of results from studies. The final estimate of a meta-analysis may not always be the result of a systematic review, and it should therefore not be considered as a type of review (http://ph.cochrane.org/en/authors.html).

Traditional literature reviews/narrative reviews describe and appraise previous work, but do not describe specific methods by which the reviewed studies were identified, selected and evaluated (http://ph.cochrane.org/en/authors.html). They give an overview with discussions and critiques of previous work and visualise the current gaps in knowledge. They are often used as rationale for new research. However, the biases that occur in selecting and assessing the literature are unknown. Traditional literature reviews cannot be replicated (http://ph.cochrane.org/en/authors.html).

Paper I is a traditional literature review.

9.1.2 Epidemiologic studies

Epidemiologic studies are based on measures of disease frequency and measures of effect (89). The simplest studies aim only at estimating a single risk, incidence rate or prevalence. More complicated studies aim at comparing measures of disease occurrence, with the goal of predicting such occurrence, learning about the causes of disease, or evaluating the impact of disease on a population (89).

9.1.2.1 Cohort studies

A cohort is defined most broadly as ”any designated group of individuals who are followed or traced over a period of time” (89). A cohort study, which is the archetype of all epidemiologic studies, involves measuring the occurrence of an event during a given time within one or more cohorts. Typically, a cohort comprises persons with a common characteristic, such as an exposure. Paper II, III and IV are true cohort studies (retrospective cohorts). The size of the cohorts, the completeness of data from the registries (the NRH, the CRN and the MBRN), the high quality of the linkage with unique national identification numbers, and the low losses to follow-up caused by emigration, add credibility to our results.

In Paper II, we measured the reproduction prevalence after natural conception or ART in patients with TC with or without SCP. In both Paper III and IV, we measured parenthood probabilities in cancer patients, as to different cancer types and different demographic variables, and in Paper IV these results were compared with those in the general population. In Paper III, we estimated the prevalence of LBW, preterm birth, congenital anomalies, caesarean sections, multiple births and perinatal losses in offspring to cancer patients compared with offspring in the general population. In Paper IV, we also estimated the prevalence of IVF, LBW, preterm births, congenital anomalies and perinatal loss in children born both before and after one of the parents`cancer diagnosis compared to children with the same birth orders in the general population.

9.1.3 Precision/Variability/Random error

Random error is variability in the data that we cannot readily explain. One possibility of error is that the observed assosciation in any population is due the role of chance (89). If a study is large, the estimation process would be comparatively precise and there would be little random error. With a large sample size, the variability will become smaller and the inference more reliable, and, thus, the estimate more likely to reflect the total population (89).

The registration in the MBRN of all births above 16 weeks of gestation is mandatory, so the records from MBRN used in the study included all registered births in the relevant time - period. Much effort is made to keep a high quality of the collected data both by manual and computer quality controls. The role of random error can thus be evaluated as neglible.

The source for selecting cases is the hospital registry at the NRH. For the vast majority of analyses, the numbers were large enough to give precise estimates. However, for some of the subgroup analyses, the sample sizes were small, and we cannot exclude the possibility of type II errors.

In Paper II-IV we used simple descriptive methods, analyses of time to event and regression analyses, the latter with adjustment for confounding factors. Especially in Paper IV, comparisons were made between relatively few cases and large numbers of controls which may have resulted in statistical differences which may have less clinical relevance. It is also important to remember that statistical significance should not be viewed as a clear-cut ”yes” or ”no” estimate, but merely a guide to action.

9.1.4 Validity

Validity is divided into internal (the degree to which the result of an observation studied is representative for the particular group studied) and external (the degree to which the results apply to other populations).

9.1.4.1 Internal validity

To estimate the internal validity, one has to consider possible bias which may influence the result. It is helpful to classify bias into three broad categories: selection bias, information bias and confounding.

9.1.4.1.1 Selection of patients/Selection bias

Selection bias results from procedures used to select the subjects of the study population. Selection bias occurs when the association between exposure and outcome differs for those who participate and those who do not participate (89).

The registration of births in the MBRN is mandatory. The entire popualtion thus participates, and there is no selection of births into the registry.

Paper II - IV are, however, based on patients treated at the NRH from 1971-1997, necessitating a discussion of selection bias. In the 1970ies, patients from almost the whole country were referred to this hospital if they needed radiotherapy, accepted as a corner stone of curative treatment applied for ie. HL, TC, cervical cancers and most loco-regionally confirmed breast cancers. Patients undergoing surgery only (gastro-intestinal cancer) and/or chemotherapy only (leukaemia) were not treated at the NRH and are therefore not included in our cohort.

From 1980 onwards, other university hospitals in the country established their own oncological units with radiotherapy-machines, and became responsible for the treatment of the patients from their geographical area. The NRH remained responsible for the curative therapy of cancer patients with the above diagnoses in individuals living in the southern part of Norway. The largest subgroups of our patients comprise patients with TC and ML, in particularly HL, in the age group 15-44 years. From 1980 onwards surgery performed at the NRH increasingly became a component of cancer therapies. Fertility saving treatment modalities have been introduced early in the 1980ies (unilateral lymph node dissection, shielding of gonads, use of sperm banks, unilateral oophorectomy) probably some years ahead of the introduction of similar strategies at other hospitals in the country.

These referral and treatment policies have most probably introduced a selection bias to our patients, which should not be neglected when interpretating our relatively favourable results of in particularly post-diagnosis paternity.

9.1.4.1.2 Information bias

Information bias results from errors in the classification of subjects. Its consequences differ depending on whether the classification error on one axis of classification (exposure or outcome) is independent (non-differential misclassification) or not (differential misclassification) of the classification on the other axis (90).

There may be sources of information bias in the present study, associated with the quality of the data registered. Registration errors are inevitable in large regtistries such as the MBRN,

the hospital registry at the NRH, and the sperm banks, and although quality control is done regularly, some errors will remain.

Some variables should be mentioned specifically:
Perinatal mortality: Perinatal mortality is one of the health indicators monitored by the MBRN. For the early neonatal deaths, the national identification number ensures double registration: both in the MBRN directly and via the civil registration from the Norwegian Population Registry. This provides an additional opportunity for controlling the data. Stillbirths are not allocated a national identification number by the Population Registry, and data on these births are based solely on registration in the MBRN. The quality of the data is dependent on the gestational age of the stillbirths, and under-reporting of the youngest stillbirths is acknowledged (91). The reporting of the youngest, smallest infants has, however, become more complete from around the 1980ies. Although not very likely, we cannot totally exclude the possibility that notification of stillbirths to the MBRN may depend on the present or previous health status of the mother (and possibly also the father). This could thus represent a possible differential misclassification bias.

Congenital anomalies: Diagnoses of congenital anomalies are made during the infant´s stay at the maternity ward, and registered directly in the MBRN notification form. In general, it is found that birth defects are underreported, especially minor birth defects, and especially in the earliest period of the MBRN. Some congenital anomalies are not visable at birth, and especially congenital heart defects are to some extent lacking in the registry. After 1998, congenital anomalies are also notified from neonatal intensive care units for infants transferred to such units after birth. This has supposedly resulted in better ascertainment of several congenital anomalies: for instance, the prevalence of registered congenital hearth defects in the MBRN increased significantly from 1998 to 1999 (MBRN, 2007, personal communication). When analysing congenital anomalies as an outcome, it is therefore important to evaluate the year of birth (time period) as a source of bias. Further, it might be that a more thorough examination is performed after the birth of infants to cancer patients than of infants to healthy parents in the general population. This could thus represent a source of differential misclassification bias

IVF: Since 1988, information about the use of IVF has been registered in the MBRN for pregnancies lasting more than 16 weeks of gestation. Underreporting of this information may

also be present, and again, it is possible that this is dependent on parent's previous cancer diagnosis, with (most likely) less underreporting for cancer patients than for healty parents in the general population.

Gestational age: Due to the problems connected to both menstrual dates and ultrasound measurements as basis for measuring the length of gestation, the measurement of "true" gestational age is problematic, and misclassifiacation of gestational age may be a source of information bias. The largest problem quantitatively involves misclassifiacation of preterm births. In addition 5-6% of the infants have missing gestational age registrations. It is, however, not very likely that this misclassification is dependent on whether the parents have been treated for cancer or not. Thus, the misclassification is most likely non-differential, which usually leads to an attenuation of the effect measures.

Birth weight: The quality of the registered birth weight is considered to be good. Only a very small number of births are registered with weights that are obviously erroneous, and there are few missing values.

We also lack information as to the number of attempted pregnancies as well as spontaneous abortions < 12 weeks, and the patients´psychosocial and economical concerns influencing fertility could not be measured. Further, we do not have DNA analyses and only assume that the registered father is the true biological father. Data suggest, however, that the previously much-quoted nonbiological paternity rate of about 10% is exaggerated, and really is about 1-2% (92-94). Whether this frequency is different among cancer survivors is not known. Finally, the information on treatment remains superficial, even in the hospital registry, in particular with respect to treatments applied at other hospitals.

9.1.4.1.3 Confounding

Confounding results from a mixing of effects, and is present when the estimate of the effect of the exposure of interest is distorted because it is mixed with the effect of an extraneous factor (89).

The variables consistently evaluated as potential confounders in the present work were parents' age, education (socio-oconomic level), inter-birth interval (in the sibling analyses) and time period (year of birth). All these variables were adjusted for in the logistic regression

analyses in Paper IV. Maternal age and time period are known to be associated with a variety of pregnancy outcomes and exposures. Among the most stable findings in perinatal epidemiology are the associations between socio-economic level and key pregnancy outcomes, as growth restriction, preterm delivery and perinatal mortality (95;96). Education is the dimension of socio-economic level that is most strongly and consistently associated with health among women and their children (96). The above mentioned factors could also be associated with being a cancer patient/cancer survivor. Paper IV also evaluates the inter-birth interval as a potential confounding variable, as the main focus was on relations within sibships, where time period between births is a central factor (97). With the use of sibship files (Paper IV) we could indirectly use the pre-diagnosis births as "controls" for unmeasured confounding by comparing effect measures for first and second siblings born to parents who had a cancer diagnosis between these births.

9.1.4.2 External validity

External validity refers to whether the results and conclusions in a study may be generalised to other populations than the study population (89).

SCP is free of cost for the patients in our country, making it advantageous compared to other Western countries (USA). Studies have shown that the cost-issue seldom is the reason for limited use of SCP and ART, even in the USA (98), making our results valid for Western populations outside the Norwegian.

The MBRN is a compulsery population based notification system which makes our results as to the general population applicable for the whole Norwegian population. The general conclusion about siblng associations in birth weight and gestational age are probably partly due to biological factors also reported in other countries and ethnical groups than the Norwegian.

The question remains open whether young adult cancer patients treated at the NRH are representative for young adult cancer survivors in the Norwegian population. At the NRH the issue concerning fertility of clinical practice and research has been an important field for at least 2.5 decades, probably more and earlier than in other Norwegian hospitals. This interest together with the early introduction of fertility-saving strategies makes the external validity of our fertility-results somewhat doubtful. Our patients were to some extent negatively selected

as they were referred to the NRH because they needed multimodal treatment including combined and intensive cytotoxic treatment. Cancer patients who could be cured by surgery only were to a large degree not treated at the NRH. The reproduction probability for these latter non-NRH cancer survivors may thus even be better than the results presented in Paper III and IV. A planned national study comprising all patients reported to the CRN will probably shed some light on this uncertainty, and thus indicate the external validity of our results for cancer patients aged 15-45 years at diagnosis.

9.2 Appraisal of main findings

9.2.1 Paper I

On the background of increasing survival rates in young adult cancer patients, post-diagnosis parenthood has become a growing issue of the oncological community. Cancer and male sub-/infertility may have shared etiology, which seems to be the case for men with TC (16;99). The development of a malignancy may also lead to sub-/infertility due to reduced spermatogenesis along with a progression of the malignancy. This has been discussed for patients with ML(100-102). Finally large pelvic tumours may directly destroy the function of genital organs and thus be a cause of sub-/infertility. However, cancer therapy leading to transiently or persistent primary or secondary hypogonadism is the most common physical cause of post-treatment male infertility due to:

1) Reduction of germ cell epithelium (i.e by unilateral orchiectomy).
   Generally, unilateral orchiectomy halves the amount of functioning germinative epithelium. However in TC patients this operation may remove more than 50%, as the remaining testicle often is not normally functioning (103) (due to maldescent or prior orchiectomy, is atropic or contains premalignant changes). Qualitative disturbancers in spermatogenesis is seen in the contralateral testis in 25% of males with unilateral TC (99). In such cases testicle-sparing surgery (104) should be considered in case of small tumours.

2) Ablation of spermatogenesis, at least transiently, by radiation or cytostatic drugs.

3) Reduced transport of sperm cells, either by disturbed anatomy or disturbed innervation.

The review documents that cancer therapy threatens male fertility depending on the type and intensity of treatment. However, treatment strategies have been effectively devolped which preserve or allow recovery of fertility, in connection with the most frequent cancer diagnoses in males aged 15-44 years. Nerve-sparing operations, SCP and TESE/MESA represent possible steps forward for post-treatment involuntarily infertile men. Even if the risk of post-diagnosis infertility seems low in the individual patient, he should be offered SCP if there is ongoing spermatogenesis, not at least based on psychological considerations (99). Ongoing research on in vitro maturation of sperm cells opens new perspectives for very young men, but is still in its infancy.
In addition, and beyond the scope of the present study, socio-economical aspects influence on post-diagnosis parenthood in cancer patients (105).

9.2.2 Paper II

Our findings in this large series of consecutive young and middle-aged TC patients indicate that referral to SCP has become an option for about half of the patients referred to the NRH. A new finding is that the probability of "natural" post-treatment paternity is 47% during the first two decades after the diagnosis in the SCP group compared to 34% in the patients without SCP. This is most probably explained by inter-group differences in attempts to achieve pregnancies. Finally, type of treatment for TC and either low sperm concentration or elevated serum FSH at the time of diagnosis are identified as independent predictors of reduced "natural" fertility.We confirm previous observations of infrequent use of cryopreserved semen (46;86;106), with only 7% of the patients using their frozen semen.

Schover et al(98) reported that only 25% of males aged 14-40 years when diagnosed with cancer receiving potentially gonadotoxic treatment have pre-treatment SCP in the US. There may be several reasons for low referral to SCP. Long travelling distances to the nearest sperm bank, as was the case for NRH patients before 1994, and personal expenses related to SCP and ART (in USA), are some of the non-medical reasons. Azoospermia and unjustifiable delay of treatment have been the only medical reasons for not offering SCP, but are debatable today. A few days delay of treatment have probably no unfavourable impact on the course of the disease (except emergency cases). In some patients with azoospermia, sperm cells can be obtained by TESE (107). It is the responsible clinician who has to initiate the discussion about SCP when seeing new TC patients. Our institution's scientific long-term interest in fertility in

cancer patients may be one explanation of the relatively high frequency of SCP referral in this study.

The infrequent post-treatment use of frozen semen may be due to the financial burden related to ART. In most health care services, ART attempts have to be paid for by the patient himself, at least in part. The main reason for lack of use of cryopreserved semen seems, however, to be the patient's experience of "natural" post-treatment fatherhood. Of clinical interest is our observation that two men achieved "natural" paternity some years after unsuccessful ART with cryopreserved semen. We also find that one third of men presenting with azoospermia at the time of diagnosis became fathers after treatment due to recovery of spermatogenesis, particularly those presenting with normal FSH (108). Several explanations can be offered for this favourable observation. Even in presumptively healthy men, sperm counts may vary considerably related to transient physical or psychological stress factors (109). Therefore, analysing only one semen sample, as generally done in our patients, may insufficiently mirror the man´s potential spermatogenesis. Furthermore, metastatic TC may be related to a transiently reduced spermatogenesis which recovers if the patient is rendered tumour-free. Finally, depending on treatment-type and intensity and time since diagnosis, the post-diagnosis recovery of spermatogenesis after cytotoxic therapy may vary, as shown for survivors of TC and HL (92;110;111).

Not more than half of the couples undergoing ART with cryopreserved semen from TC patients have become parents, often after multiple attempts. This experience is in agrement with other reports in TC patients (46). Increased experience and technical developments of ART will certainly improve the rate of pregnancies with postthaw semen. Furthermore, as better sperm counts are generally demonstrated before orchiectomy (21;46), SCP should preferably more often be offered before this operation (21).

As expected, treatment strongly determined the probability of post-treatment paternity. In contrast to other findings (112), chemotherapy seems to be more deteriorating than radiotherapy in our series. This may be due to the fact that the NRH always has tried to apply optimal testicular protection during abdominal radiation (32;113). The cumulative dose of chemotherapy is important. Pont et al (114) have shown that spermatogenesis almost always recovers after two cycles of chemotherapy. Our limited number of patients receiving two cycles of chemotherapy in this series did not allow a detailed analysis of this subgroup. On the

other hand, patients who had chemotherapy at cumulative doses above 850 mg had the lowest probability of post-treatment paternity. In this group, the chemotherapy effect could, however, not be separated from the importance of dry ejaculation resulting from extensive post-chemotherapy RPLND. Having at least two children before orchiectomy reduced the post-treatment paternity rate, most probably due to the couple's decision not to have more children. Elevated serum FSH or low sperm concentration also remained significant predictors of low probability of post-treatment fatherhood. Our results clearly indicate that at least patients with elevated serum FSH or low sperm counts at time of diagnosis, and in those where cytotoxic treatment and/or extensive RPLND is planned, should have SCP if they consider future paternity.

The strength of our study is its large number of patients with and without SCP observed during more than 20 years. Limitations are the lack of information on the number of attempts of post-treatment paternity, on abortions and on the number of patients who became fathers by ART using fresh post-treatment semen.

9.2.3 Paper III

Paper III provides the preliminary results of our analyses on fertility (without comparison with the general population) and obstetric and perinatal outcomes including congenital anomalies (though without considering sibship relations). The main discussion will be performed in the section of Paper IV.

We found that the cumulative 10 year probability of post-cancer parenthood was 14% for both genders combined, respectively 23% in males and 8% in females, depending on the different diagnoses and demographic variables. In these analyses we considered any post-diagnosis first childbirth as an event independent on the pre-diagnosis parities. Gender and type of cancer and age at diagnosis influenced the probabilities, and our results also indicated that pre-diagnosis childless patients had a higher post-diagnosis parenthood probability than those who had at least one child prior to diagnosis.

After a cancer diagnosis, the chance of fatherhood significantly exceeded that of motherhood, but the female cancer survivors´chances of having a child significantly increased after 1980. In addition, females with choriocarcinoma displayed the highest 10 year probability of post-

diagnosis parenthood (64%). Several explanations exist for these findings. In patients with gestational trophoblastic tumours (choriocarcinoma), it has been known for a long time that treatment with anti-metabolites does not lead to permanent ovarian failure(115). In addition, these women represent a selected group who we know want to have children.Young women with the most frequent other gynaecological malignancies still represent a major challenge for the oncologist: Except for choriocarcinoma, germ cell ovarian cancer and early cases of invasive cancer of the ovaries and cervix where fertility-saving procedures are possible (116;117), treatment for most cases of gynaecological cancer consists of hystero-oophorectomy (117;118). In addition, the female cancer patients were older than the male cancer patients at diagnosis, and more often had children prior to their cancer diagnosis, thus reducing both the chance of and the desire for post-diagnosis parenthood.

The study design used in Paper III demonstrated an increased risk of LBW children, preterm deliveries and caesarean sections in female cancer survivors. No significant increase of congenital anomalies or perinatal deaths among offspring to cancer patients compared to offspring in the general population was demonstrated. Multiple births were observed more frequently in post-diagnosis offspring to both male and female cancer survivors, but particularly if the father was a cancer survivor.

The combinations of psychological and obstetric considerations have probably led to the high frequency of caesarean sections in singletons to female cancer survivors. The increased frequency of multiple births may be related to the use of ART, but this is not analysed in this paper. The risks of LBW children and preterm deliveries can be related to both the malignancy itself and its treatment, such as scattered radiation from abdominal radiotherapy (3;7;8;37).

The increase of preterm delivery, together with the increased proportion of multiple births, indicates that pregnancies in cancer survivors require a particularly high level of obstetric and perinatal surveillance and care.
This preliminary study has several limitations; 1) Specific treatment modalities have not been taken into account in this preliminary report. 2) The study design did not consider any sibling-relations in birth weight, obstetric or perinatal complications or congenital anomalies (119;120). 3) No direct comparison with the general population was done when estimating parenthood probabilities.

9.2.4 Paper IV

At the age of 35 years, the probability of first-time paternity in male cancer survivors is approximately 60%, with similar figures among males from the general population. For the majority, these figures represent natural conception without the use of ART. First-time motherhood probability in females aged 35 years is significantly reduced compared to females in the general population (66% vs 79%). Of the first-time childbirths, 26% of the children to male cancer patients and 43 % of infants to females were born prior to the cancer diagnosis. Compared to the situation in the general population, pregnancies and childbirths in women with a prior cancer diagnosis are associated with increased risks of LBW, preterm delivery and perinatal death. Male cases, childless at diagnosis, display an increased risk of congenital anomalies in their first post-diagnosis offspring.

Several explanations can be provided to explain our observations of first-time parenthood probabilities in 35 year old cancer patients, in particular the surprisingly favourable probability in males. Most of our patients had been treated from 1985-90 and onwards when fertility-saving treatments had been introduced at the NRH for TC and ML (39;87;121), the most frequent cancer types in young adulthood in men. The introduction of ABVD chemotherapy and the reduced use of pelvic radiotherapy after 1980 are probably the main reasons for this development in survivors after HL (39;111;122). Recovery of spermatogenesis usually occurs after ABVD chemotherapy for HL and after cisplatin-containing combination chemotherapy in patients with TC, thus allowing fatherhood in most patients with retained antegrade ejaculation (123;124). These therapeutic strategies have contributed to the relatively high post-diagnosis parenthood probability in male patients with these malignancies.The introduction of pre-treatment sperm banking (86) along with improvement of ART overcome major infertility problems in permanently infertile men, though only few patients use their frozen semen for ART (92;110;125;126). Our reduced parenthood probability in female cancer patients must also be viewed on the background of the limited technical possibilities of ART among females (127;128). Though cryopreservation of fertilised oocytes is possible and is in clinical use in many hospitals, cryopreservation of non-fertilised oocytes and ovarian tissue was still experimental at the time of this study (129;130). Further, the possibility exists that additional young females who were childless at the end of our observation period may become pregnant with longer follow-up, thus reducing the demonstrated difference. On the other hand, the possibility of future parenthood also exists for female controls childless at the cut-off date of our study, as well as for male controls

that might have been too young to have attempted reproduction at the end of our study period. The latter point may be one explanation for the favourable result demonstrated in male cancer patients.

Previous reports (2;12;65-69;71;72;131) have not been able to prove an increased risk of congenital anomalies in cancer survivors` offspring, in spite of post-treatment chromatin alterations in sperm cells (132). It is known that defective paternal genome can be transmitted to the offspring (133;134), and increased aneuploidy in sperm samples after radiotherapy and chemotherapy has been shown (135;136). ART allows fertilisation with semen of poor quality, and this has raised concern about the long-term consequences of testicular damage caused by radio-and/or chemotherapy. A study evaluating 96 TC patients found that radiation induced an increase in the number of sperm with DNA damage lasting for at least 1-2 years post-therapy, whereas more than two cycles of chemotherapy reduced the proportion of sperms with impaired DNA integrity, possibly caused by elimination of spermatozoa with DNA-damage by apoptosis (134). The study also indicated that the use of cryopreserved sperm does not constitute an increased risk of transmitting damaged DNA compared with ICSI candidates without TC (134).

The question about the risk of congenital anomalies is, however, still one of the most frequent issues raised by cancer patients who plan post-treatment parenthood, and when discussing congenital anomalies, the impact of ART should be considered. Our finding of an increased risk of congenital anomalies in the post-diagnosis first-born offspring of male cancer patients is unexpected. More cancer patients than controls used IVF to achieve parenthood, but in contrast to a recent study (74), we could not demonstrate any associations between ART and adverse outcomes in pregnancy in our admittedly small group of cancer patients.

The congenital anomalies reported in our study were observed after all types of treatment, and in some patients, in infants born 15-20 years after the cancer diagnosis. Due to limited numbers, in-depth analyses could not be performed for elucidation of etiological relations. Both the treatment and an inherent genetic instability in the male cancer survivors should be considered as possible etiological factors for these congenital anomalies. We can not, however, exclude the possibility of a diagnostic bias influencing our results, with the possibility of more thorough examination of offspring to cancer survivors than of offspring to healthy parents. Also, ascertainment of congenital anomalies has improved during the time

period of the MBRN, especially after 1998, when notification from neonatal care units was introduced. However, the increased odds remained statistically significant when adjusting for time period, as well as for maternal age, paternal age and education.

In line with previous observations (7;8;14), pregnancies in female cancer patients were high-risk pregnancies ending with an increased percentage of preterm childbirths, LBW and, for first-born post-cancer births, perinatal loss. The findings of a decreased birth weight after the mother's cancer became even more evident when the mean weight of the second sibling, born as the first post-diagnosis child, was compared with the weight of the older sibling and with second siblings in the general population: the acknowledged parity effect in birth weight from the first to the second birth (in general around 150 grams´ increase) was lacking in offspring to females who had one child before and one after their cancer diagnosis (Figure 6). Rather, there was a decrease in the second sibling´s mean birth weight relatve the first. Neither LBW nor preterm delivery were significantly more frequent than in the general population for the pre-diagnosis births (first siblings), providing an indirect control for confounding by unmeasured socio-economic variables that tend to be stable between births. Post-diagnosis preterm birth and LBW children may in part be due to the altered anatomy after abdomino-pelvic surgery and due to fibrotic changes in the irradiated myometrium (3;37).

We consider it advantageous that we were able to analyse the first- time reproduction rate in all consecutive patients referred to a tertiary cancer centre for malignancies which are typical for young adulthood. The comparison with the general population is also a strength. In addition, by using sibship files, by comparing the effect measures for first and second siblings born to parents who had a cancer diagnosis between these births we could indirectly use the pre-diagnosis births as "controls" for unmeasured confounding. Finally, most of our patients in Paper IV have been treated from 1985-90 and onwards, thus reflecting modern treatment policies.

The study has several limitations: Post-treatment fertility after cancer may be decreased due to the malignant process itself, as suggested for TC (16), its treatment or, not at least due to psychosocial and economical concerns (105). This registry-based study can only address selected demographic and medical variables, and specific treatments could not be taken into account. Further, we did not have any information on the number and timing of attempts of post-treatment parenthood. Some of our groups were in addition too small to rule out type II

errors. Finally, half of our patients and controls were only 35 years or younger in 2004, the cut-off date of our observation time, and some individuals childless at that time may become parents during prolonged follow-up.

10. Conclusions

- Cancer and its treatment threaten male fertility and may reduce the chances of post-treatment paternity (Paper I).

- The application of fertility-saving treatment is the first option together with pre-treatment SCP for prevention of post-treatment infertility. With developing technology in mind, today's experimental techniques of harvesting gonadal tissue may be considered in young males, though unrealistic expectations for future fertility should be avoided (Paper I).

- About 50% of the young and middle-aged patients newly diagnosed with TC are interested in pre-treatment SCP if offered (Paper II).

- For some survivors of TC, ART with cryopreserved sperm offers the only chance of post-treatment paternity, but less than 10% use their frozen semen for ART, probably due to the experience of "natural" paternity (Paper II).

- First-time post-diagnosis parenthood probabilities vary with gender, age and type of diagnosis, being highest for young male cancer survivors. Survivors of choriocarcinoma, ML or TC display more favorable 10-year post-diagnosis parenthood probabilities (25-65%) than other malignant diagnoses (Paper III and IV).

- Male cancer survivors aged 35 years have an approximately 60% probability of overall first-time parenthood, similar to the general population. The comparable figure is 66% in female cancer survivors, significantly reduced compared to 79% in the general population (Paper IV).

- Pregnancies in women with a prior cancer diagnosis have a 2-3-fold increased risk of LBW children and preterm births, and an increased use of cesarian sections. These pregnancies should be considered as high-risk pregnancies.There might be increased risk of congenital anomalies in the first post-diagnosis infant fathered by male cancer

survivors and increased perinatal mortality in first births to female cancer survivors, but this needs confirmation in larger series (Paper III and IV).

11. Future perspectives

The present study warrants further clinical and epidemiological research addressing the following items:

- Reproduction after cancer should be studied in a national cohort thus avoiding the selection bias associated with the present investigation. Nordic cooperation dealing with the issue of reproduction and cancer would result in further improvement.

- More and larger studies are needed to confirm or reject our findings of increased risk of perinatal loss and congenital anomalies in post-diagnosis offspring to cancer patients.

- Our results emphasize the need of fertility-saving treatment, especially in young females diagnosed with cancer. In addition, more research is needed regarding new technology on cryopreservation and assisted reproductive techniques in both males and females. Safety aspects regarding both transplantation of cryopreserved tissue and the methods used for fertilization need future investigation.

- The present study deals with patients aged 15-45 years at diagnosis. National cohort studies regarding fertility and reproduction in survivors of childhood cancer should be performed, as the prepubertal gonads may respond different to cytotoxic treatment from what is the case in post-pubertal cancer patients.

- There is uncertainty regarding *health risks* for a female diagnosed with cancer during pregnancy or if she has a child after a cancer diagnosis. Relevant data from the Nordic population-based registries should be analysed with cancer-specific mortality or obstetric complications as the endpoints.

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13. Errata

The following errors have escaped corrections in proof:

Paper II: Page 781: Table 1 should read "≤ 850 mg" instead of "? 850 mg."
Page 783: Table 2 should read "≥ 5 mill/ml" instead of "? 5 mill/ml."
Page 783: First column, first paragraph, line 11, should read "Each of 12 men achieved one pregnancy" instead of "Each of 12 men had one child." (There were 3 set of twins and one set of triplets).

Paper IV: Page182: Figure 2, title should read: Substudy I: "First-time parenthood probability", not "Probability of first-time parenthood probability."
Page184: Table V, Female controls, perinatal mortality should read n= 12676, not n= 28182.