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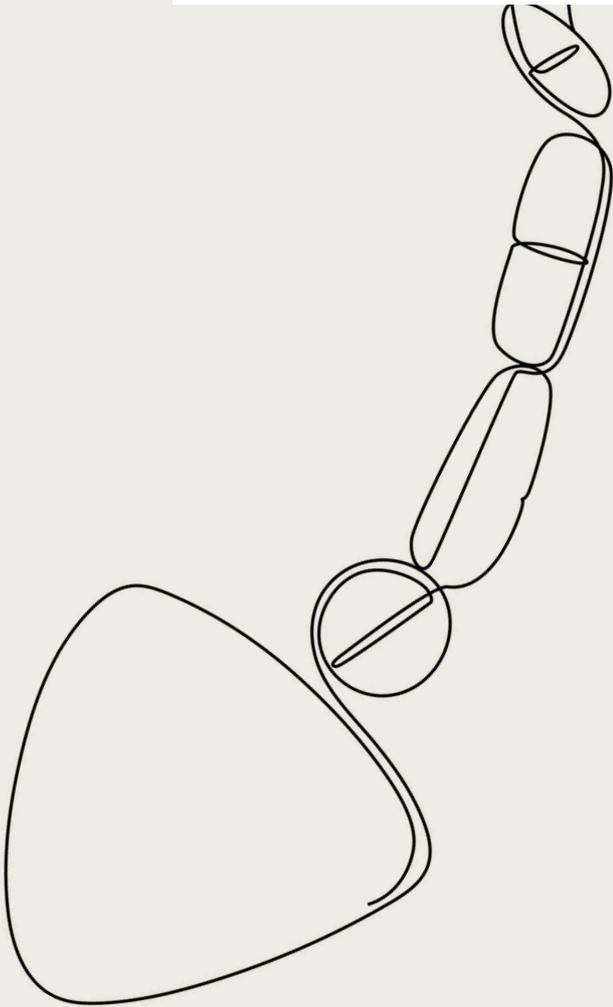
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The health of the children in relation to paternal age, cancer, and medication

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DEPARTMENT OF TRANSLATIONAL MEDICINE | LUND UNIVERSITY



The health of the children in relation to paternal age, cancer, and medication

Yahia Al-Jebari



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DOCTORAL DISSERTATION

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To be defended at Kvinnoklinikens Aula, Jan Waldenströms gata 47, Malmö.

Friday 22nd of January 2021, at 13:00

Owing to the governmental prohibition of public gatherings to limit COVID-19 spread, the public can only partake in the defence via the Zoom broadcast

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Faculty opponent

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Cancer Registry of Norway

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Abstract Even though half of a child's genome is inherited from the father, little is known about the effects of paternal disease and medications on offspring. There are concerns and anticancer therapies, due to their mutagenicity, lead to congenitally malformed children. Similarly, the effects of disease, such as cancer, paternal age, and paternally consumed prescribed drugs remain to be studied and might detriment offspring perinatal health. In this thesis, I aim to study the malformation risk associated with chemo- and radiotherapy, with cancer per se, to investigate preconception prescribed drug consumption and links with offspring preterm birth, and how paternal age affects adverse perinatal health risks. To achieve those aims, a large Swedish register database was utilized, with excerpts from the Medical Birth Registry, Swedish Cancer Registry, Swedish and Norwegian Testicular Cancer Group data, and Swedish Prescribed Drug Registry (in tandem with IBM MarketScan Research Database), among others. The studies included around 2M children born in Sweden between 1994 and 2014, and their parents. I found that the offspring that were conceived prior to paternal cancer diagnosis had a statistically significantly increased risk of being born with a congenital malformation (odds ratio (OR) = 1.08, 95% CI=1.01-1.15, P=0.02, 3.8% vs 3.4%), as compared to offspring without paternal cancer. When studying fathers with testicular germ cell cancer (TGCC), I found no statistically significant increased risk of birth defects for being conceived after radio- or chemotherapy, as compared to before those therapies. However, the children fathered by men with TGCC had a statistically significantly increased risk of birth defects, as compared to offspring without paternal TGCC diagnosis (OR=1.28, 95%CI=1.19–1.38, p= 0.001, 4.4% versus 3.5%). I studied 688 paternally prescribed preconception medications and of those 31 were statistically significantly ($p < 7.3 \cdot 10^{-5}$) associated with offspring preterm birth. These medications clustered in groups by similar chemical classifications such as cardiovascular drugs and analgesics. Advanced aged fathers (≥ 55 years) had a statistically significantly increased risk of all evaluated adverse birth outcomes as compared to reference (25-34 years). Infants fathered by older men had an increased risk of being preterm (OR=1.22, 95% CI=1.11-1.34), having low birth weight (OR=1.29, 95% CI=1.15-1.44), being small for gestational age (OR=1.24, 95% CI=1.15-1.34), and having low Apgar (OR=1.08, 95% CI=0.91-1.26). Further, children born to older fathers (≥ 45 years) had an increased risk of childhood all-cause mortality (HR=1.31, 95% CI=1.15-1.49). In conclusion, paternal factors as age, disease, and medications have large effects on the health of children.			
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The health of the children in relation to paternal age, cancer, and medication

Yahia Al-Jebari



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*I called the doctor "My wife is going into labour!
What should I do?"*

"Is this her first child?" he asked.

"No, this is her husband."

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- I. **Al-Jebari Y**, Rylander L, Ståhl O, et al. Risk of Congenital Malformations in Children Born Before Paternal Cancer. *JNCI Cancer Spectr* 2018;2. doi:10.1093/jncics/pky027
- II. **Al-Jebari Y**, Glimelius I, Berglund Nord C, et al. Cancer therapy and risk of congenital malformations in children fathered by men treated for testicular germ-cell cancer: A nationwide register study. *PLOS Med* 2019;16:e1002816. doi:10.1371/journal.pmed.1002816
- III. **Yahia Al-Jebari**, Chiyuan A. Zhang, Shufeng Li, Aleksander Giwercman, Michael L. Eisenberg. Paternal preconception prescribed drug exposure and associations with offspring preterm birth risk: drugs-wide association study (Manuscript unpublished)
- IV. He Zhang, Aleksander Giwercman, Mårten Härning-Nilsson, **Yahia Al-Jebari**. Paternal age and risk of adverse perinatal outcomes and childhood mortality: a Swedish population-based cohort study (Manuscript unpublished)

Excluded from the thesis

- V. **Al-Jebari Y**, Elenkov A, Wirestrand E, et al. Risk of prostate cancer for men fathering through assisted reproduction: Nationwide population based register study. *BMJ* 2019;366. doi:10.1136/bmj.15214
- VI. Elenkov A, **Al-Jebari Y**, Giwercman A. More Prevalent Prescription of Medicine for Hypertension and Metabolic Syndrome in Males from Couples Undergoing Intracytoplasmic Sperm Injection. *Sci Rep* 2018;8:14521. doi:10.1038/s41598-018-32813-4
- VII. Elenkov A*, **Al-Jebari Y***, Giwercman YL, et al. Testosterone replacement therapy in men who conceived with intracytoplasmic sperm injection: nationwide register study. *Eur J Endocrinol* 2020;182:423–8. doi:10.1530/EJE-19-0734 (*Equal contribution.)
- VIII. Dizeyi N, Trzybulska D, **Al-Jebari Y**, et al. Cell-based evidence regarding the role of FSH in prostate cancer. *Urol Oncol Semin Orig Investig* 2019;37:290.e1-290.e8. doi:10.1016/j.urolonc.2018.12.011

Introduction

Prologue

Around half of a child's genome is inherited from the father. Yet comparatively little is known about the effects of the paternal factors – the father's health and his medical treatments – on his children. Even for many established maternal risk factors such as advanced age and illness, chemical and pharmaceutical exposure, the analogous paternal risk is either assumed to be negligible or is unknown.

For decades, the question has been studied whether the anticancer therapies, due to their mutagenicity, have detrimental health effects on the offspring of cancer survivors. The question first arose as more sophisticated therapies came into use, with sharply increasing survival rates for many types of cancer [1–3]. As a result of this positive development, there were now many survivors of cancer, who had their cancers cured in childhood, adolescence, and adulthood. Most will want to father children even though many will struggle with infertility and fears of genetic disease caused by the harsh treatments that cured their malignancy [4].

Both chemotherapy and radiotherapy regimens lead to, dependent on type and dose, damage to the reproductive system and result in, sometimes permanent, infertility [4,5]. In mouse models, both classes of anticancer therapies, when given in doses equivalent to those given as cancer treatments, have also been shown to lead to malformed offspring [6]. And both have been shown to lead to germ cell DNA damage in humans [7–12]. Therefore, fears of increased risk of congenital malformations among offspring of men treated with anticancer therapies are warranted. There is also epidemiological evidence indicating an increased risk of birth defects for men who have had cancer and are likely to have been treated with these mutagenic treatments. However, the effects of treatment versus that of possible underlying characteristics such as the cancer itself could not be elucidated [13].

Much remains unknown regarding the effects of paternal factors on the health of offspring. If mutagenic treatments, or possibly the underlying cancer, is associated with birth defects among the offspring, then knowing which anticancer regimens or which cancer types lead to increased risks is of vital importance. Further, if the father does, independently of maternal factors, have an impact on the health of the offspring, then are there other risks imparted by the father, such as risks associated

with advanced paternal age, disease, and concomitant medical treatments? Could these paternal characteristics extend risks to other measures of perinatal health such as preterm birth? These questions are becoming more and more pertinent as the age at fatherhood is increasing year by year, with a proportional increase in the prevalence of paternal age-related disease and medicinal consumption [14].

By extension through the foetal origins of adult disease hypothesis, perinatal disease also increases the risk of later morbidities in adult life. Being born with low birth weight is now established to be associated with increased rates of coronary heart disease, strokes, hypertension, and diabetes [15,16]. The *foetal origins* of adult disease hypothesis posit that an unfavourable intrauterine environment and its constriction on foetal growth is the origin of multiple diseases later in life. However, if paternal preconception factors affect foetal development and infant health, then some disease might originate from a time before foetal development.

To delve into these questions, we first must take a step back and examine how paternal information about his disease and treatments, is transmitted to the next generation. All the genetic information that the father contributes toward the child's genetic makeup is all encoded within the one sperm that fertilized the oocyte. If the child is afflicted by, for example a birth defect attributable to the father, then that birth defect must have originated within that fertilizing sperm. So, what went wrong during that sperm's development, during spermatogenesis, that caused it to carry the elements for a birth defect?

An overview of spermatogenesis

Spermatogenesis is a specialized and complex sequence of cellular differentiation occurring within the testes that results in genetically variable haploid spermatozoa. Sperm production starts at puberty and continues throughout life with around 200 million sperm produced per day. It is a continuous process meaning that the adult testes contain all cellular stages from spermatogonial stem cells to fully differentiated spermatozoa. It takes 90 days (in humans) to go from spermatogonia to mature sperm, a process that is split in 3 roughly equally long phases [17].

The testes are composed of seminiferous tubules (tightly coiled tube-like structures). The sperm cells are produced within these tubules, in a process starting at the basement membrane (outer wall of the tubules) and continues toward the central lumen (Figure 1). In the first phase of spermatogenesis, the proliferative phase, spermatogonial stem cells (type A) located at the basement membrane start spermatogenesis by mitotically dividing. Some of the progenitor cells will remain as spermatogonial stem cells (type A), maintaining the pool of stem cells, while some (type B) undergo differentiation becoming sperms.

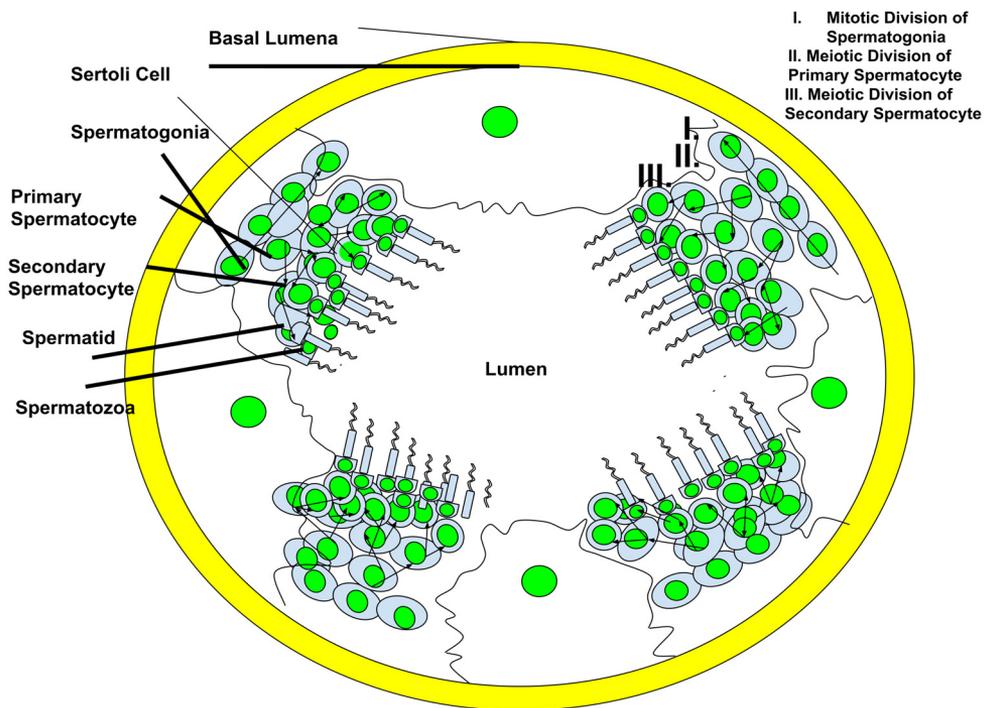


Figure 1 Schematic of germ cell development within a seminiferous tubule with cellular stages, divisions, and positions of germ cells. Image is sourced under CC BY-SA 4.0 from Jessica Atkinson, commons.wikimedia.org/w/index.php?curid=69624624

In the second phase, type B spermatogonia undergo two meiotic divisions and with only one round of DNA replication, thereby halving the diploid genome of the differentiating cells to haploid. The first meiotic division (Meiosis I) is different from Meiosis II and from previous mitotic cycles as cross-over recombination between homologous chromosome pairs takes place. This meiotic recombination, together with de novo mutations are the main sources of genetic diversity in sperms [18]. Errors in meiotic recombination, such as abnormal recombination levels or abnormal positioning of cross-overs events, have been implicated in the origin of human trisomies [19].

And in the final phase, morphological changes further mature spermatids into spermatozoa. Most of the germ cell cytoplasm is expelled together with most of the sperms cellular machinery. Within this stage, sperm DNA is packed with transition proteins in place of regular histones (Figure 2). These transition proteins are replaced by protamines at a later stage to compress the sperm DNA. This enables the sperm nucleus to be around six fold smaller than the nucleus of an interphase somatic cell [20]. The tight packaging both helps the sperms swimming ability and

protects the DNA from exogenous genotoxic factors [21]. However, sperm DNA damage can still occur during all phases of spermatogenesis.

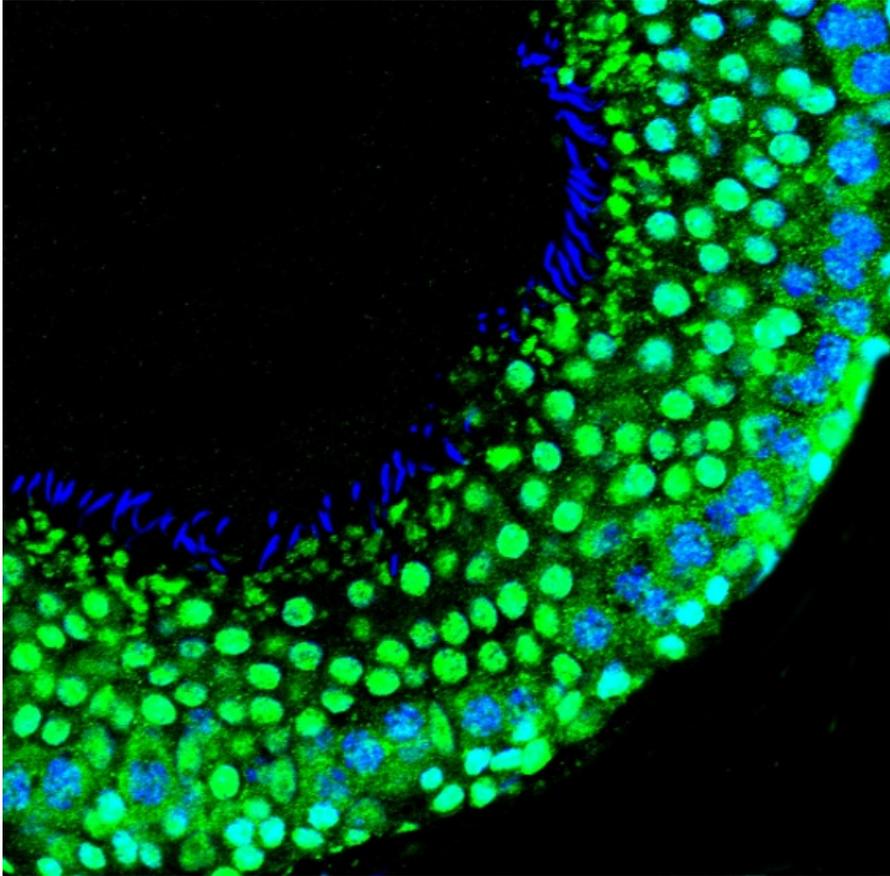


Figure 2 Seminiferous tubule section. The image shows H3K4me3 expression, an epigenetic histone modification, in seminiferous tubules of adult rat testis. The expression is seen in multiple testicular cell types: spermatogonia, spermatocytes, and spermatids. Image is sourced under CC BY-SA 4.0 from Sharvari.deshpande996, commons.wikimedia.org/w/index.php?curid=64695879

Sperm DNA damage and repair

There are multiple types of sperm DNA damage such as single strand DNA breaks, double strand DNA breaks, base modifications, abasic sites, and DNA protein cross links. These occur through three main mechanisms: through mutagens like reactive oxygen species, through aberrant sperm chromatin packaging and through abnormal apoptosis.

DNA damage is controlled through several mechanisms. There are preventative agents like detoxifying peptides and proteins, and oxyradical scavengers, for example vitamins E and C. If the DNA damage is too great in extent or severity, the damaged cell may be eliminated by spontaneous death or apoptosis. Cellular apoptosis does occur in all cell types involved in normal spermatogenesis, more so in spermatocytes and spermatids, and few in spermatogonia [22]. Testicular cells are particularly sensitive to apoptotic stimuli such as high-dose chemotherapy [23].

DNA damage in the genome can also be detected and corrected by multiple different DNA repair pathways to reduce the amount of mutations. Nevertheless, any potential health repercussions for the next generation also depends on when in the spermatogenic cycle this damage occurs.

Spermatogonial stem cell DNA damage

In the first proliferative phase of spermatogenesis, spermatogonial stem cells undergo cycles of DNA replication and mitoses. This process is continuous throughout life, and therefore any DNA degradation in one spermatogonial stem cell, if left unrepaired, could persist, and be replicated into all descendant spermatogonial stem cells and ultimately the sperms derived from those stem cells. Because spermatogonial stem cells replicate throughout life, the total number of DNA replications increases with age. Sperms of a 20 year old man has completed an estimated 160 chromosome replications, whilst sperms of a 40 year old has completed 610 chromosome replications [24]. It is therefore imperative that spermatogonial stem cells are protected against high mutation rates during the proliferative phase [25]. This especially applies to errors in the DNA synthesis step. When copying the genome, the DNA replication machinery makes 1 mistake for every 10M nucleotides, or around 600 mistakes per cell per division. Most mismatched DNA nucleotides are corrected by proofreading DNA polymerase and the process of strand directed mismatch repair. However, an estimated 0.1% of mistakes escape repair and these mutations accumulate in the genome every division [26]. Further, during this phase, mitotic cells are particularly vulnerable to double stranded DNA breaks, as DNA double-strand break repair is inactivated to prevent aberrant chromosome telomere fusion [25].

Both endogenous and exogenous genotoxic factors can cause different types of DNA damage in spermatogonial stem cells. These can cause DNA lesions as oxidative damage, mismatched bases, intra-strand crosslinks, or bulky adducts (pyrimidine dimers are generally caused by ultraviolet irradiation and therefore unlikely to be a major factor in the testes). These lesions distort the helical structure of DNA and are repaired by the nucleotide excision repair, base excision repair, and DNA mismatch repair pathways. However, knowledge about these repair pathways in spermatogonial stem cells is limited [25].

Large genomic studies have shown that most mutations among offspring are derived from the father with around 80% of de novo mutations occurring in the paternal germline. The number of mutations in offspring increases by approximately three mutations per additional year of parental ages. Further, the rate of de novo mutations that are passed on to offspring differs by more than 2-fold between fathers. This suggests that there is variation among men in the turnover rate of spermatogenic stem cells or in the rate of mutation per cell division [27]. The latter could be due to a systemic genomic instability caused by genetic, environmental or lifestyle factors. Speculatively, if this genomic instability manifests as some men experiencing more mutations in their germ cells and in their somatic cells, then this genomic instability might predispose to cancer among the fathers and genetic disease among their children.

These de novo mutations, that some fathers seem to pass on to their offspring to a higher extent, do have health implications for the offspring. They have been linked to congenital malformations, schizophrenia, and autism [28–30].

Spermatocyte DNA damage

While spermatogonial stem cells are sensitive to DNA breaks, spermatocytes induce DNA breaks as part of homologous recombination. Homologous recombination repair is an error free DNA repair pathway that is activated during homologous recombination, and is used as a powerful DNA repair pathway in other cell stages and cell types [31,32]. As homologous recombination repair is active during homologous recombination, spermatocytes are likely more resilient to DNA double breaks and other forms of DNA damage. There is, however, DNA damage that can occur during this stage. If the DNA damage repair machinery is overburdened by DNA damage, some might be left unrepaired. Further, if the homologous repair pathway malfunctions or is prematurely stopped, DNA breaks can be left in the spermatocyte [33,34].

Although spermatocytes do have efficient DNA repair machinery, it has been shown in mouse models that melphalan (a bifunctional alkylating agent used in chemotherapy) exposure induces DNA damage during meiosis. These DNA lesions persist unrepaired as the spermatocytes progress through spermatogenic development. The same study showed that this type of genetic damage can have profound detrimental impacts of the next generation as these unrepaired sperms DNA lesions can, upon fertilization, be faultily repaired into chromosomal structural aberrations in the zygote (Figure 3) [35]. Chromosomal structural aberrations could manifest as a wide variety of genetic disease, including congenital malformations.

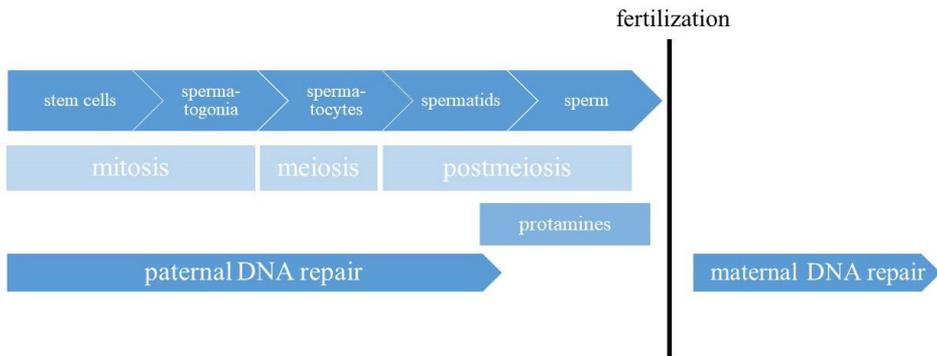


Figure 3 Schematic showing the presence of DNA repair during mitosis and meiosis, and the lack of it in mature sperms.

Besides homologous recombination repair, there is also evidence that mismatch repair plays an active role during meiosis, in repairing mismatched nucleotides when comparing non-sister chromatids. Mismatch repair is also active in meiotic chromosome pairing and recombination, suggesting that impediments to mismatch repair could manifest in chromosomal aberrations [36].

Any transient exposure causing DNA damage in spermatocytes or the subsequent spermatogenic differentiation steps is limited to only damaging those cells already committed to sperm differentiation. This means that a transient mutagenic exposure, by for example chemotherapy, can only lead to damaged sperm DNA during a limited time. This also applies to sperm DNA damage occurring in following spermatozoal maturation stages. For this reason, that there might be a danger of transient genetic disease, men are often recommended to try to avoid fathering children in the 6 months post anticancer therapies [37].

Post-meiotic germ cell DNA damage

As spermatids develop into mature spermatozoa, they become transcriptionally silent, meaning they do not have any DNA repair machinery. Thus, any sperm DNA damage that occurs during this phase of sperm development will be transmitted to the zygote. One of the main reasons for sperm DNA to be densely packed with protamines instead of histones is to protect the sperm DNA from damage within the hostile environment of the female genital tract [38], but conceivably also protect from damage during the long phase of sperm maturation. This makes sources of spermatozoa DNA damage especially hazardous as it might have detrimental health effects on the offspring by directly transmitting defective DNA.

Sperm DNA damage can also occur in the latter stages of spermatid maturation. Reactive oxygen species, irradiation and chemical, mutagens have all been shown to damage post-meiotic sperm DNA [38,39]. For instance, reactive oxygen species (highly chemically reactive compounds containing oxygen) can cause oxidative damage in the DNA of spermatozoa. This creates base adducts called 8-hydroxy 2'-oxoguanine and can cause base transversion mutations [40]. In somatic cells these lesions are repaired by the base excision repair pathway. 8-oxoguanine glycosylase 1 cuts the base adduct and leaves an abasic site, then the next enzyme in the pathway, apyrimidinic endonuclease 1, incises the DNA phosphate backbone preparing it for the insertion of an undamaged nucleotide. However, apyrimidinic endonuclease 1 is absent in spermatozoa meaning that the repair stops after creation of the abasic site [25,40,41]. Instead, these lesions are corrected in a round of DNA repair prior to initiation of S-phase of first mitotic division of the fertilized zygote [42]. However, if the oocyte incorrectly repairs the lesion, a mutation will be created which may have profound effects on the future health of the developing oocyte.

Similarly to how sperm DNA damage that occurred before the spermatid stage can be transmitted and mis-repaired into chromosome structural aberrations, sperm damage that occurs in the spermatid stage can likely lead to the same mechanistic pathway. Recently, it has been shown in a bovine model that irradiated sperm, with irradiation causing sperm DNA damage in a dose-response manner, leads to chaotic mosaicism in embryos. This chaotic mosaicism results in the wide range of chromosomal aberrations seen in humans, including aneuploidies, segmental changes, and abnormal ploidy states [43].

Post-fertilization

Fortunately, sperm DNA damage that is transmitted to the oocyte can be repaired by the DNA repair machinery of the zygote. Interestingly, this repair machinery, the partaking enzymes and molecular components, are produced by and modelled after the mother's genome meaning that it is the mother's ability to repair DNA that dictates the efficiency of DNA repair in this step [44–46]. So interindividual variation in *maternal* repair ability, due to environmental, lifestyle or genetic factors, might contribute to the efficiency of which paternal sperm DNA damage is repaired. This aligns with studies showing that the success of IVF treatments for men with high levels of sperm DNA damage also depends on the quality of the oocyte, as measured by the proxy serum anti-Mullerian hormone in maternal serum [47].

There is also a selection pressure occurring during pregnancy. Most aneuploid conceptuses spontaneously terminate in early pregnancy. Although 90% of aneuploid pregnancies are of maternal origin, the remainder are due to paternal factors [48,49]. Severe birth defects or foetuses with severe (non-aneuploid) genetic disease are likely to experience similar early pregnancy termination selection. Therefore, sperm

DNA damage might present clinically as infertility or repeated early pregnancy loss. It is possible that the amount/severity of sperm DNA damage affects how it manifests clinically. Very severe sperm DNA damage might rarely lead to live-born children with failure to fertilize or pregnancies failing shortly after implantation. While less severe sperm DNA damage might allow pregnancy to continue, but detrimentally affect foetal development or growth in other ways, resulting in for example low birth weight.

Embryonic mosaicism, potentially caused by sperm DNA damage, might also be associated with adverse perinatal health outcomes. Mosaicism in embryos is relatively common, afflicting 15-90% and 30-40% of cleavage and blastocysts stage embryos, respectively. In studies looking at using mosaic embryos in IVF treatments, mosaic embryos retain the ability to implant and can result in the birth of healthy offspring. However, embryonic mosaicism in IVF treatments does impact clinical outcomes such as implantation, clinical pregnancy and live-birth rates [50]. This suggests that sperm DNA damage (often seen in infertile men needing assisted reproduction) can affect prenatal development and potentially perinatal health. DNA fragmentation, a measure of DNA double-breaks, is routinely assessed in fertility clinics. The most sensitive method, the comet assay, can detect cells with more than 100 double strand breaks per cell. However, studies on irradiated sperm show that even low exposure, corresponding to far fewer than 100 DNA-breaks, can induce embryonic genomic instability [43,51]. It is also unknown how mosaic embryos correct their genetic makeup, as mosaic embryos can result in healthy offspring without evidence of mosaicism. Some suggested pathways include preferential growth of euploid cells or preferential allocation of euploid cells to the inner cell mass. It is unknown whether this process is metabolically costly to the embryo or whether it affects placental function, which might increase risk of clinical outcomes of low birth weight, preterm birth, and pre-eclampsia. It is also unknown whether any level of mosaicism persists in some individuals throughout life.

Sperm epigenetics

If this thesis was written 15 years ago, then genetics and sperm DNA damage would be the only described scientifically feasible pathway for paternal information on disease and treatments to be transmitted to offspring [52]. In recent years, more and more research on the sperm epigenome has clarified multiple epigenetic pathways for such information to be transferred, with potential effects on prenatal development and infant health [53].

There are now several established mechanisms for paternal characteristics to be transmitted to offspring that are not mediated through the encoded information in

the nucleotide sequence of DNA. These include mechanisms mediated through DNA methylation, chromatin modifications, RNAs and sperm proteins.

Sperm DNA methylation

DNA methylation is vital in governing gene expression throughout life and plays an important role in the development of germ cells. DNA methylation is involved in the establishment of the primordial germ cells, in the erasure and reestablishment of germline-specific patterns during embryonic development, and in the formation of the sex-specific patterns (imprinting) in male/female gametes [54].

In gametes, DNA methyltransferases imprint sex-specific differential DNA methylation on certain DNA segments called imprinting control regions. In the gamete, these differentially methylated regions escapes one or both rounds of reprogramming, and will be maintained in the conceptus throughout life dictating monoallelic (parent specific) expression of those genes [55]. Imprinting only pertains to a small number of genes and most are maternally imprinted [56]. Studies have shown links between abnormal sperm DNA methylation, especially in imprinting control regions, and male infertility [57]. Other studies on infertile men showed that abnormal DNA modifications were correlated to defects in sperm morphology and high sperm DNA fragmentation, suggesting that DNA fragmentation might arise together with abnormal sperm epigenetics [58]. Interestingly, an increase in imprinting disorders in children conceived through in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) has been noted, suggesting intergenerational transmittance of abnormal imprinting [59]. DNA methylation has also been shown to transmit paternal information to offspring in animal models. Male rats given altered diets conceived offspring with glucose intolerance and weakened insulin secretion [60]. Generally, environmental changes during early paternal development has a major role on germline methylation patterns [61].

Studies on humans have also demonstrated a link between paternal characteristics and epigenetic changes in the offspring. Paternal obesity was associated with low methylation of several imprinted genes important in normal embryonic growth and development, for instance *Mest* and *Peg5* [62,63].

Other exogenous factors such as toxins and lifestyle factors could also affect the sperm DNA methylation profile. In mice, exposure to vinclozolin (a pesticide) caused an increase of reactive oxygen species within the testes, which in turn has a large impact on DNA methylation. This aberrant DNA methylation profile was inherited and increased the levels of DNA mutations (copy number variations) in the descendant mice [64,65]. This suggests that some interplay between inherited genomic instability and abnormal gamete DNA methylation.

Sperm chromatin

Most histones associated with sperm DNA are replaced by protamines during the post-meiotic maturation of sperm. But some small portion, 5-10%, of histones do remain. This was initially thought to be remnants from an inefficient histone replacement process. There is now scientific consensus that the persisting histones carry paternal information that is passed to the embryo [56].

Histones also carry a multitude of post translational modifications that are involved in the extensive chromatin replacement process in spermatids. These persistent sperm histones also have post translational modifications, such as lysine acetylation, methylation, and phosphorylation. These are transmitted to the conceptus, but their effects are undetermined. Some studies have shown that promoters of gene involved in embryonic development are enriched in persisting histones [56].

Histone acetylation is important for proper chromosome separation within developing gametes. Experiments in mice which chemically inhibited histone deacetylation, lead to half of embryos being aneuploid, and likely therefore not be carried to term. This process of histone acetylation is thought to be important to why trisomy 21 increases with maternal age [66]. However, less is known regarding the epigenetics of sperm and the effects it might have on chromosomal aberrations of the offspring.

Sperm RNAs

During the maturation stage of spermatogenesis, most of the germ cell cytoplasm is expelled together with most of the sperms RNAs. However, some RNAs do remain and are transmitted to the oocyte upon fertilization [56]. Therefore, transgenerational inheritance is also speculated to be mediated through sperm-borne RNA [67]. Non-coding RNAs are involved in the regulation of epigenetic modifications in germ cells, as DNA methylation and histone post translational modifications. Their role as mediators of inheritance has been investigated, and it's been shown in mice that sperm RNAs have the potential to influence embryogenesis [68]. Using mice models, paternal obesity impair offspring glucose tolerance and induces offspring obesity, seemingly mediated through sperm microRNAs [69]. This finding that sperm RNAs can mediate inheritance was supported by further experiments injecting sperm RNAs from mice given an altered diet into a normal zygote, resulting in offspring with metabolic disorders [70].

Genetic factors

Many of the exposure – outcome mechanisms we discuss herein might allude to non-mendelian genetic mediation (like epigenetics or genetic damage), when often an orthodox genetic mechanism could be involved. An example is the relation between paternal cancer therapy exposure and birth defects among the offspring. Having received cancer therapy is a good proxy for having had cancer so the relation might be between paternal cancer itself and offspring birth defects. If a gene variant predisposes to cancer and causes disruptions to the reproductive system or causes genomic instability, then that gene variant might also predispose to birth defects. A possible example of this is the retinoblastoma gene, with defects in this gene being linked to osteogenic sarcoma, retinoblastoma and bladder cancer [71]. Men with an inactive retinoblastoma gene present with infertility, reduced DNA damage repair, as well as sperm microsatellite instability [72,73].

Similarly, parental exposures that might be thought of as environmental or linked to lifestyle factors (for instance obesity or metabolic syndrome) also have a genetic component. It is possible that some genetic traits predispose to obesity and to adverse offspring perinatal outcomes. In fact, such an association has been described for mothers, where genetically elevated maternal body mass index and systolic blood pressure was linked to higher and lower offspring birth weight, respectively. However, no (or very weak) associations were seen with paternal alleles [74].

Perturbations to spermatogenesis

Anticancer therapies

Experimental evidence

Chemotherapy and radiotherapy are used in anticancer treatment for their ability to kill cancer cells. While radiation can be targeted at tumours and specific areas of the body, chemotherapy kill fast-growing cells throughout the body. Anticancer therapies are (often by design) exceptionally toxic to cells. Anticancer therapies can damage or interfere with cellular processes. They can damage the DNA, disturb DNA replication or repair, or hinder other vital parts of the cell cycle [75]. In somatic cells, cytotoxic chemotherapeutics and radiation have been shown to cause DNA mutations, DNA breaks, DNA copy number variations and aberrant ploidies. In fact, anticancer treatments are so mutagenic that cancer survivors having been treated with these sometimes go on to develop secondary malignancies related to their treatment [76,77].

Radiotherapy and cytotoxic drugs have a large effect on the male reproductive system. Infertility, sometimes permanent, is a long-known side-effect of chemotherapy [78]. Their large detrimental impact on the reproductive system together with their proven mutagenicity leads to concerns that these treatments might cause genetic damage in germ cells, which potentially might affect the health of the offspring conceived after exposure. Particularly, genetic disease and birth defects are of concern.

Chemotherapy and radiotherapy, especially high dose treatments, can damage post-meiotic sperm DNA [35,43]. This has been detected in animal and in vitro studies. In rodent models, males exposed to mutagens before mating induces partial and full chromosomal abnormalities in the offspring [79]. Ionizing radiation induces instability of repeated DNA sequences in mice descendants, though doses given in human radiotherapies might be too low to cause instability [80,81]. In animal breeding studies looking at male rodents exposed to chemotherapeutic agents and then mated with unexposed females, a multitude of detrimental effects on reproduction and the offspring was noted. This included infertility, pregnancy loss, heritable chromosomal aberrations, malformed offspring and cancer among offspring [6,82]. In humans, transient cytogenetic damage and decreased sperm DNA integrity following exposure to chemotherapy and radiotherapy has been noted [7–12].

As sperm are non-DNA-repair-competent in final maturation stages, this damage will be transmitted to the oocyte. As this damage only affects the germ cells already committed to sperm differentiation, only sperms produced during a limited time-window can be affected. This is the basis for the recommendation to men undergoing mutagenic anticancer therapies to avoid conceiving a child in the 6 months after completion of treatment [37].

However, not all the potentially transmissible genetic damage from mutagens, such as chemotherapeutics, is transient. Spermatogonial stem cells might also be damaged by these mutagens, and incurred lesions could become permanent mutations that are propagated in the stem cell pool, and to future generations. Low-level long term exposure, which allows cells to accumulate damage without inducing apoptosis, is speculated to be particularly dangerous to sperm stem cells [35]. However, animal breeding studies have found that few chemical mutagens cause mutations in spermatogonial stem cells [83].

Epidemiological evidence

Most studies investigating health effects on the offspring of male cancer survivors that have been treated with mutagenic radio- and chemotherapies have not found increased risk of chromosomal abnormalities, genetic disease, nor of childhood cancer [84–95]. While these findings are reassuring, these studies are also limited in a multitude of ways. They often do not differentiate between those having

received mutagenic and non-mutagenic treatments, or encompass a broad range of cancers and treatments, including any number of chemotherapy cycles, surgery only treatments, or multimodal treatments. The factor that is most limiting for these studies is the small number of children included, leading to insufficient statistical power and inability to detect small risk increases, especially for rarer outcomes such as birth defects.

A 2011 study overcame the issue of low statistical power due to small numbers by using data from large Swedish and Danish national registries [13]. This was the largest study of its kind at the time. They included 1 777 765 children of which 8670 were fathered by cancer survivors. They found no association with common measures of adverse perinatal health such as preterm birth or low birth weight. However, the most important finding was that children to fathers with a history of cancer had a 17% increased risk of severe congenital malformations. This risk increase is modest, but possibly suggestive of a link between cancer therapy and birth defects. Curiously, when examining the data in depth, the evidence points to a different narrative. In this study, they did not have access to cancer treatment data for the fathers. As a proxy for cancer treatment data, different subgroups were formed based on cancer type, as specific cancers generally receive similar treatment. For example, haematological cancers are generally treated with chemotherapy, while skin cancers are generally treated solely by a surgical excision. Surprisingly, this study showed a 40% increased risk of birth defects associated with being conceived after paternal skin cancer, and no risk associated with haematological cancer. Contradictory to the original notion that anticancer therapies are linked to offspring birth defects, these results suggest that the increased risk might be due to some underlying paternal characteristic, such as the cancer itself.

Cancer

Some experimental evidence points to cancer per se affecting spermatogenesis, sperm parameters and fertility. Pre-treatment cryopreserved sperm from men having cancer shows increased sperm DNA damage [8,96–98]. This suggest that the cancer itself is influencing the reproductive system. This might be mediated through increased reactive oxygen species (from cancer, infections, or inflammation), which has been associated with aberrant spermatogenesis and infertility [99]. It is possible that preclinical stages of cancer, especially testicular cancer due to its proximity, has a detrimental effect on the genome or epigenome of spermatozoa and cause congenital malformations. Other possible mechanisms could be that some men have a genetic predisposition, lifestyle or environment that increases their risk of cancer and their risk to father children with congenital malformations. Closely related, some men might, for a variety of reasons, have higher rates of genomic instability manifesting as cancer and offspring birth defects.

Parental disease and medical treatment

At conception, a large portion of fathers to-be have some medical diagnosis and might receive medical treatment. In the US, around 4 in 10 men aged 20–59 consumed a prescribed drug during a sampling interval of 30 days [100]. A Norwegian and a Danish/Dutch study, found that 1 in 4 and 1 in 3 of fathers, respectively, consumed a prescribed drug in the time preceding conception [101,102]. While many chemical and drug exposures have been investigated on the maternal side, the potential mechanisms mediated through sperm has been relatively unexplored. Paternal environmental and occupational hazards have been studied for their effects on perinatal health [103–109], yet medical treatments that are often in much higher concentrations in the body and are known to have (on-target and off-target) physiological effects, have received little interest. Similarly, lifestyle, diet and diseases are associated with metabolic, endocrinological or immunological changes that might leave epigenetics alterations [110,111]. Though little is known regarding how disease and prescribed drugs affect the sperm epigenome, and the potential offspring perinatal health effects.

Some studies have described relationships between certain drugs and detrimental perinatal health. A study found that fathers dispensed diazepam had increased risk of offspring perinatal mortality and growth retardation [101]. Cyclosporine, mainly used for patients undergoing organ transplants, has been linked to offspring preterm birth [112]. High-dose folic acid supplementation alters sperm epigenetic profiles, with unknown effects on offspring [113]. With all the epigenetic transmission mechanisms that have been described in recent years, it is possible that other paternal factors, including disease/treatments could affect future generations.

Paternal age

Men produce sperm into old age which has led to the assumption that male fertility is maintained throughout life [114]. In recent years, a flurry of studies has shown that advanced paternal age has large detrimental consequences on fertility and offspring health. Epidemiological studies show that elderly men have lower fecundity as they take longer to impregnate their partners and they have increased risk for pregnancy loss [115–117]. Elderly fathers has also been linked to offspring preterm birth, neonatal intensive care admission and adverse maternal factors as preeclampsia [118]. Dissemination of these risks is particularly important when put in the context of the on-going trend to delay parenthood in developed countries.

The aforementioned disorders associated with advanced paternal age are believed to be linked to the increasing number of spermatogonial stem cell divisions that accrue as men age, resulting in increasing transmissible de novo mutations. This is supported by large genomic studies have shown that most mutations among offspring are derived from the father with around 80% of de novo mutations

occurring in the paternal germline with the number of mutations in offspring increasing proportionally with parental ages [27].

Although mutations occur relatively rarely in the spermatogonial stem cell population, some selfish germline mutations affect the growth characteristics of stem cell causing them to outgrow their non-mutant kin. This is believed to occur with the mutations causing Apert syndrome, achondroplasia, and Costello syndrome by de novo gain-of-function mutations in the genes FGFR2, FGFR3, and HRAS. Even though these mutations might only occur in a single spermatogonial stem cell, this stem cell clonal expansion, which likely takes place in most men, leads to the stem cell pool being enriched so that 1 in 10 000 sperms is afflicted and some cases leads to the formation of testicular tumours. The selfish gene mutation theory explains why the syndromes associated with these mutations are so common among older fathers [119].

However, accumulated de novo mutations might not alone explain the associations with offspring perinatal ill health. A recent seminal paper showed that advanced male age is linked to a wide range of changes in sperm. They found that with increasing age, sperm telomeres lengthened, sperm DNA stability deteriorated (increasing DFI to pathological levels), and saw evidence of sperm DNA methylation changes in genes involved in embryogenesis. And some of the age-dependent differentially methylated genes can potentially escape epigenetic reprogramming [120].

Aims

While the overarching aim of this thesis is to investigate the health of the children in relation to paternal age, cancer, and medication, this is achieved by the following specific aims:

- Estimate the malformation risk in new-borns conceived by men who were subsequently diagnosed with cancer
- Investigate whether antineoplastic therapy implies any increase in malformation risk in children fathered by men treated for testicular germ cell cancer
- To investigate whether testicular germ cell cancer per se is associated with risk of congenital malformations
- To screen all prescribed drugs consumed by fathers before conception for their associations with offspring preterm birth risk
- To investigate whether advanced paternal age increases the risks of infant and childhood morbidity and mortality

Methods

Data sources

Studies I-IV used a database based on excerpts from Swedish national registries and will be covered in depth. In addition, study III also used the IBM MarketScan Research Database where the most pertinent parts will be discussed.

Swedish register database

The Swedish register database that we gained access to, which the studies in this thesis are based on, contains excerpts from many national registries. Some of the excerpts were not used in any of the studies in this thesis. We will only cover those parts that were used extensively to conduct the research.

Data extraction

In collaboration with the Swedish Board of Health and Welfare, we gained access to a research database containing excerpts from national registries. The database was defined as including all children born alive in Sweden between 1994 and 2014, together with their mothers and fathers. Statistics Sweden, a Swedish governmental agency responsible for producing official statistics, was tasked by the Board of Health and Welfare to identify the cohort of subjects, and to create a cipher key between all the subjects' personal identity number (a 12 digit unique number assigned to each Swedish resident) and a deanonymized unique serial number. This allows us to work with data from multiple registries and to link data from different registries together without having access to the personal identity number, maintaining the privacy of the subjects in the database. Statistics Sweden also provided a linkage file so that the family relations of the children, fathers, and mothers, could be discerned. Statistics Sweden used the Swedish Total Population Register and the Swedish Multigenerational Register to identify the children and their parents.

For the children, data from the Swedish Medical Birth Register, the Swedish Cancer Register, the Prescribed Drug Registry, and the Cause of Death Register was obtained. For all these registries, data from 1994 to the latest data at date of extraction (2016) was obtained.

For the parents, data from the Swedish Cancer Register (founded 1958 until latest data), the Prescribed Drug Register (July 2005 until latest data), and the Cause of Death Register (1994 until latest data) was obtained. For the mothers, the data from the Swedish Medical Birth Register (1994- to latest data) was also obtained.

For the register excerpts, not all variables stored by the registers were given. Similarly, some data variables were partially redacted to further maintain subject anonymity. Some examples of this partial redaction are the parental ages which were given to a resolution of 1 year (only year of birth, without specific date). Similarly, the children's date of birth is also given to a 1-month granularity.

The Swedish Medical Birth Register

The Swedish Medical Birth Register, founded in 1973, includes data on virtually all children born within Sweden. The data is collected from prenatal, delivery, and neonatal care records from health care providers. All care and treatment given to patients during pregnancy within the framework of maternal health care is free of charge in Sweden and is offered universally. It is mandatory for every public and private health care provider to report to the Medical Birth Register.

For the studies in this thesis, perinatal data on the children was sourced from the Medical Birth Register. This included data on sex, diagnoses of congenital malformations, date of birth, gestational duration, and birth weight. Many maternal factors are recorded in the Medical Birth Register and data on those factors were used in the studies herein, mainly as covariates in adjusted statistical models. These include maternal weight and height (as measured at first prenatal maternity clinic visit), maternal smoking (self-reported during prenatal visits), maternal parity, and mode of conception (For the years 1994 - 2007. For the subsequent years data on mode of conception was collected from Q-IVF, the Swedish national quality register for assisted reproduction). Maternal body mass index (BMI) was calculated from maternal height and weight. Date of child conception was estimated by using the date of birth and subtracting the gestational duration.

No data on the father is included in the Medical Birth Register.

The Swedish Cancer Register

The Swedish Cancer Register, founded in 1958, covers the whole population in Sweden with an estimated coverage rate close to 100% [121,122]. Around 60 000 cancer diagnoses are recorded on a yearly basis in Sweden. It is mandatory for every health care provider, public and private, to report new cancer diagnoses to the Swedish Cancer Register. Every cancer diagnosis detected at clinical, morphological, autopsy, or other assessments must be reported according to Swedish law.

Study I used data on paternal cancer diagnoses to identify which children were born to fathers with cancer. The date of cancer diagnoses was compared to the date of

child conception to ascertain when the child was conceived in relation to paternal cancer diagnosis.

The Swedish and Norwegian Testicular Cancer Group Register

The Swedish and Norwegian Testicular Cancer Group (SWENOTECA), founded in 1981, is a group of Swedish and Norwegian physicians working to ensure that patients with testicular cancer receive optimal diagnoses, care, and treatments. This includes thorough management programs for staging, treatment, and follow-up of testicular germ cell cancer (TGCC). All Swedish and Norwegian health care providers treating testicular cancer partake in SWENOTECA.

While the Swedish Cancer Register contains data on essentially all cancer diagnoses in Sweden, it lacks information on what anticancer therapies the patients received. Fortunately, SWENOTECA also maintains a national quality register of all testicular cancer treatments given in Sweden (for seminomas since 2000 and non-seminomas since 1995). This data was used in Study II to identify which children were born to fathers that had a TGCC diagnosis (yes/no), received chemotherapy (yes/no and number of chemotherapy cycles), had received radiotherapy (yes/no), or had only been treated with orchiectomy only (surveillance only, therefore no potentially mutagenic treatments).

The Swedish Prescribed Drug Register

The Swedish Prescribed Drug Register was established in July 2005 and records all prescribed drugs dispensed at all pharmacies in Sweden. This includes any prescription filled, prescribed by public or private health care providers and pharmacies. Each year, more than 100 million prescriptions are recorded by the register.

In study III, paternal prescribed drug consumption data from the Swedish Prescribed Drug Register was used. For a specific list of drugs that were outputs from analyses using IBM MarketScan data, Swedish paternal prescriptions were tabulated. For each drug, the fathers that had consumed that drug were identified according to their serial number. The children's estimated date of conception was used to see whether the father's prescription had been filled within the interval of interest (0-6 months preconception).

The Swedish Cause of Death Register

The Swedish Cause of Death Register has data on all deaths registered in Sweden since 1952. It contains date of death, the main, and secondary causes of death.

Study II & III did not use death data whatsoever. Study I used death data in a sensitivity analysis (following fathers in a cancer-survival analysis). Study IV used childhood death as an outcome, where date of death and cause of death information was utilized.

The Swedish Education Register

Statistics Sweden maintains the Swedish Education Register. It contains such information as the highest attained education level and the type of education for people in Sweden. While the data given is detailed, for most studies this information was collapsed to 3 categories of education level, to be used as a covariate to adjust for socioeconomic factors. The levels were defined as ≤ 10 years, >10 – ≤ 14 years, ≤ 15 years of formal education.

Cohort and linkage

The number of children in the cohort was 2 108 569. There were 1 181 492 unique fathers and 1 192 658 unique mothers in the cohort (Figure 4). Some fathers and mothers had multiple children, so the number of unique parents was lower than the number of children.

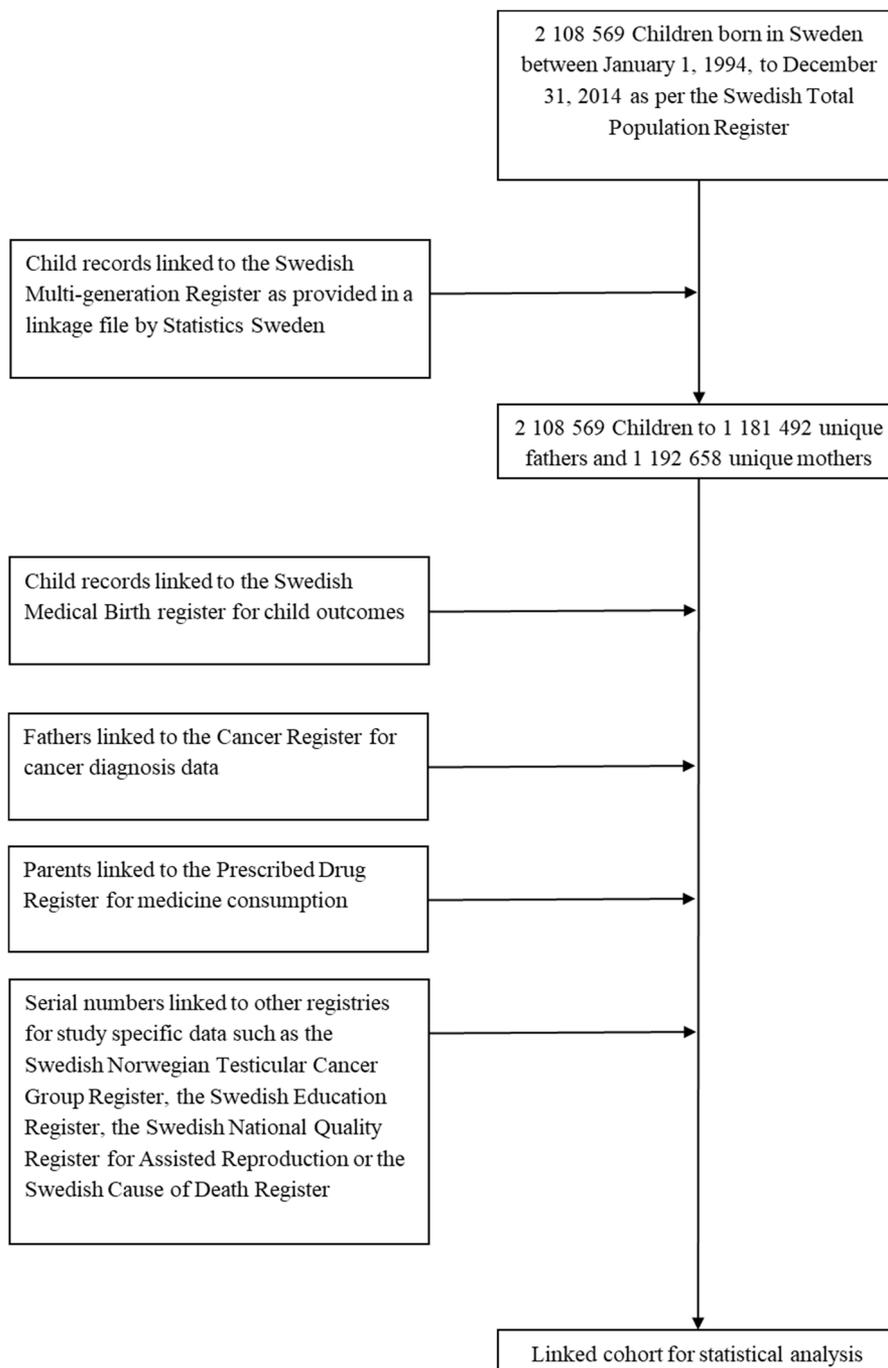


Figure 4 Generalized flowchart of the register linkage process.

For all studies in this thesis, the main statistical analyses have outcomes that pertain to the health of the children. Therefore, the final database should be structured in such a way so that each row is one unique child. However, the exposures (and covariates) in all studies pertain to the fathers (and mothers). As the same man can father multiple children in the cohort, and some exposures are dependent on the relation between when the father was exposed (pre-/post cancer diagnosis/treatment, preconception prescribed drug consumption, age at conception), attention must be given to ensure that the parental exposures are correctly estimated, and correctly merged with files containing the child outcomes. For example, adding the linkage data, which defines intergenerational kinships, to the data excerpt from the Medical Birth Register allows the identification of the fathers to the children in the Medical Birth Register. Merging information from, for example, the Cancer Register by the father's serial number (preprocessed so the file contains maximum one cancer diagnosis per father) adds information on paternal cancer diagnoses.

IBM Marketscan Research Database

The IBM Marketscan Research Database contains health care claims records for patients insured through their employers. The database includes 150 million health insurance recipients. Paper III used a previously defined cohort based on this database. The cohort was originally defined for the purpose of investigating the association of preconception paternal health, in terms of chronic disease diagnoses, and effects on perinatal outcomes [123]. We used this cohort to investigate paternal prescribed drug consumption and associations with preterm birth. As the two research questions are related, substantial work from the previous project could be used and built upon. This included the definition and assembly of the cohort, and the characterization of the outcome. Abridgedly, data from 2007 to 2016 was used. Women aged 20 to 45 years and their infants were identified from in- and out-patient records and linked to fathers. Preterm birth, and ultimately date of conception, was estimated by ICD-9, ICD 10, Diagnosis Related Group and Current Procedure Terminology diagnoses codes together with date of birth data.

By linking to pharmaceutical claims data, preconception (0-6 months) of prescribed drug data could be quantified for each parent. This allowed us to screen all drugs prescribed to fathers within the database to be investigated for associations with preterm birth.

Outcomes

Congenital malformations

There are many kinds of congenital malformations, and they vary in severity from essentially harmless to incompatible with life. Therefore, we decided to classify the congenital malformation diagnoses into severe and non-severe groups. For this purpose, we used a diagnosis guide by the European Registration of Congenital Anomalies and Twins (EUROCAT) organisation, which is a European network of population-based registries for the epidemiological surveillance of congenital anomalies. The diagnosis guide gives a list of congenital malformations in International Classification of Diseases (ICD) 10 that are considered “minor”, with the rest considered severe or “major” [124].

According to the prespecified format of the data excerpts from the Swedish Board of Health and Welfare, we obtained perinatal diagnoses that were recorded in the Swedish Medical Birth Register. These include a main diagnosis and up to four secondary diagnoses. If a child did not have any diagnosis that is considered a birth defect, as defined by diagnoses codes ICD-9-SE 740-759 and ICD-10-SE Q00-Q99, then that child’s perinatal diagnoses were redacted by the Swedish Board of Health and Welfare. This resulted in data excerpts containing diagnoses only for children with birth defects. However, those children that had congenital malformation diagnoses could have other perinatal diagnoses recorded that were not birth defects. A further complication, the children in the cohort were born over such a long time, the ICD system to describe the diagnoses switched from ICD-9-SE to ICD-10-SE within the cohort interval, meaning earlier codes are in an earlier version of the classification system.

To attain a classification for diagnoses into minor and major, first all diagnoses had to be screened to determine if they were a congenital malformation diagnosis. This could be done by checking whether the first 2 digits of the code were in the following set[Q0-Q9, 74,75]. ICD-9 codes had to first be translated to ICD-10 codes by translating each code manually (some are straight-forward while others do not have direct counterparts). Then the ICD-10 codes, and the translated ICD-9 codes could be compared to the list from EUROCAT to classify all the congenital malformation diagnoses into minor and major.

Preterm birth and gestational duration

Preterm birth is generally defined as gestational duration of less than 37 weeks. In the Swedish Medical Birth Register gestational duration is given by weeks and by days, and therefore generating a dichotomous variable denoting preterm birth is straightforward. Gestational duration itself can also be used as an outcome, as was done in paper III.

Low birth weight

Low birth weight is generally defined as less than 2500 grams. Birth weight is recorded for virtually all children in the Swedish Medical Birth Register.

Small for gestational age

Small for gestational age (SGA) is a measure to describe infants who are smaller than usual while accounting for their gestational duration. There are multiple definitions, but the most common one is being below the 10th percentile of birth weight per gestational duration (i.e. per weekly or daily interval).

Low Apgar score

The Apgar score is based on the five criteria of a newborn infant: pulse, respiration, muscle tone, irritability, and color. On each criteria a score of 0, 1, or 2 is given, with low scores denoting worse health. An Apgar score of 4 to 6 is considered moderately abnormal, and 0 to 3 is seen as low in full term infants [125].

Childhood mortality

In paper IV, childhood mortality was as an outcome and investigated in relation to paternal age. Childhood mortality was defined as death up to 5 years old.

Childhood cancer

In paper IV, childhood cancer was investigated in relation to paternal age, similarly to childhood mortality. Childhood cancer was defined as receiving any cancer diagnosis (ICD-9: 140.0-208.91) up to 5 years old.

Statistical analyses

Missing data

In paper I, missing data is handled by only analysing cases without missing data, so called full cases. This means that for every subject in each analysis, if one of the dependent (outcome) or independent (covariates) variables has a missing value, the subject will not be included in that analysis.

In paper II & IV, which overlaps in included subjects with paper I, missing data is handled by multiple imputation. Multiple imputation creates a prespecified number of imputed datasets where missing values are replaced by estimated values. The new values are estimated so that they align with the observed distribution of the known data, including amount of data uncertainty/variance. The multiple datasets are then used for statistical analyses, such as logistic regression, with the risk estimates from each dataset being pooled together for the overall risk estimate.

Logistic regression

For dichotomous outcomes (without time-dependency), logistic regression is appropriate. This means endpoints that only have two states where the time of occurrence does not affect the risk. Examples of such outcomes can be found in all papers in this thesis, such as congenital malformations in papers I & II, and preterm birth in paper III & IV, which are outcomes all present at birth. Logistic regression analyses can be unadjusted using one independent variable or adjusted for several independent (categorical or continuous) variables.

Linear regression

For continuous (or “linear”) outcomes (without a time-dependency), linear regression is appropriate. An example of an outcome analysed by linear regression is gestational duration, where gestational duration is a continuous (can take any value on a linear scale) dependent variable. Except for the type of outcome variable, the practical uses of logistic and linear regression are similar.

Survival analyses

In paper IV and as a sensitivity analyses in paper I, survival analyses were conducted. These are useful when the time taken to the outcome is important. These include Kaplan Meier and Cox regressions. Kaplan Meier curves are one of the best options to measure the proportion of subjects surviving (or analogous outcome) as

measured over time. It is also intuitive to understand graphically. However, it does not allow for adjustment for covariates. Cox regression gives risk estimates (hazard ratios) that can be adjusted for covariates. Therefore, one can combine the graphical output from a Kaplan Meier curve with the risk estimate from a Cox regression to convey the risks to the reader.

Covariate adjustment

In all 4 studies in this thesis, the main result risk estimates have all been adjusted for, depending on the study, relevant covariates. The selections of which adjustment covariates should be included in the models is mainly done by trying to determine if a covariate is confounding the measured risk of interest. One can use the following criteria to determine whether a variable should be included as it might be confounding: 1. The variable must be linked to both the exposure and the outcome. 2. The variable must be differentially distributed between the groups being compared. 3. The variable cannot be mediator in the causal mechanism between exposure and outcome [126]. Though these criteria seem clear cut, the decision to add a specific factor as a covariate can be vague. For example, in paper 1, when estimating the risk of offspring birth defects for fathers with cancer, we adjust for maternal smoking. Maternal smoking is associated with increased birth defects. But maternal smoking is not biologically linked to paternal cancer. However, one can make the convincing argument that the parents share a common social setting and it is likelier that the father smokes if the mother is a smoker. And paternal smoking, in turn, is associated with paternal cancer. See table 1 for which covariates have been included in the models.

However, this process of deciding on whether a variable is confounding and should be adjusted for is in some ways subjective, with reliance on different assumptions. Therefore, different researchers will want to adjust for different covariates. A pragmatic approach is to make several different models with less or more possible confounders, and to check and see whether adjusting for a variable has a non-negligible effect on the main risk estimate. This pragmatic approach also gives understanding of the overall structure of the data.

Table 1 study exposures, outcomes and covariates for main study analyses

	Paternal exposures	Offspring outcomes	Covariates
Study I	cancer after child conception, cancer before child conception	all malformations, major malformations	child's year of birth (five-year categories), maternal age at childbirth (five-year categories), paternal age at offspring birth (five-year categories), maternal BMI (<20, ≥20 to <25, ≥25 to <30, ≥30 to <35, ≥35 kg/m ²), maternal parity (0, 1, 2+ children), maternal smoking (nonsmoker, 1–9 cigarettes per day, ≥10 cigarettes per day, or missing data), maternal years of formal education, paternal years of formal education (≤10, >10–≤14, ≥15 or missing data).
Study II	chemotherapy, radiotherapy, testicular germ cell cancer	all malformations, major malformations	maternal age at childbirth (continuous), paternal age at offspring birth (continuous), maternal body mass index (categorical: <20, ≥20 to <25, ≥25 to <30, ≥30 to <35, ≥35 kg/m ²), maternal smoking (categorical: nonsmoker, 1–9 cigarettes per day, ≥10 cigarettes per day).
Study III	688 preconception prescribed medications	preterm birth	(univariate)
Study IV	age	preterm birth, low birth weight, small for gestational age, low Apgar score, childhood mortality	maternal age (categorical: <23, 23-29, 30-39, ≥40 years), maternal smoking (categorical: non-smokers, 1-9 cigarettes/day, 10+ cigarettes/day), maternal parity (categorical: 1, 2 and 3+), maternal education level (categorical: primary and lower secondary education, upper secondary education, university), paternal education level (same as maternal), offspring birth year

Exposures, outcomes, and covariates in secondary and sensitivity analyses are not included

A priori statistical testing

In conventional statistical inference, the null hypothesis is the default position where there is no difference between the two groups or no difference between the two observed characteristics. An alternative hypothesis that there is a difference can be proposed. If the observed distribution is unlikely to have occurred under the null hypothesis, according to a prespecified statistical significance level, then the alternative hypothesis is adopted over the null. The prespecified significance level is related to the false positive rate of the statistical test. This type of a priori hypothesis testing is not valid where one tests many factors, as the false positive rate will ensure some of those tests will be statistically significant by chance alone.

In genome wide association studies, all gene loci are tested for an association with the outcome. This multiple testing would lead to many false positives. Bonferroni correction can be used to adjust for this, where the individual test significance level

is lowered to the conventional significance level divided by the number of statistical tests. This methodology is used in paper III, allowing for all paternally consumed drugs to be tested versus offspring preterm birth risk.

Description of results

The results section has been split up into the four broad topics studied in this thesis, as based on the four papers included. Each of those topics are subdivided into:

- Study population, describing the cohort characteristics and distributions
- Main results, stating the results that most pertain the main aim of the study
- Secondary results, stating other results of the study that are of less importance than the main results, such as sensitivity analyses

Statistical software

The data handling, merging, general pre-processing, data cleaning and statistical analyses were conducted by custom Python and R scripts, in Excel sheets, and in SPSS, using a variety of versions as updates were released over the years.

Results

Paternal cancer and risk of offspring congenital malformations

Study population

In paper 1, 1 796 154 children were included as sourced from the Swedish register database. Of those children, 9926 had fathers with a cancer diagnosis prior to their conception, and 26 601 had fathers who would be diagnosed with cancer after conception. More paternal, maternal, and infant characteristics are given in Table 2

Main results

The children to men that were conceived prior to paternal cancer diagnosis had a statistically significantly increased risk of being born with a congenital malformation, as well as an increased risk of being born with a major malformation (odds ratio (OR) = 1.08, 95% CI = 1.01 to 1.15, P = 0.02, 3.8% vs 3.4%, and OR = 1.09, 95% CI = 1.01 to 1.18, P= 0.03, 2.4% vs 2.1%, respectively), as compared to those children to men who were not diagnosed with cancer.

Secondary results

Some cancer types had larger associated risks of birth defects than others. Eye and central nervous system cancers were associated with the highest risk of all malformations (OR = 1.30, 95% CI = 1.04 to 1.61, P = 0.02, 4.5% vs 3.4%). Testicular cancer was associated with an increased risk of the more severe major malformations (OR = 1.28, 95% CI = 1.00 to 1.64, P = 0.05, 2.7% vs 2.1%).

Table 2. Selected parental and perinatal characteristics for children without paternal cancer, with paternal history of cancer, and with paternal cancer after offspring conception.

Characteristic	No Cancer	Paternal history of cancer	Paternal cancer after offspring conception
Total No. of children (%)	1759627 (98.0)	9926 (0.6)	26601 (1.5)
Parental characteristics			
Mean maternal age at offspring birth, years (SD)	29.8 (5.1)	31.6 (5.0)	31.4 (5.2)
Mean maternal BMI at early pregnancy, kg/m ² (SD)	24.4 (4.4)	24.5 (4.6)	24.3 (4.3)
Mean paternal age at offspring birth, years (SD)	32.7 (6.1)	35.7 (7.1)	36.4 (7.8)
Non-smoking mothers early in pregnancy, No. (%)	1571503 (89.3)	9105 (91.7)	22839 (85.9)
Mothers smoking 1-9 cigarettes per day, No. (%)	117620 (6.7)	488 (4.9)	2121 (8.0)
Mothers smoking more than 10 cigarettes per day, No. (%)	46932 (2.7)	203 (2.0)	1131 (4.3)
Missing information regarding maternal smoking, No. (%)	23572 (1.3)	130 (1.3)	510 (1.9)
Maternal parity, No. (%)			
Nulliparous	765013 (43.5)	4061 (40.9)	9966 (37.5)
Parous, 1 child	649562 (36.9)	3814 (38.4)	9797 (36.8)
Multiparous	345052 (19.6)	2051 (20.7)	6838 (25.7)
Mode of conception			
Natural	1722595 (97.9)	9132 (92.0)	25973 (97.6)
Assisted	37032 (2.1)	794 (8.0)	628 (2.4)
Birth characteristics			
Sex, No. (%)			
Male	904143 (51.4)	5116 (51.5)	13673 (51.4)
Female	855480 (48.6)	4810 (48.5)	12928 (48.6)
Missing	4 (0.0)	0 (0.0)	0 (0.0)
All congenital abnormalities, No. (%)	60540 (3.4)	357 (3.6)	1016 (3.8)
Major congenital abnormalities, No. (%)	37785 (2.1)	230 (2.3)	629 (2.4)

*BMI = body mass index

In a post hoc analysis where we stratified malformations into subgroups, an elevated risk of chromosomal abnormalities (OR = 1.40, 95% CI = 1.08 to 1.80, P = 0.01, 0.12% vs 0.24%) was observed for children born to fathers prior to paternal cancer diagnosis, as compared to the non-cancer reference.

The risks for the children conceived before paternal cancer diagnosis and those conceived after paternal cancer diagnosis were of about the same magnitude (OR = 1.06, 95% CI = 0.92 to 1.21, P = 0.42, 3.8% vs 3.6%, and OR = 1.01, 95% CI = 0.86 to 1.20, P = 0.88, 2.4% vs 2.3%, respectively).

Sensitivity analyses did not differ in their results. These sensitivity analyses consisted of excluding children conceived by assisted reproductive technologies (to negate differences in fertility), and modeling by the generalized estimating equation (to negate possible familial effects). Another sensitivity analysis estimated, by Cox

regression, the father's risk to be diagnosed with cancer depending on if they had a child without or with a birth defect (as an exposure). This analysis found that fathering a child with a congenital malformation was associated with a statistically significant increased risk of being diagnosed with cancer (hazard ratio (HR) = 1.10, 95% CI = 1.01 to 1.19, P = 0.02).

The congenital malformation rate was also visualized with the time difference between child conception and paternal cancer diagnosis on the x-axis. This gives an overview of how the malformation rate changes depending on when the child is conceived as compared to when the father is diagnosed with cancer. The curve showed an apparent peak in the years preceding paternal cancer diagnosis, and no such peak after diagnosis (Figure 5). The curve also shows high malformation rates for children conceived to men who much earlier in life, more than 20 years earlier, have been diagnosed with cancer. In our cohort, this corresponds mainly to men who have had cancer in their childhood and would later go on to father children in adult age.

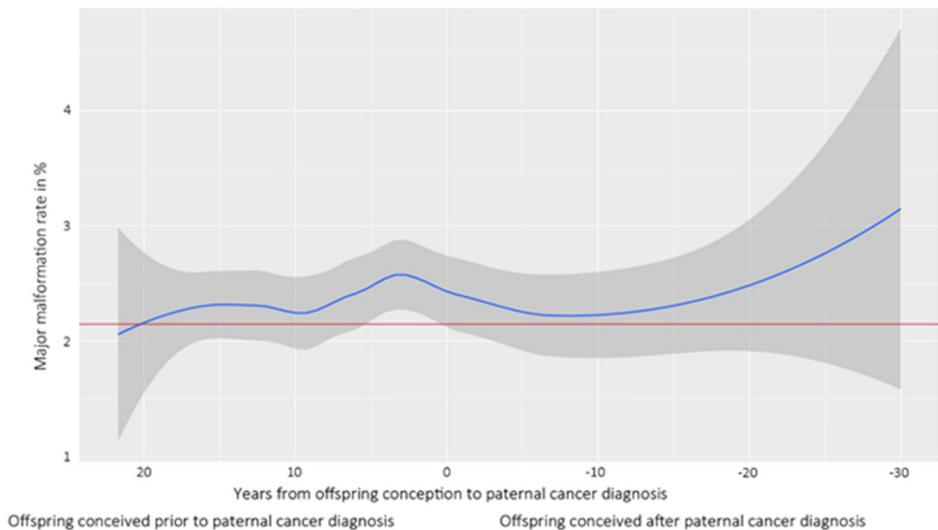


Figure 5 The rate of major malformation vs. the time in years from offspring conception to paternal cancer diagnosis. Presented as major malformation rate (%) with 95% prediction interval (in grey). Horizontal line (in red) indicates control level malformation rate. Zero on the x axis indicates the date of paternal cancer diagnosis.

Paternal anticancer therapies and risk of offspring congenital malformations

Study population

In study II, 2 027 997 children were included. Of those, 4 207 (0.2%) had fathers who were diagnosed with TGCC at some time point. Grouping the children by whether they were conceived before or after paternal TGCC resulted in 2 770 (65.8%) conceived before and 1 437 (34.2%) after diagnosis. Further parental and infant characteristics are given in table 3

Table 3. The distribution of children according to paternal TGCC diagnosis with parental characteristics and birth outcomes.

Characteristic	No paternal TGCC	Conceived prior to paternal TGCC diagnosis	Conceived after paternal TGCC diagnosis
Total No. of children	2023790	2770	1437
Parental characteristics			
Maternal age at offspring birth, years, mean (SD)	29.9 (5.1)	29.0 (4.9)	31.1 (4.6)
Maternal BMI at early pregnancy, kg/m ² , mean (SD)	24.4 (4.4)	24.2 (4.2)	24.5 (4.6)
Paternal age at offspring birth, years, mean (SD)	32.8 (6.2)	30.9 (4.9)	33.8 (4.8)
Non-smoking mothers early in pregnancy, No. (%)	1826916 (90.3)	2466.6 (89.0)	1372 (95.5)
Mothers smoking 1-9 cigarettes per day, No. (%)	139791.2 (6.9)	210.8 (7.6)	47.2 (3.3)
Mothers smoking more than 10 cigarettes per day, No. (%)	57082.8 (2.8)	92.6 (3.3)	17.8 (1.2)
Mode of conception			
Assisted	42521 (2.1)	71 (2.6)	201 (14.0)
Birth characteristics			
Sex, No. (%)			
Male	1040460 (51.4)*	1428 (51.6)	723 (50.3)
Female	983324 (48.6)*	1342 (48.4)	714 (49.7)
Congenital Malformations			
All congenital abnormalities, No. (%)	69920 (3.5)	125 (4.5)	59 (4.1)
Major congenital abnormalities, No. (%)	43714 (2.2)	80 (2.9)	42 (2.9)

Abbreviations: TGCC, testicular germ cell cancer; No, number; SD, standard deviation; BMI, body mass index. Values are pooled over five imputed data sets. *Excluding six children for which sex was missing.

The most observed anti-TGCC treatment was chemotherapy. The largest group of children born to men were those conceived by men treated with chemotherapy as seen in table 4.

Table 4. Parental and perinatal characteristics for groupings based on when conception occurred in relation to paternal treatment regimen.

Characteristic	Paternal treatment regimen					
	Surgery only		Chemotherapy		Radiotherapy	
	Conceived before	Conceived after	Conceived before	Conceived after	Conceived before	Conceived after
Total No. of children	947	393	1639	894	203	157
Mode of conception						
Assisted	29 (3.1)	39 (9.9)	40 (2.4)	139 (15.5)	4 (2.0)	26 (16.6)
Birth characteristics						
Sex, No. (%)						
Male	504 (53.2)	196 (49.9)	838 (51.1)	444 (49.7)	98 (48.3)	86 (54.8)
Female	443 (44.8)	197 (50.1)	801 (48.9)	450 (50.3)	105 (51.7)	71 (45.2)
Congenital Malformations						
All congenital abnormalities, No. (%)	45 (4.8)	18 (4.6)	75 (4.6)	37 (4.1)	6 (3.0)	5 (3.2)
Major congenital abnormalities, No. (%)	27 (2.9)	11 (2.8)	50 (3.1)	28 (3.1)	4 (2.0)	4 (2.5)

There were 19 children conceived to fathers prior to treatment with both chemotherapy and radiotherapy, among them, one had a major malformation. Similarly, 7 children were conceived after both treatment modalities, with one major malformation among them.

Main results

To ensure that groups were comparable in terms of presence of paternal TGCC and anticancer therapies, children conceived to fathers before a specific anti-TGCC therapy acted as reference for the children conceived after that same therapy. When comparing in this strict manner, we found no statistically significant increased risk of congenital malformations for being conceived after radio- or chemotherapy:

- radiotherapy, all malformations: OR = 1.01, 95% CI = 0.25–4.12, p=0.98, 3.2% versus 3.0%
- radiotherapy, major malformations: OR = 1.37, 95% CI = 0.27–7.05, p=0.70, 2.5% versus 2.0%
- chemotherapy all malformations: OR = 0.82, 95% CI = 0.54–1.25, p=0.37, 4.1% versus 4.6%
- chemotherapy major malformations: OR = 1.01, 95% CI = 0.62–1.65, p=0.97, 3.1% versus 3.1%

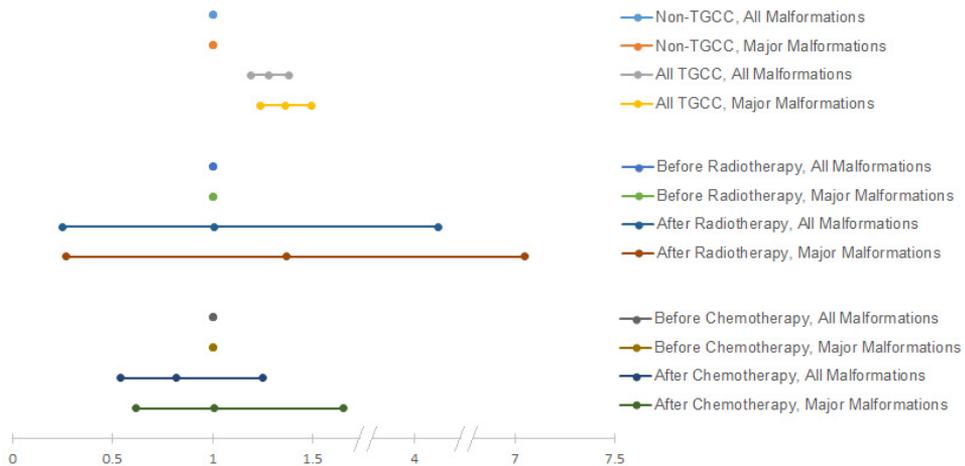


Figure 6. Forest plot of ORs and 95% CIs for risk of all and major malformations. Calculations were made according to the presence of TGCC or not and according to before or after treatment with radiotherapy or chemotherapy. CI, confidence interval; OR, odds ratio; TGCC, testicular germ-cell cancer.

However, the children fathered by men with TGCC (post- or preconception) had a statistically significantly increased risk for all and for major birth defects, as compared to children fathered by men without a TGCC diagnosis (all malformations: OR = 1.28, 95%CI = 1.19–1.38, $p=0.001$, 4.4% versus 3.5%; major malformations: OR = 1.36, 95% CI = 1.24–1.49, $p<0.001$, 2.9% versus 2.2%, Figure 6).

Secondary results

When stratifying the children to father's who had received chemotherapy by the number of chemotherapy cycles, no pattern of increased risk could be discerned among the children conceived after as compared to before treatment. In fact, in 5 of 6 chemotherapy cycle categories, the risk estimate was below one for the post-treatment children as compared to the pre-diagnosis group.

When grouping all the children conceived after paternal TGCC diagnosis together as compared to pre-diagnosis conceived children, no increased risk for all nor for major malformations was detected (OR = 0.88, 95% CI = 0.63–1.22, $p=0.43$, 4.1% versus 4.5% and OR = 1.03, 95% CI = 0.69–1.53, $p=0.88$, 2.9% versus 2.9%, respectively).

In performed sensitivity analyses, children conceived by assisted reproduction were excluded, and we found negligible differences in risk estimates (all malformations: OR = 1.25, 95% CI = 1.16–1.36, $p=0.004$, 4.3% versus 3.4%; major malformations:

OR = 1.37, 95% CI = 1.25–1.51, $p < 0.001$, 2.9% versus 2.1%). Further sensitivity analysis excluded the children of fathers with non-TGCC cancer from the reference group and this yielded similar risk estimates (OR = 1.28, 95% CI = 1.19–1.38, $p = 0.001$, 4.4% versus 3.5% and OR = 1.36, 95% CI = 1.24–1.49, $p < 0.001$, 2.9% versus 2.2%).

Paternal disease, concomitant prescribed drug treatment and risk of preterm birth among offspring

Study population

Study III utilized a US based cohort and a subgroup from the Swedish register-based cohort. The US cohort included 785 809 infants with 51 759 (6.6%) born preterm. The Swedish register cohort included 885 715 children of whom 51 770 (5.8%) were born preterm. In the US cohort, the mean age of the father and mother at birth were 35 and 33, respectively. While in Sweden, the corresponding ages were 33 and 30, respectively. The use of prescribed drugs was higher in the US as compared to Sweden. In the US, 319 153 (40.6 %) fathers to-be were prescribed a drug in the 6-month interval prior to conception, while in Sweden that number totalled 291,157 (32.9%). The distribution of number of prescriptions within the 6-month preconception interval also skewed higher in the US as measured by higher median, and 75th percentiles. Further characteristics on both cohorts are given in Table 5.

Table 5, Descriptive statistics and characteristics of the US and Swedish cohorts

Characteristic	US	Sweden
No. of children	785,809	885,715
No. of fathers	687,989	609,561
No. of mothers	687,992	608,673
Parental characteristics		
Paternal age at offspring birth, years, mean (SD)	35.0 (5.2)	33.3 (6.6)
Maternal age at offspring birth, years, mean (SD)	32.8 (4.2)	30.4 (5.2)
Maternal preconception prescribed drug consumption*		
No. of mothers consuming prescription drugs 0-6 months before child conception (%)	464,015 (59.0)	478,822 (54.1)
Median number of prescriptions† (IQR)	4 (2 – 7)	2 (1 – 4)
Paternal preconception prescribed drug consumption*		
No. of fathers consuming prescription drugs 0-6 months before child conception	319,153 (40.6)	291,157 (32.9)
Median number of prescriptions† (IQR)	3 (1 – 6)	2 (1 – 4)
Birth characteristics		
Sex, No. (%)		
Male	403,830 (51.4)	454,202 (51.4)
Female	381,979 (48.6)	429,465 (48.6)
Gestational duration, weeks, mean (SD)	-	39.3 (2.0)
Preterm birth (gestational age <37 weeks), No. (%)	51,759 (6.6)	51,770 (5.8)

**As parents in both cohorts can have multiple children, this number reflects the number of children that have been exposed via their parents prior to conception.
†excluding parents with zero no. of prescriptions.*

Main results

Included in the analysis were 688 paternally prescribed preconception medications. Of these, 31 were statistically significantly ($p < 7.3 \times 10^{-5}$) associated with offspring preterm birth (Table 6). Grouping of drugs by anatomical therapeutic chemical (ATC) classification, as seen in Figure 7, shows medications from the certain classes cluster above the significance threshold: Cardiovascular (ATC-C: Diuretics, Beta blockers, ACE inhibitors, Angiotensin II receptor blockers, Dihydropyridine derivatives, HMG CoA reductase inhibitors), Anti-infective (ATC-J: antibacterials) and Nervous (ATC-N: Anilides, Opioid and Benzodiazepine derivatives, Antiepileptics). In contrast, medications from blood (B), dermatological (D), or respiratory (R) groups were rarely or not associated with preterm birth.

Table 6 Numbers of exposed children and risk estimates of statistically significant candidate drugs.

English Drug Name	Total number of children to fathers with exposure (% of cohort)	Children born preterm (% of exposed)	Preterm Birth, Odds ratio per step, univariate	Odds Ratio* for highest exposure versus reference, univariate	p value, preterm, univariate†	Preterm Birth, Odds ratio per step, adjusted*	Odds Ratio* for highest exposure versus reference, adjusted††	p value, preterm, adjusted††
Doxycycline Hyclate	11462 (1.5)	1489 (13.0)	2.01	4.02	4.12×10^{-151}	1.46	2.13	1.17×10^{-41}
Clomiphene Citrate	1442 (0.2)	213 (14.8)	1.45	6.52	2.25×10^{-22}	1.37	4.82	1.00×10^{-15}
Azithromycin	42612 (5.4)	3193 (7.5)	1.16	1.34	2.84×10^{-15}	1.08	1.17	5.18×10^{-5}
Anastrozole	460 (0.1)	74 (16.1)	1.57	9.39	4.23×10^{-12}	1.54	8.56	3.46×10^{-11}
Doxycycline Monohydrate	522 (0.1)	70 (13.4)	2.12	4.48	6.05×10^{-10}	1.79	3.22	2.43×10^{-6}
Lisinopril	12043 (1.5)	987 (8.2)	1.11	1.67	2.72×10^{-9}	1.09	1.51	2.11×10^{-5}
Zolpidem Tartrate	6558 (0.8)	554 (8.4)	1.14	1.91	1.16×10^{-8}	1.09	1.54	1.81×10^{-4}
Ciprofloxacin	9399 (1.2)	747 (7.9)	1.22	1.50	5.75×10^{-8}	1.18	1.40	7.54×10^{-5}
Omeprazole	12125 (1.5)	955 (7.9)	1.11	1.66	7.34×10^{-8}	1.09	1.57	1.61×10^{-6}
Metoprolol Succinate	3027 (0.4)	290 (9.6)	1.21	2.17	7.40×10^{-8}	1.18	1.96	3.76×10^{-5}
Cialis	2145 (0.3)	225 (10.5)	1.21	2.63	1.46×10^{-7}	1.18	2.26	1.19×10^{-5}
Pravastatin Sodium	2368 (0.3)	220 (9.3)	1.20	2.54	5.60×10^{-7}	1.17	2.17	3.53×10^{-5}
Allopurinol	2695 (0.3)	254 (9.4)	1.19	2.36	7.78×10^{-7}	1.16	2.11	2.04×10^{-5}
Losartan Potassium	2111 (0.3)	215 (10.2)	1.20	2.45	1.09×10^{-6}	1.17	2.22	1.58×10^{-5}
Citalopram Hydrobromide	4815 (0.6)	384 (8.0)	1.14	1.91	1.79×10^{-6}	1.13	1.80	1.39×10^{-5}
Furosemide	395 (0.1)	46 (11.6)	1.42	5.68	4.02×10^{-6}	1.37	4.78	3.61×10^{-5}
Hydrocodone Bitartrate-Acetaminophen	35727 (4.5)	2588 (7.2)	1.07	1.29	4.50×10^{-6}	1.03	1.14	1.56×10^{-2}
Diazepam	2955 (0.4)	246 (8.3)	1.22	2.24	4.86×10^{-6}	1.17	1.86	5.38×10^{-4}

Lisinopril Hydrochlorothiazide	3902 (0.5)	337 (8.6)	1.14	1.93	5.54×10 ⁶	1.12	1.75	1.25×10 ⁻⁴
Axiron	137 (0.0)	21 (15.3)	1.79	10.19	1.22×10 ⁵	1.73	8.89	4.01×10 ⁻⁵
Cefdinir	4178 (0.5)	344 (8.2)	1.27	1.62	1.44×10 ⁵	1.26	1.58	4.36×10 ⁻⁵
Tramadol HCL	5680 (0.7)	462 (8.1)	1.14	1.72	1.48×10 ⁵	1.12	1.58	2.63×10 ⁻⁴
Gabapentin	2323 (0.3)	214 (9.2)	1.17	2.21	1.61×10 ⁵	1.15	2.05	1.05×10 ⁻⁴
Crestor	3723 (0.5)	318 (8.5)	1.14	1.92	2.21×10 ⁵	1.1	1.61	2.19×10 ⁻³
Cyclobenzaprine HCL	11171 (1.4)	844 (7.6)	1.15	1.33	2.36×10 ⁵	1.14	1.31	6.62×10 ⁻⁵
Amlodipine Besylate	4268 (0.5)	361 (8.5)	1.16	1.78	2.90×10 ⁵	1.11	1.54	1.84×10 ⁻³
Valsartan Hydrochlorothiazide	223 (0.0)	34 (15.2)	1.46	6.62	2.94×10 ⁵	1.41	5.55	1.64×10 ⁻⁴
Nifedipine	224 (0.0)	26 (11.6)	1.45	6.50	2.99×10 ⁵	1.42	5.74	1.01×10 ⁻⁴
Lorazepam	3367 (0.4)	274 (8.1)	1.18	1.93	3.88×10 ⁵	1.15	1.74	5.46×10 ⁻⁴
Colcrys	699 (0.1)	73 (10.4)	1.36	3.41	4.95×10 ⁵	1.31	2.99	3.24×10 ⁻⁴
Bystolic	930 (0.1)	99 (10.6)	1.25	2.43	5.45×10 ⁵	1.23	2.25	2.50×10 ⁻⁴

* These odds ratios are interpolated by taking the $OR_{step}^{(Number\ of\ steps - 1)}$. Interpolated ORs are subject to less variance due to small group sizes.

† Univariate p values dictate candidate status.

‡ Adjusted for paternal age (continuous variable), maternal age (continuous variable), and for maternal use of the same investigated drug (continuous, processed as paternal drug). For some drugs, too few mothers (<200) were exposed to those drugs during the same exposure interval. Those drugs were adjusted for parental ages, but not maternal drug use.

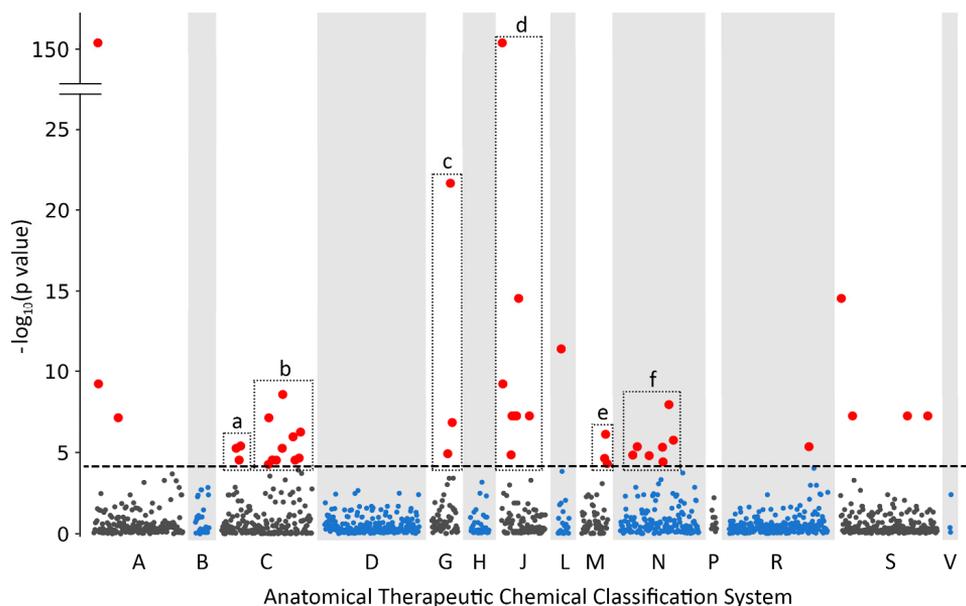


Figure 7 Manhattan plot of ATC code of drug against $-\log_{10}(p \text{ value})$. ATC codes give structure as the drugs are classified by indication. This hierarchal structure can be leveraged as drugs used for similar indications are in proximity on the x-axis, analogously to chromosomes. Within each first level group, drugs are further clustered by increasingly specific indications. As many drugs have multiple indications, translation of national drug codes to anatomic therapeutic codes causes some data points to appear multiple times along a horizontal line.

Abbreviations: A, Alimentary tract and metabolism; B, Blood and blood forming organs; C, Cardiovascular system; D, Dermatologicals; G, Genito-urinary system and sex hormones; H, Systemic hormonal preparations, excluding sex hormones and insulins; J, Antiinfectives for systemic use; L, Antineoplastic and immunomodulating agents; M, Musculo-skeletal system; N, Nervous system; P, Antiparasitic products, insecticides and repellents; R, Respiratory system; S, Sensory organs; V, Various.

a. Diuretics (C03: Lisinopril/Hydrochlorothiazide, Valsartan/Hydrochlorothiazide, and Furosemide).

b. Beta blocking agents, selective (C07AB: Bystolic, and Metoprolol Succinate), Dihydropyridine derivatives (C08CA: Amlodipine Besylate, and Nifedipine), ACE inhibitors, plain (C09AA: Lisinopril, and Lisinopril/Hydrochlorothiazide), Angiotensin II receptor blockers, plain (C09CA: Losartan Potassium, and Valsartan/Hydrochlorothiazide), HMG CoA reductase inhibitors (C10AA: Crestor, and Pravastatin Sodium).

c. Sex hormones and modulators of the genital system (G03: Axiron, and Clomiphene Citrate), and Drugs used in erectile dysfunction (G04BE: Cialis). Anastrozole is classified as an aromatase inhibitor (L02BG) but is used off-label to treat hypogonadism and infertility and can therefore be included in this group.

d. Antibacterials for systemic use (J01: Doxycycline Hyclate, Doxycycline Monohydrate, Cefdinir, Ciprofloxacin [as J01EA, J01EC, J01EE, and J01MA], and Azithromycin).

e. Antigout preparations (M04A: Allopurinol, and Colcris). Cyclobenzaprine HCL (M03BX), a muscle relaxant, which is in proximity on the x axis can be excluded due to differing indication.

f. Analgesics (N02: Tramadol HCL, Hydrocodone Bitartrate-Acetaminophen), Antiepileptics (N03A: Gabapentin), Benzodiazepine derivatives and Benzodiazepine related drugs (N05BA: Diazepam, Lorazepam; N05CF: Zolpidem Tartrate), Selective serotonin reuptake inhibitor (N06AB: Citalopram Hydrobromide).

Secondary results

Some drugs could not be tested for replication in the Swedish cohort as they lack approval from Swedish Medical Products Agency, were duplicates with same primary active ingredient, or had 10 times or lower prevalence of fathers exposed in Sweden. Thus 17 drugs could be tested for replication. Of those, Tramadol, Tadalafil (Cialis), and Allopurinol were statistically significant in both cohorts for preterm birth, and Tramadol, Omeprazole, Metoprolol, Gabapentin, Citalopram, Valsartan Hydrochlorothiazide, Tadalafil, and Losartan were statistically significant for a decrease in gestational duration in the Swedish cohort (Table 7)

Table 7 Candidate drugs replicated in the Swedish cohort. Preterm birth and gestational duration regressions in the Swedish data.

Swedish generic drug name	Total number of fathers with exposure 0-6 months pre-conception (%), Sweden	Number of children born preterm of exposed	p value, preterm birth, adjusted	OR-step, adjusted	OR, highest exposure versus reference	p value, gestational age, adjusted*	Days difference per step, adjusted*	Gestational duration in days, difference between highest exposure and reference, adjusted
Tramadol	11848 (1.3)	821 (6.9)	4.73E-05	1.10	1.46	6.39E-11	-0.55	-2.18
Omeprazole	15277 (1.7)	913 (6.0)	6.94E-01	1.01	1.03	4.29E-04	-0.32	-0.97
Metoprolol	3358 (0.4)	210 (6.3)	2.02E-01	1.08	1.25	2.78E-03	-0.57	-1.72
Gabapentin	520 (0.1)	39 (7.5)	1.45E-01	1.13	1.83	4.30E-03	-0.89	-4.43
Citalopram	6554 (0.7)	418 (6.4)	2.78E-01	1.04	1.17	5.57E-03	-0.33	-1.33
Valsartan Hydrochlorothiazide	247 (0)	19 (7.7)	9.41E-02	1.35	2.47	9.58E-03	-1.82	-5.45
Tadalafil	2462 (0.3)	180 (7.3)	1.26E-03	1.14	1.89	1.36E-02	-0.37	-1.84
Losartan	1303 (0.1)	74 (5.7)	4.27E-01	0.92	0.78	3.92E-02	-0.63	-1.90
Allopurinol	743 (0.1)	56 (7.5)	4.55E-02	1.28	2.11	5.68E-01	-0.26	-0.77

*Adjusted for paternal age (continuous variable), maternal age (continuous variable), and for maternal use of the same investigated drug (continuous, processed as paternal drug). Tadalafil had too few mothers (<200) exposed was adjusted for parental ages, but not maternal drug use.

For US data, most drugs had an associated odds ratio per categorical step (OR_{step}) of dose (no. of prescriptions, categorized) of about 1.1-1.2, or an interpolated 1.5-2.5 times higher risk for the highest exposed groups. Multiple drugs were only statistically significant for a decrease in the continuous measure of gestational duration in the Swedish cohort, but not for preterm birth.

To give an indication of whether it is the disease or the medication that was associated with preterm birth, we graphed the preterm birth rate in relation to time between first paternal prescription to offspring conception. These showed medications associated with PTB before, during, and after conception. Valsartan, Tramadol, and Gabapentin showed a peak of increased rate of PTB just before conception (Figure 8).

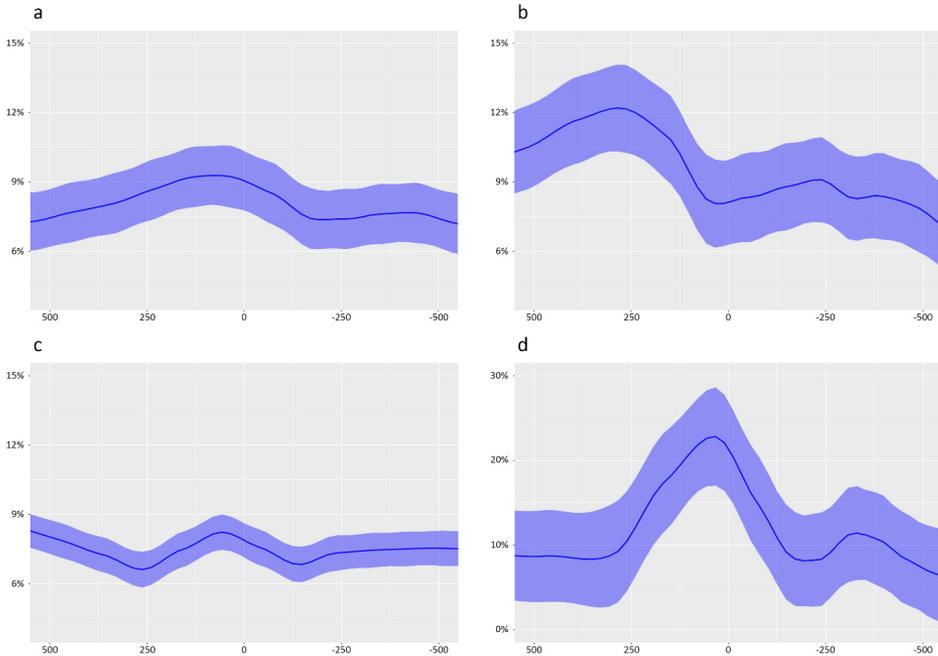


Figure 8 Locally estimated scatterplot smoothing plots for a. Gabapentin, b. Losartan, c. Tramadol and d. Valsartan. The rate of preterm birth is plotted against the difference in days between date of first paternal prescription of drug to date of conception of offspring. Positive time difference (left side) denotes paternal prescribed treatment started before conception. Note that all panels cover the same time interval (± 500 days), and the same range of preterm birth rate (4-15%), except Valsartan which covers 0-30%. All locally estimated scatterplot smoothing plots use $\text{span}=0.4$. All first prescriptions were included in the analysis regardless of how long before or after conception they were prescribed. However, only the 500 days before and after conception are displayed.

Paternal age and risk of detrimental perinatal offspring health events

Study population

In study IV, using data from the Swedish register database, 2 108 570 children to 1 181 492 fathers and 1 192 658 mothers were included. The mean paternal age at infant birth age increased between 1994 - 2014, from 31.2 to 33.1. The maternal age increased from 28.6 to 30.4 years during the same interval. Infant, paternal and maternal characteristics are given in Table 8.

Table 8. Descriptive statistics for paternal, maternal, and infant characteristics by paternal age group in Sweden during 1994-2014.

Characteristic	Paternal age (years)					
	<25	25-34	35-44	45-54	≥55	Unknown
Live births, N(%)	1906531 (9.1)	12451591 (59.6)	5784461 (27.7)	675771 (3.2)	67651 (0.3)	19970
Paternal characteristics						
Education level, N(%)						
Low	86979 (46)	436690 (35)	222427 (39)	30693 (46)	2996 (46)	
Medium	90572 (48)	503160 (41)	186799 (32)	19435 (29)	1747 (27)	
High	11140 (5.9)	297753 (24)	165617 (29)	16830 (25)	1838 (28)	
Unknown	7556	1962	3603	619	184	
Maternal characteristics						
Maternal age (years), N(%)						
<23	92642 (49)	64228 (5.2)	6627 (1.1)	678 (1.0)	97 (1.4)	3165 (16)
23-29	87994 (46)	631959 (51)	91830 (16)	8128 (12)	922 (14)	6330 (32)
30-39	9742 (5.1)	540464 (43)	438666 (76)	44008 (65)	4162 (62)	8846 (44)
≥40	275 (0.1)	8502 (0.7)	41323 (7.1)	14763 (22)	1584 (23)	1627 (8.1)
Unknown	6	0	0	0	0	2
Highest achieved education, N(%)						
Low	72087 (38)	283629 (23)	138473 (24)	21270 (32)	2250 (34)	7733 (42)
Medium	90331 (48)	506750 (41)	206484 (36)	22286 (33)	2062 (31)	6036 (32)
High	26097 (14)	447883 (36)	229706 (40)	23181 (35)	2314 (35)	4822 (26)

Unknown	6897	2138	3783	840	139	1379
Smoking, N(%)						
Non-smoker	120000 (65)	999309 (84)	476644 (86)	54652 (85)	5692 (89)	14086 (76)
1-9 cigarettes/day	31840 (17)	105570 (8.8)	38959 (7.1)	4550 (7.1)	338 (5.3)	2034 (11)
>9 cigarettes/day	31688 (17)	90366 (7.6)	36869 (6.7)	5138 (8.0)	395 (6.1)	2446 (13)
Unknown	49914	7125	25974	3237	340	1404
Parity, N(%)						
First	569017 (46)	135840 (71)	164213 (28)	20759 (31)	2497 (37)	12439 (63)
Second	479035 (38)	43754 (23)	223022 (39)	22009 (33)	2193 (32)	4386 (22)
Third or higher order	197103 (16)	11057 (5.8)	191198 (33)	24808 (37)	2075 (31)	3054 (15)
Unknown	4	2	13	1	0	91
Infant characteristics						
Gestational age (week)	39.27 (1.98)	39.33 (1.93)	39.26 (1.98)	39.16 (2.10)	39.14 (2.12)	38.94 (2.94)
Birth weight (g)	3469 (576)	3532 (581)	3533 (601)	3484 (621)	3466 (617)	3319 (698)
Apgar score at 5 min	9.73 (0.78)	9.74 (0.77)	9.74 (0.78)	9.72 (0.84)	9.73 (0.85)	9.53 (1.38)
Adverse birth outcomes, N(%)						
Preterm birth (<37w)	12185 (6.4)	71559 (5.8)	34905 (6.0)	4698 (7.0)	494 (7.3)	1929 (9.7)
Unknown	693	218	368	59	14	119
Low birth weight(<2500g)	8475 (4.5)	48826 (3.9)	25461 (4.4)	3668 (5.4)	368 (5.5)	1676 (8.4)
Unknown	3450	519	1487	197	25	132
Low Apgar score (<8)	4359 (2.3)	26530 (2.1)	12823 (2.2)	1738 (2.6)	176 (2.6)	1126 (5.7)
Unknown	3450	519	1487	197	25	132
SGA (<10 percentiles)	21914 (12)	119315 (9.6)	55709 (9.7)	7618 (11)	798 (12)	3296 (17)
Unknown	4065	727	1807	253	36	241
Diagnosis of Cancer, N(%)	182 (<0.1)	1292 (0.1)	591 (0.1)	67 (<0.1)	5 (<0.1)	23 (0.1)
Mortality, N(%)	649 (0.3)	3705 (0.3)	1946 (0.3)	278 (0.4)	34 (0.5)	878 (4.4)

N (%) for categorical variables, one decimal was kept for those <10%; mean (SD) for continuous variables, two decimals were kept for SD. Unknown values were excluded for computing %, mean or SD.

Main results

The advanced paternal age (≥ 55 years) group has a statistically significantly increased risk of all evaluated adverse birth outcomes as compared to reference (25-34 years). Infants fathered by older men had an increased risk of being preterm (OR=1.22, 95% CI=1.11-1.34), having low birth weight (OR=1.29, 95% CI=1.15-1.44), being small for gestational age (OR=1.24, 95% CI=1.15-1.34), and having low Apgar (OR=1.08, 95% CI=0.91-1.26). Infants to fathers aged 45-54 and fathers aged 35-44, also had increased risk of investigated adverse events, but to a smaller magnitude (see Figure 9A).

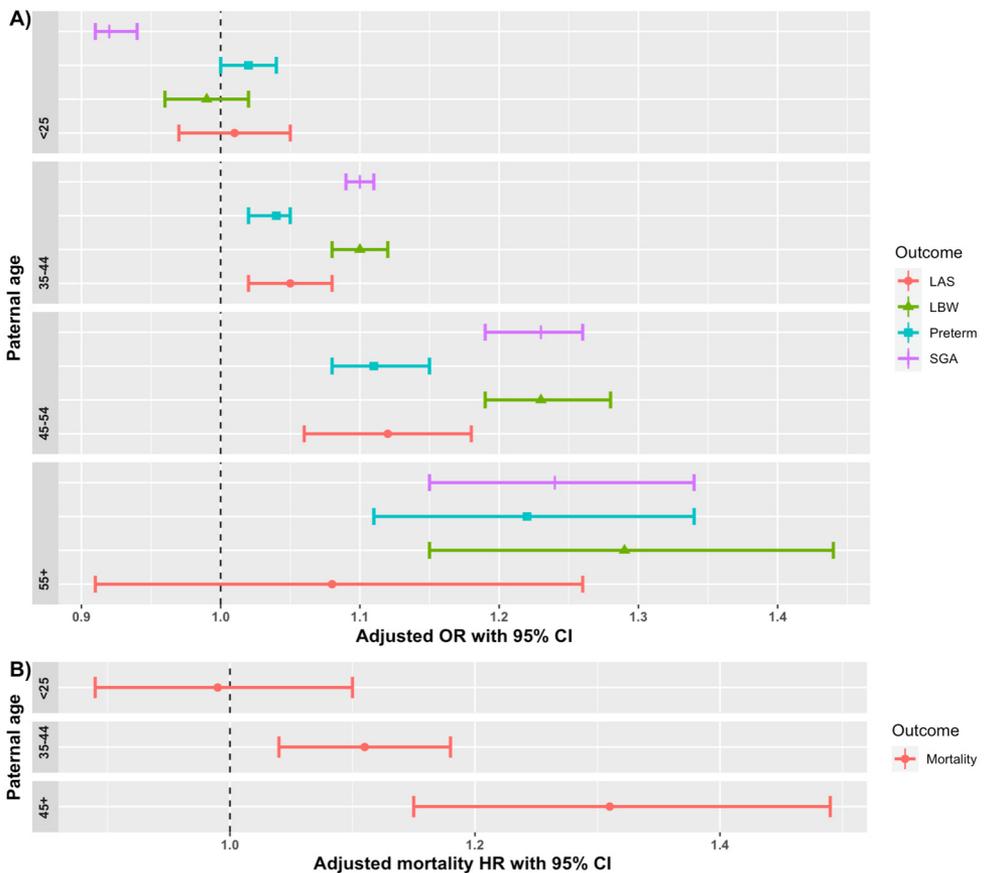


Figure 9 A&B Adjusted odds ratio of being preterm, low birth weight (LBW), SGA and low Apgar score (LAS) and B) adjusted hazard ratio of childhood mortality for different paternal age groups (25-34 years of age as reference). The analyses were based on complete cases i.e. any missing outcomes or covariates were removed. For childhood mortality (**B**), paternal age groups 45-54 and 55+ were merged into 45+ due to few cases. OR = Odds Ratio, HR = Hazard ratio, CI = Confidence Interval.

Children born to older fathers (≥ 45 years) had an increased risk of childhood all-cause mortality (HR=1.31, 95% CI=1.15-1.49, Figure 9B). Offspring to fathers aged 35-44 also had an increased mortality risk but to a lower extent (HR=1.11, 95% CI=1.04-1.18). By further adjusting for each adverse perinatal outcome in the Cox regression model, children to fathers aged ≥ 45 still had an increased risk (HR=1.21, 95% CI= 1.05-1.39). Childhood mortality for these paternal age groups is visualized in Figure 10

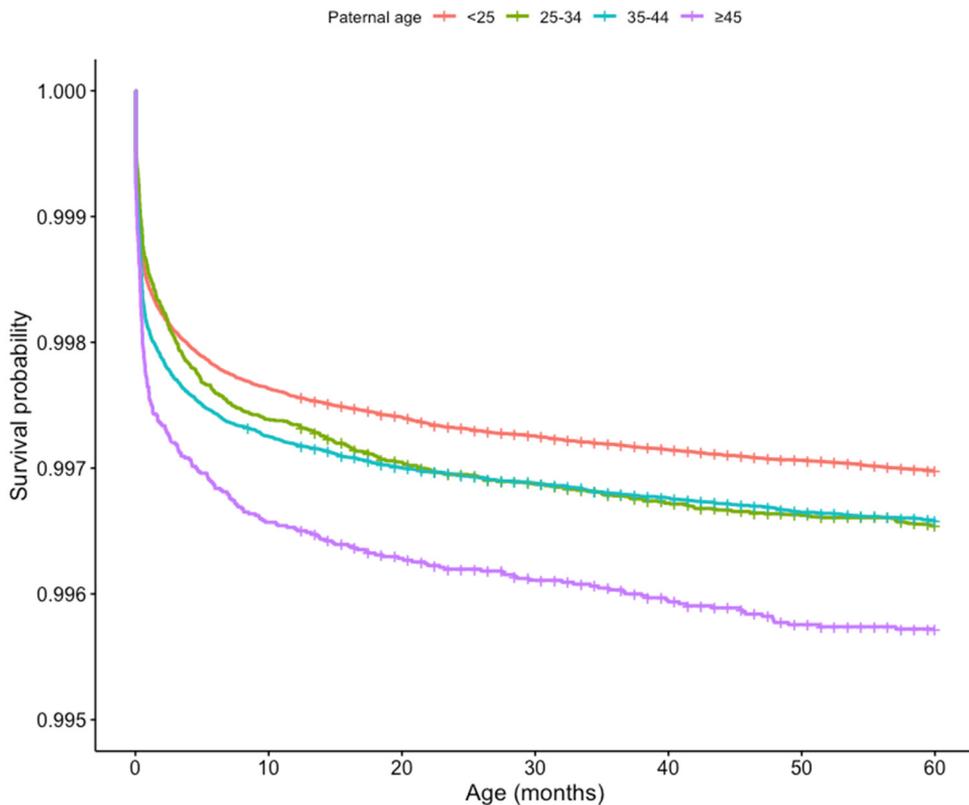


Figure. 10 Kaplan-Meier survival probability among children to fathers of different paternal age. P <0.0001 by log-rank test. Paternal age groups 45-54 and ≥ 55 were merged into ≥ 45 due to few cases.

Secondary results

There was no association between paternal age and the risk of developing cancer among offspring during the first five years of life (fathers aged ≥ 45 versus reference: HR=1.00, 95% CI=0.77-1.28).

Congenital malformation and infectious disease were overrepresented as causes of death among children to older fathers as compared to reference-aged fathers (cause of death as congenital malformations HR=1.58, 95% CI=1.26-1.96, 0.152% vs. 0.090% events, cause of death as infectious disease HR=2.23, 95% CI=1.03-4.82, 0.011% vs. 0.007% events) as compared to reference.

Sensitivity analyses adjusting for the mothers age as a continuous nonlinear variable to negate possible residual confounding within the wide maternal age groups lead to attenuated risks. But risk estimates of preterm birth (OR = 1.14, 95% CI = 1.03 - 1.26), low birth weight (OR = 1.19, 95% CI = 1.07 - 1.33) and being small for gestational age (OR = 1.21, 95% CI= 1.11 - 1.30) all remained statistically significant (but not low Apgar) for the ≥ 55 age group as compared to reference. Analyses using complete cases, partial (not imputing missing outcomes) or full imputation did not differ.

Discussion

Principal statement of findings

The main overarching finding of this thesis is that the father, his age, his disease, and his medications have profound impacts on the health of his children, as evaluated in several studies investigating different perinatal outcomes. Therefore, this register-based thesis provides novel data which not only adds to our biological understanding of diseases but can also be implemented in daily clinical work as well as in public health related strategies.

Regarding the initial fears that mutagenic cancer therapies might negatively affect the offspring of cancer survivors, we have shown that no such risks could be detected for the most common malignancy in young males – testicular cancer. Intriguingly, we did see that paternal cancer itself was associated with risk of congenital malformations in the offspring. This was determined by observing that children conceived before paternal cancer diagnosis, whom have not been exposed to chemo- or radiotherapy, had modestly, but statistically significantly, increased risks of birth defects. Further, children to fathers with testicular cancer specifically had about a 30% increased risk of birth defects, and this increased risk could not be attributed to antineoplastic therapies.

We have also shown that paternal characteristics can detriment children's health as measured by other health outcomes than birth defects. Risk of offspring preterm birth was elevated for fathers that were older, and for fathers consuming certain prescribed drugs. The offspring health risks were by no means inconsequential. They included a 30% increased risk of childhood mortality for older fathers, an increase which could only be partly explained by increased adverse perinatal morbidities. Commonly prescribed medications such as analgesics, selective serotonin reuptake inhibitors, and angiotensin II receptor blockers were linked to reduced offspring gestational age, results that could be replicated using a distinct external cohort. These associations, although the mechanisms remain unelucidated, show that the hitherto overlooked paternal characteristics can affect the offspring and open for many new avenues of study.

Mechanisms of action

Infertility

Although the effects of paternal characteristics on the health of the offspring is not a field that has been studied extensively, there is one paternal characteristic that is established to affect infant health, namely paternal infertility. Children conceived through in vitro fertilization and through intra cytoplasmic sperm injection, the need for which is determined by both parents combined fertility, have higher risk for birth defects, preterm birth, and low birth weight. The contribution of risk from the father as compared to the mother regarding birth defects seems about equal, which can be deduced by looking at the risks after IVF (which skews more toward female infertility), and ICSI (used for male factor infertility). However, the risks of preterm birth and low birth weight do seem to skew toward female factors [127]. It is also generally understood that these risks do not originate from the assisted reproductive treatments themselves; they instead reflect the parental characteristics [128]. So, it seems that men with infertility have higher risks of offspring perinatal morbidities. In the context of this thesis, cancer, disease, concomitant medications and aging all detriment fertility, and were linked to offspring morbidities.

We also see a link between testicular cancer per se and infertility, in the literature [129], and in this thesis. The data from study II shows that 9.9% of men having undergone orchiectomy *only* as treatment conceived their children through assisted means (Results, Paternal anticancer therapies and risk of offspring congenital malformations, Table 4). If one compared that to those also treated with chemotherapy or radiotherapy, more than half of the increased rate of assisted reproduction use is seemingly related to the cancer and/or orchiectomy, instead of the presumed gonadotoxic treatments. Some studies have also indicated sperm parameter changes and sperm DNA damage in patients after diagnosis of cancer but prior to treatment, which the orchiectomy cannot explain [8,96–98]. Furthermore, study III shows that cardiovascular drugs, male sex hormones, erectile dysfunction, and antidepressants (among others) are associated with offspring preterm birth in the US cohort. These medications or the underlying indications have been associated with infertility as well [130–132]. And advanced male age also deteriorates fertility and is associated with adverse perinatal events among offspring [118,120].

One can therefore consider that paternal infertility and offspring perinatal morbidities are both forms of reproductive failure that often occur together. It is not clear if one phenomenon causes the other, or if they both are manifestations of underlying paternal reproductive characteristics such as high sperm DNA damage or abnormal DNA methylation.

Genetic damage

The works in this thesis were originally prompted by the fears that mutagenic antineoplastic therapies might cause genetic disease in the offspring of exposed men, as evidenced in experimental and animal studies. We do not detect such risks.

A possible explanation is the pairing of the high sensitivity of germ cells to apoptotic stimuli (such as antineoplastic therapies) and the high rates of pregnancy loss in humans [23,133]. Hypothetically, this could mean that major injuries to germ cells result in severe infertility, and the highly damaged sperms from a short post-treatment interval that fertilize an oocyte might only lead to an increase in essentially undetectable early pregnancy loss. However, smaller injuries to germ cells caused by chronic disease, aging or medications affect fertility to a lesser extent and might cause damage to sperms that is not so high as to lead to pregnancy loss, and instead manifest as perinatal morbidities. This could explain why we see a modestly increased risk of birth defects in the years preceding cancer diagnosis.

It is, however, more difficult to include preterm birth and low birth weight in mechanism. It could be mediated through sperm-DNA-damage induced embryonic mosaicism and the possible subsequent metabolically costly clearing of aneuploid cells, clinically manifesting as preterm birth. This would require that paternal chronic disease, medications, cancer, and aging be linked to higher levels of DNA damage (fragmentation), which is feasible, possibly through higher levels of reactive oxygen species. Further, this aligns with the above observation that infertility, which is highly related to sperm DNA damage, also is associated with preterm birth and low birth weight (and birth defects).

Epigenetic damage

Another possible mechanism for the mediation of risks associated with paternal characteristics that manifest as perinatal morbidities is through sperm epigenetics. Studies in humans have shown that paternal characteristics such as age affects the sperm epigenome [120]. Studies in rodents show intergenerational epigenetic effects linked to paternal obesity and diet [60]. Likewise, in humans, paternal obesity has been linked with low methylation of several imprinted genes important in normal embryonic growth and development [62,63]. If relatively harmless dietary changes can affect sperm epigenetics, then medications that directly affect metabolism (i.e. lipid lowering drugs), and others indirectly affect metabolism (selective serotonin reuptake inhibitors [134]) might also affect sperm epigenetics. High-dose folic acid supplementation alters sperm epigenetic profiles, which can be considered another proof of concept [113].

In broad terms, there is some evidence that paternal characteristics affect the sperm epigenome. There are theoretical and animal experiments that suggest that the sperm epigenome could affect embryonic development. As there are only two broad

transmission mechanisms that are most likely, through genetic damage and through epigenetics damage/alterations, both should be considered and studied. If one sees increases in preterm birth or other perinatal morbidities for a subset of fathers without them having high sperm DNA damage, then that would point to an epigenetic mechanism. There could be some degree of interplay between genetic damage and epigenetic alterations, and just like how many things can cause infertility, multiple mechanisms might act to mediate how paternal characteristics affect the next generation.

Clinical implications

Cancer and cancer treatment

We do find somewhat increased risk of congenital malformations in children fathered by men with cancer and with testicular cancer specifically, but the increases are in both cases rather modest and pertain to conditions that are rare. This is reassuring, and this information can be passed on to patients.

More reassuring information is that our data does not support the presence of the thus far assumed increased risk of malformations in offspring of men treated with irradiation and chemotherapy. Similarly, although we had few children born to fathers exposed to high numbers of chemotherapy cycles or to radiotherapy, we see no evidence that these treatments might increase the risks of birth defects.

Taken altogether, our results indicate that there is no cause for concern for men having had cancer or undergone cancer treatment regarding the health of their offspring. Nevertheless, with 8% of cancer survivors and 14% of testicular cancer survivors conceiving children by assisted reproductive techniques, presumably due to high levels of infertility after cancer and gonadotoxic treatments, *pretreatment sperm cryopreservation is still indicated* since no tools for reliable prediction of post-treatment recovery of spermatogenesis are available.

Meistrichs recommendation

In 1993, Meistrich published an opinion paper with the recommendation that men undergoing mutagenic antineoplastic therapies should refrain from conceiving children in the 0-6 months post therapy [37]. This was based on the experimental and animal studies that showed that there might be risks of birth defects during this window of time. Theoretically, these risks might be applicable to humans. Although we do detect any risks post cancer treatment, we have not specifically studied the short post treatment interval. We did see that in the 3 years preceding cancer diagnosis, and the 3 years post diagnosis, the rates of all and major malformations were essentially identical (published paper I, Table 2). We cannot deduce that it is

safe to father children during the short 6-month time interval, but on the other hand, there is no epidemiological evidence that suggests increased risks either. There could still be specific treatment types that are linked to risks. Or that in our studies the included number of children was too low in to detect any risk increases. However, in the latter case these risk increases are not substantial. Nevertheless, based on the results of this thesis, there is no support for cessation of pregnancy in the case that conception occurred shortly after termination of cancer therapy.

Abstaining from conceiving children during this relatively short time, might not substantially negatively impact patients. However, some patients do lose their fertility post treatment, sometimes permanently, so abstaining for short time might eliminate their last chance for natural conception, and if assisted reproduction by banked sperm fails, any conception. If sperm production resumes post treatment and the patient wants to achieve pregnancy (and having waited six months), then there is no evidence that natural conception is less safe than using assisted reproduction using either fresh or pre-treatment sperm, especially as spontaneous pregnancy avoids the potential risks associated with assisted methods. These factors should be considered and discussed with patients.

Paternal prescribed drug consumption

In study III, we see that some paternal medications are associated with increases in preterm birth. Preterm birth has a large effect on infant health. The majority of neonatal and infant morbidity and mortality is attributable to preterm birth [135]. It is linked with impaired childhood development and increased risk of future chronic disease [136–138].

The associations between paternal prescribed drugs can be used to better identify parents at risk of preterm birth and possibly of other adverse perinatal events. By stratifying antenatal care early in pregnancy, one could give better targeted advice, follow-up, and possibly tocolytic treatment to couples with higher risks of preterm birth and its many associated morbidities.

Paternal age

Advanced paternal age is not risk-free regarding offspring morbidities. With the trend of increasing parental ages worldwide, this issue is becoming more important from a public health perspective. Parents should be informed of the risks that delaying parenthood entails so that they can make cognizant decisions

Methodological issues

Study designs

My work on the effects of paternal cancer and the risk of congenital malformations was prompted by the Danish-Swedish register study that showed that children to fathers with a history of cancer had a 17% increased risk of severe congenital malformations. This study showed that paternal skin cancer, usually treated by a surgical excision, had a 40% increased risk of birth defects [13]. This was an early sign that inspired the hypothesis the increased risks observed might not be related to the anticancer therapies, but to some underlying factor such as the cancer itself.

Several study designs can be selected to test this hypothesis. Ideally, one would have access to data on anticancer therapies for men with cancer. One could then quantify and compare the risks of birth defects for the children to men who have not had cancer, to those who have had cancer - with and without - mutagenic treatments. Ideally, one could compare fathers with similar characteristics, such as those with the same type of cancer, but treated with or without chemo- and radiotherapy. However, if one does not have access to treatment data for the fathers with cancer, then these studies designs are not possible. Paper 1 was based on a database where treatment data was not available. Instead we only had access to paternal cancer diagnoses. That dwindles down the options one has for study designs.

One could, as done in the Danish-Swedish study, investigate the risk of birth defects for children fathered by men who have been diagnosed with different cancers forms, using the cancer diagnosis as a proxy for the different treatments the fathers receive (assuming that specific cancer types are generally treated similarly). The disadvantages with this approach are twofold. Firstly, even for cancers that generally are not treated with mutagenic treatments, such as skin cancer, some fathers in this group will still have received mutagenic treatments. Grouping by cancer type requires assumptions on an unmeasured primary exposure. Consequently, if a risk increase is detected associated with any cancer type, one will not be able to confidently distinguish between the effect of the cancer and the effect of the potential treatment. Secondly, such a study will likely not yield more information than the already conducted Danish-Swedish study.

Another option is to investigate the risk for children fathered by men who would after conception go on to be diagnosed with cancer. This approach has the strength that one can be certain that the fathers have not received any radio- or chemotherapy before conceiving their children. These father-children pairs are quite similar in characteristics to post-cancer father-children duos. There are, however, some differences in parental ages, infertility, and lifestyle factors (such as smoking), that is inescapable when looking at families per- and post paternal cancer diagnosis. The distribution of cancer types for men who have a history of cancer and those who

will be diagnosed with cancer after child conception will likely differ. This can be mended by comparing within the same cancer type as opposed to within the entire broad and heterogeneous disease of cancer.

There are some other drawbacks of this study design. The exposure (paternal cancer) occurs after the outcome (offspring birth defects). This is unconventional and can negatively affect the interpretability of the study. A counterargument is that we are investigating an underlying paternal quality such as cancer, or genomic instability, that might afflict the father many years before the conception of the child, and it's the link between that quality and subsequent birth defects that we are investigating. As there is no viable scientific mechanism of how an offspring birth defect might lead to subsequent paternal cancer, the arrow of causality likely flows from the father to the child. Therefore, paternal cancer should be the exposure, regardless if that event is recorded after the detection of the outcome. Conversely, one could instead view the offspring birth defect as a measurement of a paternal characteristic, analogous to a paternal infertility diagnosis (apt as it can be considered a failure of reproduction), and then investigate the risk of subsequent paternal cancer. We did perform this type of Cox regression analysis in paper I, though only as a sensitivity analysis as comparisons of risks for children conceived before and after paternal cancer are not possible through this Cox method.

Handling missing data

Paper I and II have similar research aims and use overlapping data. In paper I, almost 300 000 or 14% of children had missing data were excluded due to the use of full case analysis. In study II, these children were included by utilizing multiple imputation to impute missing values.

Full case analysis has the advantage that it is quick and easy to perform. If there is very little missing data, and if it is randomly scattered throughout the dataset, then full case analysis likely does not add significant biases. However, there are multiple disadvantages to using full case analysis. One is reducing the number of subjects in the analysis, which yield lower statistical power, as seen in paper I. If some subgroup of subjects is more likely than others to have missing data, then by excluding those one is introducing bias. There are more sophisticated methods, such as multiple imputation, that do not have these disadvantages, which are extensively used in epidemiological studies.

Multiple imputation as a method for dealing with missing data allows more subjects to be included, boosting statistical power with less bias. Though, multiple imputation is more complex and therefore the chance of human error increases. It is also more computationally expensive. One of the major pitfalls of multiple imputation is data that is not missing at random (an assumption one needs to make to justify the analysis). If there is a covariate that is included in the multiple

imputation model, that predicts the missingness of another covariate, than the model can correct for such missingness [139]. Problematically, if there is a systematic missingness to the data, then multiple imputation can give misleading results. There would be no way to correct for such a bias by multiple imputation.

Strengths and weaknesses of the studies

Registries

A major strength of the studies was the use of national Swedish registries with their impeccable quality. Reporting to these registries is mandatory according to law in Sweden, with many registries maintained by governmental agencies such as the Swedish Board of Health and Welfare. The ability to link Swedish residents using the Swedish personal identity number allows the data from vastly different sources to be collated, even linking across generations, and allowing the use of data on events occurring decades apart. The extent of the linking abilities, together with the quality and breadth of material in the registries make the Swedish (and other Nordic) registries a unique and exceedingly valuable research asset. For our use cases, these qualities allowed the use of high-quality data and gave sufficient statistical power to study even rarer exposures and outcomes. Study II was possible to conduct as we had access to complete and detailed anticancer treatment data from SWENOTECA, data that other studies on offspring birth defects following paternal cancer have been lacking [13,85–92,94].

A strength of study III was use of two large population-based cohorts, sourced from different contexts, with differing healthcare systems and differing health, genetic and lifestyle population profiles. It can also increase the certainty of the findings by using a robust statistical and analytical methodology that is extensively used in genome wide association studies. By first using the IBM MarketScan Research Database to generate possible candidates and then replicate those findings in the distinct Swedish cohort, one can be more confident that the associations found are not merely spurious.

A weakness for studies I & II were the short follow up of congenital malformation diagnoses from the Medical Birth Register. This could have been remedied with data from other registries. The Register of Congenital Malformations and the Hospital Discharge Register could have been used to identify children with malformations that were not diagnosed at birth during pediatric or neonatal examinations. We did have access to the Hospital Discharge Register, but the diagnosis codes were partly redacted by the Swedish Board of Health and Welfare for privacy concerns, and therefore did not allow for classification according to minor/major malformations. We opted, therefore, to not include those diagnoses in

the studies. It would have been possible to include them for the outcome “all malformations”, but at the expense of study clarity and simplicity. Furthermore, severe malformations (possibly apart from cardiac birth defects) are likelier to be diagnosed at birth, and therefore included in the Swedish Medical Birth Register. There will be some children that will have birth defects that will be misclassified as without malformations, but this likely doesn’t lead to bias as misclassification would have to be differentially distributed between fathers with and without cancer.

Another weakness in study II was that data on seminoma patients are lacking for the period 1995–2000. However, this misclassification results in the inclusion of children of men with seminomas in the control group and would therefore diminish the difference in malformation risk between TGCC offspring and the non-TGCC offspring. As the non-TGCC reference group was orders of magnitude larger than the TGCC group, the risk diminishing effect is likely small.

Confounding by indication

Study I, II, and III all showcase the quandary of deducing whether the measured risk is due to the paternal indication or the paternal treatment. It can be considered a strength of study I and II that careful study and analysis designs permit conclusions to be drawn on whether it is the cancer or antineoplastic therapies that is causing the increased risk of offspring birth defects. This was accomplished by keeping comparison groups as similar as possible. For example, comparing children to fathers who have been treated with chemotherapy to children to fathers that would later be treated with chemotherapy. The same methodology could be used to control for the severity of disease, by using the intensity of treatment (number of chemotherapy cycles) as a proxy for severity and comparing children to fathers with the same treatment intensity.

Conversely, a weakness of study III is that no such distinction on indication versus treatment could be made. With access to specific data on indication and treatment it would be possible to use the same types of analyses as in papers I and II to try to infer if the risks are linked to indication or treatment. Adjusting for currently uncontrolled confounding of general health characteristics (such as disease diagnoses) of the father could give more accurate results on whether it is the specific drug or the underlying general health of the father that is associated with preterm birth. But, for paper III, this was considered outside of the scope. Checking hundreds of prescription medications versus offspring preterm birth and using an external cohort to replicate findings is already a large undertaking. And using paternal medication status as a proxy for underlying disease without adjustment, elucidates the risks regardless of mechanism.

Another weakness of study III, that is related to the indication discussion above, is unmeasured confounding in general. This also pertains to maternal health

characteristics. Prior research has focused on maternal factors and the association with preterm birth. Maternal traits such as obesity, smoking and general health are well-known to affect neonatal health [140–142]. Maternal medications, which often overlap between preconception and gestation, have been studied with several associations noted [143]. As these maternal characteristics and medications are not biologically linked to paternal health and paternal use of prescribed drugs, and at most are transferable through a shared social setting, the reasoning for adjusting for these factors is unclear. We have, however, included maternal prescribed drug use in the adjusted models and in replication, which for most drugs lead to only a small attenuation of risk magnitude. It is unknown how well adjusting for maternal use of the same drug captures the overall state of maternal health.

Unanswered questions and future research

We see an association between paternal cancer and offspring birth defects. The biological mechanism this effect is mediated through remains to be elucidated. However, it is noteworthy that the observed risk is small in magnitude, and therefore likely difficult to study. Further, it can be asked if elucidating this mechanism will have any impactful clinical ramifications. After all, we conclude that there is no cause for concern regarding offspring malformations for men with cancer. Conversely, the same mechanism might be involved in other associations of interest, such as the risks of preterm birth, infertility, or pregnancy loss, and might therefore clarify the overall biological foundations of these factors.

An epidemiological study to investigate the risk of birth defects during the short time interval right after cancer treatment would be difficult to conduct. In paper 1, which included all children born in Sweden during two decades (almost 2M), had among them only around 500 children conceived in the 6 months post paternal cancer diagnosis. With an assumed 2.4% major malformation rate, 12 of those would have a major malformation. And that does not consider paternal cancer type or treatment type, only the presence of a cancer diagnosis. One would have to source exposed subjects from an astronomical population, with detailed treatment data, to be able to get sufficient statistical power to study risks during this short time interval.

Sperm DNA damage could be what mediates some or all the observed risks of perinatal morbidities. Therefore, it would be informative to see what the risks of different offspring perinatal morbidities are in relation to paternal sperm DNA damage levels. Likewise, do paternal factors influence sperm DNA damage levels? For example, do prescribed medications or disease progression change sperm DNA damage levels?

Sperm DNA damage could possibly affect the risk of preterm birth and other perinatal outcomes by increasing the rates of embryonic mosaicism. Not much is known about this phenomenon. Could some low level of mosaicism (as opposed to in utero growth restriction) persist throughout life and affect general health, analogous to the effect of senescent cells in aging?

One of the most exciting scientific questions that might be relevant to the works herein is whether there is epigenetic intergenerational heritability in humans. We see that some medications are associated with preterm birth. This could be mediated through epigenetic effects, such as alterations on sperm DNA methylation. Finding prescribed drugs that alter sperm DNA methylation in paternally imprinted regions, and then investigating these methylation regions in offspring could confirm such a mechanism.

In study III, the observed risks of preterm birth associated some paternal drugs were generally higher, in some cases much higher, in the US as compared to in Sweden. This could be due to differing parental characteristics in the two countries. Especially as many drugs associated with metabolic syndrome were significant in the US cohort, the extent and severity of obesity might explain some of the difference in risks observed.

Study III also prompts the question of how much of the observed paternal risks are due to unmeasured maternal factors. Some diseases like metabolic syndrome are to a large extent due to lifestyle (through diet, exercise, smoking and sedentary behavior). These factors are likely shared within a social setting as within a couple. So, associations between prescribed drugs for metabolic syndrome might just capture the risks of mothers with suboptimal lifestyle. However, this might apply to medicines for metabolic syndrome, but is more difficult to apply to other classes of drugs not “transmissible” through a shared social setting.

If prescribed paternal medications increase risks of preterm birth and such effects are mediated through similar mechanisms that also increase the risks of birth defects, then it is possible that some paternal medications increase the risks of congenital malformations. A similar screen of all drugs as conducted in study III, but using birth defects as the outcome, might yield important results.

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The health of the children in relation to paternal age, cancer, and medication



Yahia Al-Jebari holds a Master of Engineering from University College London, United Kingdom. His doctoral thesis pertains to the impact of paternal characteristics on the health of the next generation. The main overarching finding is that the father, his age, his disease, and his medications have profound effects on the health of his children, as evaluated in several studies investigating different perinatal outcomes.

