1 Introduction

1.1 History of DES

The history of Diethylstilbestrol (DES) goes back to the year 1938 when DES was first synthesized by the biochemist Dodds\(^1\). DES is a nonconjugated estrogen and resembles the action of steroidal estrogens. It was the first estrogen that could be orally administered, without loss of its activity, and moreover it was relatively inexpensive to produce. In 1948, the use of DES as a drug to prevent fetal loss was investigated in a study among 632 pregnant women\(^2\) and the researchers concluded that DES was effective against miscarriages. Based on the outcome of this study, DES was introduced into clinical practice for the prevention of pregnancy complications (like a history of abortion, and threatened complications as abortion, toxemia, intra-uterine death and premature delivery) and became widely accepted in a short period of time. Subsequently, DES was prescribed to several millions of pregnant women worldwide from the late 1940s to the early 1970s. In the United States and Europe (especially in the United Kingdom, Germany, France and the Netherlands) several million women have used DES during pregnancy. The general belief was that DES was harmless, and that its beneficial effects might even result in “bigger and stronger babies” (see figure 1.1). DES therapy usually started with five milligram per day during the sixth or seventh week of pregnancy, increasing the dose by five milligram every 2 weeks until the 15th week and with a further dose increase by five milligram thereafter, until the therapy stopped at 150 milligram per day in week 35, according to the Smith and Smith protocol\(^2\). However, many alternative treatment regimens have been described. In 1953, Dieckman et al conducted a double blind placebo-controlled trial in the United States among 840 women who received DES and 806
women who received a placebo, aimed at determining the effectiveness of DES in reducing the risks of abortion, prematurity, postmaturity, prenatal mortality or pregnancy toxemia. The conclusion was that DES was not effective. Remarkably, despite these findings, DES continued to be used. It was not until 1970 that the first adverse health effects of DES exposure became evident. Herbst and colleagues reported on a cluster of seven young women (aged 15 to 22 years) with adenocarcinoma of the vagina who had been seen at the Massachusetts General Hospital between 1966 and 1969. Six of these tumors appeared to be clear cell adenocarcinomas (CCAs) and one was an endometroid tumor. This clustering of cases was remarkable since vaginal cancer is a rare cancer and, at that time, predominantly a disease of older women. Subsequently, Herbst et al conducted a matched case-control study including the same CCA cases and one extra case (8 in total). Each case was individually matched to 4 controls by hospital- and date of birth (within 5 days). A striking finding was that seven of the eight mothers of patients with CCA had used DES during pregnancy, whereas no mothers in the control group had used DES. In the years following, many other transplacental adverse health effects of DES in DES daughters became manifest. In the same year (1971), shortly after these alarming findings, DES was contra-indicated for the use during pregnancy by the US Food and Drug Administration. Remarkably, in the Netherlands it lasted till 1975 before the Dutch authorities officially banned DES for the use in pregnant women. In the Netherlands, the first Dutch publication on adverse health effects of DES was a report of a meeting by the Dutch Organization of Obstetrics and Gynecology (NVOG) on March 24, 1973, published in the “Nederlands Tijdschrift voor Geneeskunde”6. The results of the study of Herbst were discussed by Vooijs and other gynecologists (among whom was Plate, who was known to be an important advocate of the prescription of DES). In 1975, a paper was published by Stolk and Vooijs in the same journal with recommendations for surveillance of DES daughters7.

Foundation of DES Action group

In response to all public commotions about negative effects of DES use in the breeding of cattle and the surprising observation that merely no attention was paid to the DES problem in women, the DES Action group was founded in the Netherlands in 1981. Despite the fact that Dutch medical journals had paid some attention to the adverse effects of DES, the majority of general practitioners and gynecologists had a lack of knowledge about the management of DES daughters. In some patients this lack of knowledge has lead to mistreatment (e.g. patients with
adenosis who were treated with cryosurgery, with cervical stenosis as a consequence. So, one of the important spearheads of the DES Action group was to educate both medical doctors and the public about the negative health effects of DES. Two milestones in this respect were the set up of the Working Group “Beleidsbepaling DES-problematiek” (as part of the NVOG) and the organization of a network of gynecologists specialized in the counseling and treatment of DES daughters in 1982 (“DES netwerk”). Another main ambition of the DES Action group was the tracing of DES-exposed individuals. Through large media campaigns women born between 1947 and 1975 were called on to check with their mothers whether they had possibly been exposed to DES and, if positive or in case of doubts, to trace their mothers’ medical file. Currently, the DES Action group, in the meantime transformed from an action group to an organization for the protection of DES interests (the DES Center), still plays an important role in supporting DES-exposed individuals and physicians and to encourage scientific research of the long-term health effects after DES exposure.

**DES registry**

In 1992, a change in civil law shortening the period of liability urged the need for registration of all DES-involved individuals in order to guarantee future health claims for compensation by the pharmaceutical industry. A big media campaign in 1992 resulted in registration of more than 17,000 DES-exposed individuals (among whom were 13,500 DES daughters). All DES-exposed persons were advised to register, not only individuals with potentially DES-related problems, but also healthy individuals because of possible health problems in the future (at that time, DES daughters were still quite young and registration was required to be able to receive compensation for future health claims). In the following years, registration was still possible for people who newly discovered that they were DES-exposed. At registration it was advised to trace the mother’s medical file but documented DES exposure was not required. Data were collected on name, address, date of birth and date of registration. In many cases, the mother’s medical file at time of registration was already untraceable or destroyed. In the Netherlands, DES had been prescribed not only by gynecologists, but also on a large scale by general practitioners, which made the efforts to trace the medical file quite laborious, or even infeasible.
DES Fund
In 1986, six DES daughters with CCA of the vagina started a lawsuit against ten pharmaceutical industries that had marketed DES. Because it appeared impossible for each woman to prove which specific company produced the medicine their mother had been taking, the Supreme Court decided that the pharmaceutical industries had collective liability. Subsequently, in order to avoid long-lasting lawsuits, a settlement was made with a couple of pharmaceutical companies, leading to the foundation of the DES Fund in 2000. In the settlement different categories of “damage” (DES-related adverse health outcomes) were defined and for each category a specific allowance was decided upon\(^9\).

The NCI-DES study
In 1975, a cohort study was started in the United States to provide DES daughters with yearly gynecological examinations in order to check upon potential health outcomes, cervical and vaginal abnormalities in particular, and cancer (the the national cooperative Diethylstilbestrol Adenosis (DESAD) cohort)\(^10\). In 1990, this cohort and three other cohorts (female offspring of the women treated with DES in the Dieckmann trial\(^11\), female offspring of DES-exposed women participating in the Women’s Health Study\(^12\) and offspring of mothers treated in a private obstetric clinic, the Horne cohort) were combined into one overall cohort: the National Cancer Institute’s Combined cohort study, later referred to as NCI-DES study. In the most recent paper on the NCI-DES study, the cohort consisted of 4,817 DES-exposed daughters and 2,073 unexposed controls (including the Horne cohort)\(^13\). The NCI-DES follow-up study is the only cohort of DES daughters in the world. DES exposure was validated by the mother’s medical record for all cohort members.
1.2 Adverse health effects in DES daughters

Clear cell adenocarcinoma of the vagina/cervix
In the United States, shortly after the first publications on the strongly increased risk of CCA of the vagina/cervix risk of DES daughters, the Registry for Research on Hormonal Transplacental Carcinogenesis was established to study the clinical, histopathological and epidemiological characteristics of patients with CCA born after 1948 (including both women with and without prenatal DES exposure)\(^{14,15}\). The risk of CCA before age 34 was estimated at 1 per 1,000, based on the absolute number of CCA patients and sales figures in the US\(^{16}\). Several risk factors for DES-associated CCA were identified, among which were maternal history of spontaneous abortion, early timing of exposure to intrauterine exposure to DES (before week 12), fall season of birth and prematurity\(^{17,18}\). Up to 2007, 760 CCA patients have been recorded in the registry, two-third of whom were DES-exposed (http://obgyn.bsd.uchicago.edu/registry.html). In 1975, a registry of CCA patients was started in Nijmegen, the Netherlands\(^7\) and was recently updated (n=140 patients in 2005 of whom at least 76 women could be proved to be exposed to DES in utero)\(^{19}\).

Anatomic abnormalities
Soon after the finding of an increased CCA among very young DES daughters, other non-malignant anomalies became apparent, like the occurrence of glandular cells in the vagina (adenosis)\(^{20}\) and anatomical malformations of the genital tract, including transverse vaginal ridges, cockscomb (ridge on anterior cervix), cervical collars, hoods, polypoid defects, hypoplastic cervix and uterine cavum malformations (like a T-shaped uterus, which was typically observed in DES daughters)\(^{21-25}\).

Cancer other than CCA of the cervix/vagina
Besides the increased risk of vaginal and cervical CCA, a slightly increased risk of breast cancer was observed among DES daughters older than 40 years compared to unexposed women participating in the NCI-DES study (Relative risk (RR)=1.83, 95% Confidence interval (CI)=1.1-3.2)\(^{26}\). The risk of others cancers was not increased. So far, risks for squamous cell cervical cancer and melanoma have not been evaluated, since information on cancer in the NCI-DES study was based on self-reported data and only partially verified by medical records\(^{26,27}\). The
agreement between self-reported cancers and medical verification for cervical cancer and melanoma appeared to be poor (22% and 58%, respectively) and therefore these tumors were not included in the reports of the NCI-DES study\textsuperscript{27}.

**Cervical dysplasia**

A two-fold risk of cervical dysplasia was observed in the NCI DES Follow-up study\textsuperscript{28,29}. A recent analysis of the same cohort, with longer follow-up, confirmed this finding, with a nearly three-fold increased risk among DES daughters who also had typically DES-related vaginal epithelial changes, such as adenosis and metaplastic squamous epithelium\textsuperscript{13}. The latter anomaly is considered as the physiological transition from glandular tissue (adenosis) to normal epithelial tissue in the vagina and ectocervix (enlarged ectropion, wider transformation zone). It has been suggested that the areas of vaginal adenosis and the wider transformations zone in DES daughters may be more susceptible to carcinogenic factors such as HPV infection\textsuperscript{29}.

**Reproductive dysfunction**

Related to the malformations of the reproductive tract, reduced fertility and unfavorable pregnancy outcomes were observed in DES daughters\textsuperscript{11,30-33}. Whereas earlier reports showed conflicting results regarding infertility in DES daughters\textsuperscript{33,34}, a recent follow up of the two cohorts previously studied (Dieckmann and DESAD) showed an increased risk of infertility in DES daughters compared to unexposed women (RR=1.8, 95CI%=1.6-2.1)\textsuperscript{31}. The risk of infertility was highest among women with uterine and tubal problems as cause of infertility (RR=7.7, 95%CI=2.3-25 and RR=2.4, 95%CI=1.2-4.6). In this analysis women had a median age of 42 years. Unfavorable pregnancy outcomes more often occurred in DES daughters than in unexposed women\textsuperscript{32,35}. The most recent analysis on reproductive outcomes among participants of the NCI-DES study was conducted in 2000 and the average age at the end of follow-up was 45 years of age, so the majority of women was at the end of their reproductive life-span\textsuperscript{32}. In this analysis 1,269 DES daughters identified through prenatal records and 1,240 DES daughters identified through referral (see previous section for more details about this study design) were compared to 838 unexposed women. Compared to the unexposed women, DES daughters identified through record review had an increased prevalence of preterm delivery (7.5%, and 19.4% RR=2.93, 95%CI=2.23-3.86, respectively), more first-trimester spontaneous abortions (23.5% and 29.6%, respectively, RR=1.31, 95%CI=1.13-1.53), more often second-trimester
pregnancy loss (1.6% and 6.3%, respectively, RR=4.25, 95%CI=2.36-7.66) and more ectopic pregnancies (1.9% and 7.1%, respectively, RR=3.84, 95%CI=2.26-6.54). Similar results were observed for the DES referral group compared to the unexposed women⁴².

**Third generation effects**

Animal studies have suggested that the teratogenic effect of DES might be transmitted to next generations³⁶. Subsequently, a few studies examined transgenerational adverse effects of DES in humans. In a study examining the risk of reproductive tract abnormalities among adult daughters of women exposed to DES in utero, not a single malformation was detected³⁷. A higher risk of hypospadias among sons of DES daughters had been suggested³⁸-⁴¹ but one study with the largest number of verified DES-exposed grandsons found no effect⁴². Other congenital malformations like urinary anomalies and heart defects were only examined in the NCI DES Follow-up study, with a slightly increased risk of heart anomalies observed, but these results may be flawed by reporting bias.

In general, transgenerational effects of DES exposure in utero (other than hypospadias) have been mainly studied in animal studies³⁶. Increased risks of uterine tumors have been found in both the F1 (DES daughters) and F2 (DES granddaughters)⁴³-⁴⁴, although the incidence was lower in the F2 generation. Furthermore, F1 mice differed from F2 mice, in the sense that they were subfertile whereas F2 were normally fertile⁴⁴.

### 1.3 Screening of DES daughters

Because of their increased risks of vaginal and cervical cancer, DES daughters have been advised to undergo regularly screening in order to early detect and treat (pre)cancerous lesions. In the United States, DES daughters have been recommended (since 1978) to have annual gynecological examinations existing of elaborate pelvic examination including a Pap smears (four quadrants of the vagina combined with ectocervical and endocervical smears) and iodine staining⁴⁵-⁴⁶. Colposcopy is only indicated as part of the initial examination or in case of abnormal cytology or extensive epithelial changes. The recommended age at start of screening was 14 years. No separate guidelines were given for women with or without DES-related malformations⁴⁵-⁴⁷. In the Netherlands, a guideline for the surveillance of DES daughters was developed in 1975 by a Dutch working group of gynecologists⁷,⁴⁸. The recommendations
concerned cytological examinations (four quadrants of the vagina and endo/ecto cervix), combined with colposcopy if possible. Biopsies were only indicated in case of abnormal cytological findings. Women were advised to have yearly examinations and when adenosis was present twice a year. The start of screening was set initially at age 10, but was later changed into age at menarche or age 14 if menarche had not occurred already (whichever came first). In young girls, the examinations were done under anesthesia. In 1992 the guideline was revised, with a shift in focus. Whereas the earlier guideline mainly paid attention to the prevention of cancer, the revised guideline also focused on medical counseling with respect to fertility and pregnancy, especially in women with DES-related malformations. In table 1.1 the different recommendations of the current guideline are summarized. Depending on the absence or presence of DES-related malformations and (un)certainty about DES exposure, differential recommendations have been given. At the initial examination cytological, colposcopic and gynecological examinations of the cervix and vagina have been recommended. Women with DES-related malformations have subsequently been advised to have these three examinations each year, with colposcopy left out after five years. Women without DES-related malformations have been advised to have yearly examinations during the first 5 years, with referral to the general population screening program after five years.

<table>
<thead>
<tr>
<th>DES exposure</th>
<th>DES-related malformations†</th>
<th>Cytological examination†</th>
<th>Colposcopy</th>
<th>Gynecological examination†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certain</td>
<td>Present</td>
<td>Yearly</td>
<td>Yearly during first 5 years, thereafter only by indication</td>
<td>Yearly</td>
</tr>
<tr>
<td>Certain</td>
<td>Absent</td>
<td>Yearly during first 5 years followed by general population screening†</td>
<td>-</td>
<td>Yearly during first 5 years</td>
</tr>
<tr>
<td>Uncertain</td>
<td>Present</td>
<td>Yearly</td>
<td>Yearly during first 5 years, thereafter only by indication</td>
<td>Yearly</td>
</tr>
<tr>
<td>Uncertain</td>
<td>Absent</td>
<td>General population screening†</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*DES-related malformations were defined as altered vaginal fornik, collar, transverse or longitudinal vaginal septum, cockscob, hypoplastic cervix, pseudopolyp, collar, vaginal ridges, adenosis, extensive transformation zone.
†Cytological examination: vaginal and cervical pap smears.
‡Gynecological examination: palpation of the vagina, uterus and adnexae.
The main differences with the US guidelines have been 1) separate recommendations for women with and without DES-related malformations and, 2) colposcopy during first 5 years after start screening. The US guideline recommends colposcopic examination at initial examination and thereafter only by indication.

**General population screening**

In the Netherlands, the national screening program for cervical cancer was established in the 1980s, initially inviting all women aged 35-55 every 3 years. After a reorganization of the program in 1996, the invitational age range was broadened from 30 to 60 years and the time-interval between two consecutive screening tests was extended to five years, with the cumulative number of screenings for each woman left unchanged. The Dutch DES screening differs from the national population screening with respect to the cytological screening of the vagina, which is no part of the regular screening program, the early age at start of the screening and the high frequency.

### 1.4 Aims and outline of the thesis

The purpose of the studies described in this thesis is to investigate whether women who were exposed to DES *in utero* and their offspring have long-term adverse health effects.

More specifically, these aims are:

1. to assess long-term risks of prevalent and incident cancer compared to the general population (Chapters 2.1 (DES Center prevalence study) and 2.2 (DES-net study), respectively)
2. to investigate the long-term risk of pre-invasive cervical lesions compared to the general populations (Chapter 3)
3. to examine the effectiveness of screening among DES daughters (Chapter 4)
4. to assess the risk of congenital malformations among children of DES daughters compared to children of non-exposed subfertile women (OMEGA-study, Chapter 5.1) or compared to children of non-exposed sisters and external reference data (DES-net study, Chapter 5.2)
### Table 1.2 Overview of study designs used in this thesis

<table>
<thead>
<tr>
<th>Study name</th>
<th>DES-net (Chapters 2.2,3,4,5.2)</th>
<th>OMEGA (Chapter 5.1)</th>
<th>DES Center prevalence study (Chapter 2.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study aim</td>
<td>Long-term health effects after DES exposure in utero</td>
<td>Long-term health effects after treatment with assisted reproductive techniques</td>
<td>Inventory of health problems</td>
</tr>
<tr>
<td>Study design</td>
<td>Retrospective cohort study with prospective follow-up</td>
<td>Retrospective cohort study</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Study population</td>
<td>Registered at the DES Center, n=12,182</td>
<td>Fertility clinics, n=157 DES-exposed women with n=205 sons</td>
<td>Registered at the DES Center, n= ±13,500</td>
</tr>
<tr>
<td>DES daughters</td>
<td>Registered at the DES Center, n=12,182</td>
<td>Fertility clinics, n=157 DES-exposed women with n=205 sons</td>
<td>Registered at the DES Center, n= ±13,500</td>
</tr>
<tr>
<td>Unexposed women</td>
<td>Sisters of DES-exposed participants, n=1,744</td>
<td>Fertility clinics, unexposed to DES n=6,494 women and n=8,729 sons</td>
<td></td>
</tr>
<tr>
<td>Questionnaire</td>
<td>16-page questionnaire on DES exposure, reproductive history, medical history, risk factors hormone-related cancer, congenital malformations children</td>
<td>23-page questionnaire on cause of subfertility, reproductive history, medical history, risk factors hormone-related cancer, congenital malformations children</td>
<td>8-page questionnaire on DES-related health problems</td>
</tr>
<tr>
<td>Verification DES exposure</td>
<td>Medical file DES mother (10%)</td>
<td>Self-reported DES exposure “verified” by subfertility treatment records (62%). Information on timing of exposure (for hypospadias cases only) by telephone interview</td>
<td>No verification</td>
</tr>
<tr>
<td>Outcome</td>
<td>Incidence (fup)</td>
<td>All cancers</td>
<td>Cervical/vaginal dysplasia</td>
</tr>
<tr>
<td>Prevalence</td>
<td>DES-related malformations</td>
<td>Congenital malformations children</td>
<td>Hypospadias children</td>
</tr>
<tr>
<td>Verification outcome</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reference population</td>
<td>Internal</td>
<td>Unexposed sisters</td>
<td>Unexposed subfertile women</td>
</tr>
<tr>
<td></td>
<td>External (general population)</td>
<td>NCR (cancer) PALGA (dysplasia) EUROCAT/Rotterdam-study (congenital malformations)</td>
<td>----</td>
</tr>
</tbody>
</table>

Abbreviations used: NCR=Netherlands Cancer Registry, ECR=Eindhoven Cancer Registry, PALGA=the nationwide network and registry of histo- and cytopathology in the Netherlands, EUROCAT= European Registration of Congenital Anomalies, Rotterdam-study=a study examining the prevalence of hypospadias among male newborns in Rotterdam (Pierik FH et al. Hum Reprod 2002 April;17(4):1112-5).
1.5 Study design

The present thesis is the result of analyses conducted within the framework of three different studies. The main study concerns the DES-net project, which is a nationwide retrospective cohort study of DES daughters with prospective follow-up. The second study is the OMEGA-study, a retrospective cohort study on the long-term health effects of subfertility treatment. The third study is a cross-sectional study based on a survey conducted by the DES Center in order to monitor health problems of women registered at the DES Center: the cancer prevalence study. A summary of the three study designs is listed in table 1.2 and will be discussed in more detail in sections 1.5.1-1.5.3.

1.5.1 DES-net cohort study

Background
The DES-net project (DES-net is the acronym for DES Netherlands) was initiated to examine the long-term health risks in women exposed to DES in utero. The Netherlands is particularly well suited for setting up a new cohort of DES daughters, because (1) DES has been widely prescribed (an expected number of 40,000-100,000 DES-exposed daughters), (2) a registry of daughters exposed to DES in utero, based on self-referrals, was established in 1992 (DES Center registry), and (3) the country is covered by municipal registries and the population-based Netherlands Cancer Registry (NCR, since 1989), thus enabling long-term follow-up.

Study participants of DES-net
The data collection for this cohort started in March 2000 (see figure 1.2). The DES daughters in this cohort were identified through the registry of the DES Center (see also 1.1). Preceding the mailing to all cohort members in March 2000, extensive tracing techniques were used to obtain current addresses of all participants (Fall 1999). Cohort members were traced through linkage with data of the largest mailing company in the Netherlands (Cendris). When no current address was available, we sent letters to the population office of the last known municipality of residence, requesting for information on address and vital status. In case a person had moved more three times to different municipalities, we assigned the address as missing. A total of
13,714 invitation letters and paper questionnaires were mailed in March 2000. Until 2005 women who newly entered the registry of the DES Center from 2000 onwards, were also sent a questionnaire.

![Diagram](Figure 1.2 Design of the DES-net cohort study)

**Questionnaire data**

Information on risk factors for hormone-related cancers and medical history was collected by means of a 16-page self-administered questionnaire (see thesis supplement, pages 227-242). The questionnaire included two informed consent forms: 1) to obtain permission from the DES daughter for abstracting data from the medical records and for obtaining data from population-based disease registries and 2) in case no documentation of DES exposure could be provided, to obtain permission from the mother to validate *in utero* DES exposure through her medical record. Non-responders were sent a reminder after 2 months and a second reminder after 6 months. The final response rate to the questionnaire was 61%.

**Identification of non-exposed sisters**

The internal reference group in the DES-net project consists of sisters of DES daughters who were not exposed to DES *in utero*. Sisters were chosen as a comparison group because they share part of the genetic, *in utero* and lifestyle background with their DES-exposed sisters. In
November 2004, all participating DES daughters who reported one or more unexposed sisters in the questionnaire, were asked to invite their sisters to participate in our study. Subsequently, we sent an invitation letter to the 2,094 unexposed sisters (who were reported to the researchers by the DES-net participants), accompanied by a questionnaire and a leaflet, of whom 1,889 responded by returning the questionnaire.

**Assessment of exposure**

Women were asked to provide a copy of their documented DES exposure. In case no documentation was available, the mother was asked to give written informed consent to have her medical record traced by the researchers. It appeared extremely difficult to trace mothers’ medical records since many medical files of the period 1947-1975 appeared to have been destroyed. The tracing of the files was complicated by the fact that DES has been prescribed not only by gynecologists but also on a large scale by general practitioners in the Netherlands. Based on results from pilot studies (see 6.3.3), we decided to discontinue the tracing of mothers’ medical files and to include all women in the analyses, irrespective whether DES exposure was medically verified.

**Assessment of health outcomes**

Information on cancer incidence was obtained from the Netherlands Cancer Registry (NCR) and PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands.

The NCR is a nationwide population registry which was established in 1989; information is available on all newly diagnosed invasive tumors, characteristics of the tumor (topography, morphology, stage) and primary treatment. Information on cancer prevalence was retrieved from the Eindhoven Cancer Registry (ECR), since this registry had data on cancer prevalence available from earlier years (1970 onwards).

PALGA is a nationwide database of excerpts of all histopathology and cytopathology reports, with nationwide coverage since 1989. All cancers with a histologically confirmed diagnosis are recorded in PALGA. Cancers that were reported in the questionnaire and occurred before 1989 were verified by medical file. Information on cervical intra-epithelial neoplasia (CIN) was also retrieved from PALGA. Information on type of cervical examination (smear or histology by excision or biopsy), date of examination and outcome was obtained. For each woman the first occurrence of the highest grade of histologically confirmed CIN was used.
in the analysis. Reported DES-related diseases (defined as adenosis, squamous cell metaplasia, transverse vaginal ridges, cockscmb, cervical collars, hoods, pseudo-polyps, hypoplastic cervix, uterine cavum malformations and tubal malformations), cervical dysplasia and cancer were verified by medical file. All congenital malformations reported in the questionnaire among participants’ children were verified by medical file. Information on vital status was obtained from the Central Bureau of Genealogy (CBG), a nationwide registry of all deceased Dutch citizens.

Screening data
From the PALGA database we also extracted information on all cervical and vaginal cytological and histological examinations. We collected information on date, type of examination (smear or biopsy), localization (vagina or cervix) and outcome (type of lesion, morphology). Two screening outcomes were used in analysis: 1) having had a Pap smear (vaginal or cervical) within the 5-year interval preceding diagnosis and 2) time since operationally last negative smear\textsuperscript{51,52}.

<table>
<thead>
<tr>
<th>Follow-up linkage</th>
<th>End of follow-up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCR</td>
<td>December 2005</td>
<td>Cancer, all types</td>
</tr>
<tr>
<td>PALGA</td>
<td>November 2008</td>
<td>Cancer, carcinoma in situ, dysplasia cervix/vagina, screening history (number of smears, type of smear, outcome)</td>
</tr>
<tr>
<td>CBG</td>
<td>October 2007</td>
<td>Vital status</td>
</tr>
</tbody>
</table>

1.5.2 OMEGA cohort study

Background
The OMEGA cohort study was started in 1995 to examine the long-term risks of ovarian and other hormone-related cancers in women who received IVF treatment\textsuperscript{53}. In 1983, IVF treatment was introduced in the Netherlands. It has been hypothesized that the use of fertility drugs and IVF-treatment itself might be involved in the development of hormone-related cancers\textsuperscript{54}. Triggered by two case-reports of sons born to women with a history of prenatal DES
exposure, the association between the prevalence of hypospadias among DES daughters was examined within the OMEGA cohort.

**Study participants (exposed and non-exposed)**

During the period 1980-1995, a retrospective cohort of 26,428 women diagnosed with subfertility problems was identified through all twelve Dutch IVF-hospitals with legal permission to provide this treatment. To obtain a comparison group of subfertile women who were not treated with IVF, women were selected from four (of the twelve) participating IVF-clinics that had kept a registry of all subfertile women. A total of 19,136 IVF-exposed women and 6,588 non-exposed women were included in the study. For the analyses described in this thesis (Chapter 5.1), a cohort of offspring of the OMEGA cohort members was set up. In total 463 DES daughters participated in the OMEGA study of whom 157 had at least one liveborn son.

**OMEGA children’s cohort**

For the purpose of the study described in this thesis, the OMEGA children’s cohort was established based on all reported pregnancies in the questionnaires. Miscarriages and stillbirths were excluded from the analyses, as were children with missing gender and unknown birth date. Finally, girls were excluded (because the analyses was focused on the risk of hypospadias which only occurs in boys), leaving 8,934 male live births for analysis.

**Questionnaires**

All women received a 23-page risk factor questionnaire through their gynecologists. Non-responders were sent a reminder after 4-6 weeks and were subsequently approached by telephone once. Through the questionnaires, information was collected on women’s reproductive history, history of IVF treatment, the use of exogenous hormones, medical history and lifestyle factors like smoking, alcohol use and physical activity. Furthermore, written informed consent was asked for future linkage with the Netherlands Cancer Registry and for data-abstraction from women's medical records. From the medical records detailed information was collected on type of fertility treatment, cause of subfertility, duration of subfertility and other medical conditions.
Assessment of exposure

DES exposure was verified by subfertility treatment records for 61% of the sons of participating DES daughters. Additionally, information on timing of maternal DES exposure (first trimester yes/no) was collected for the 4 DES grandsons with hypospadias.

Assessment of outcome

The reported cases of hypospadias were verified by medical file after written informed consent was obtained from the mother. Detailed information was collected on date of diagnosis, severity of the malformation and surgical procedures. The sons born to (subfertile) DES daughters were compared with non-exposed children born to subfertile women participating in the OMEGA cohort.

1.5.3 DES Center study on prevalence of cancer

Background

In 1994 a survey was conducted among all 13,500 DES daughters registered at the DES Center to make an inventory of all experienced health problems. Data from this survey were used to examine the risk of prevalent cancer other than clear cell adenocarcinoma of the vagina/cervix.

Study participants

The DES daughters in this cohort were identified through the registry of the DES Center.

Assessment of exposure

All women were registered at the DES Center. No additional data were collected with respect to DES exposure.

Assessment of outcome

Cancers were self-reported in the questionnaire and verified by medical files after informed consent was obtained. Information was collected on date of diagnosis, topography and morphology of the tumor. The numbers of prevalent cancers (diagnosed in the period 1974-
1994) were compared with age-, sex- and period-specific data on prevalence from the Eindhoven Cancer Registry.

1.6 Outline of this thesis

Chapter 2 of this thesis describes long-term cancer risk in DES daughters, examined in the DES Center prevalence study (2.1) and the DES-net study (2.2). In chapter 3 results are presented on the risk of cervical intra-epithelial neoplasia in combination with invasive cervical cancer in DES daughters compared to the general population. In chapter 4, results are reported from a nested case-control study examining the effectiveness of cervical and vaginal screening in DES daughters. Chapter 5 describes the results of two studies on the risk of hypospadias among sons of DES daughters. The first study was conducted within the OMEGA cohort (5.1) and the second study as part of the DES-net cohort study (5.2). In chapter 6 the main findings are summarized and placed in a wide context by comparison with the literature. Also, several methodological issues concerning the performed studies are discussed, conclusions are drawn and recommendations for future research are given.
References


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