Long-term health effects after DES exposure in utero

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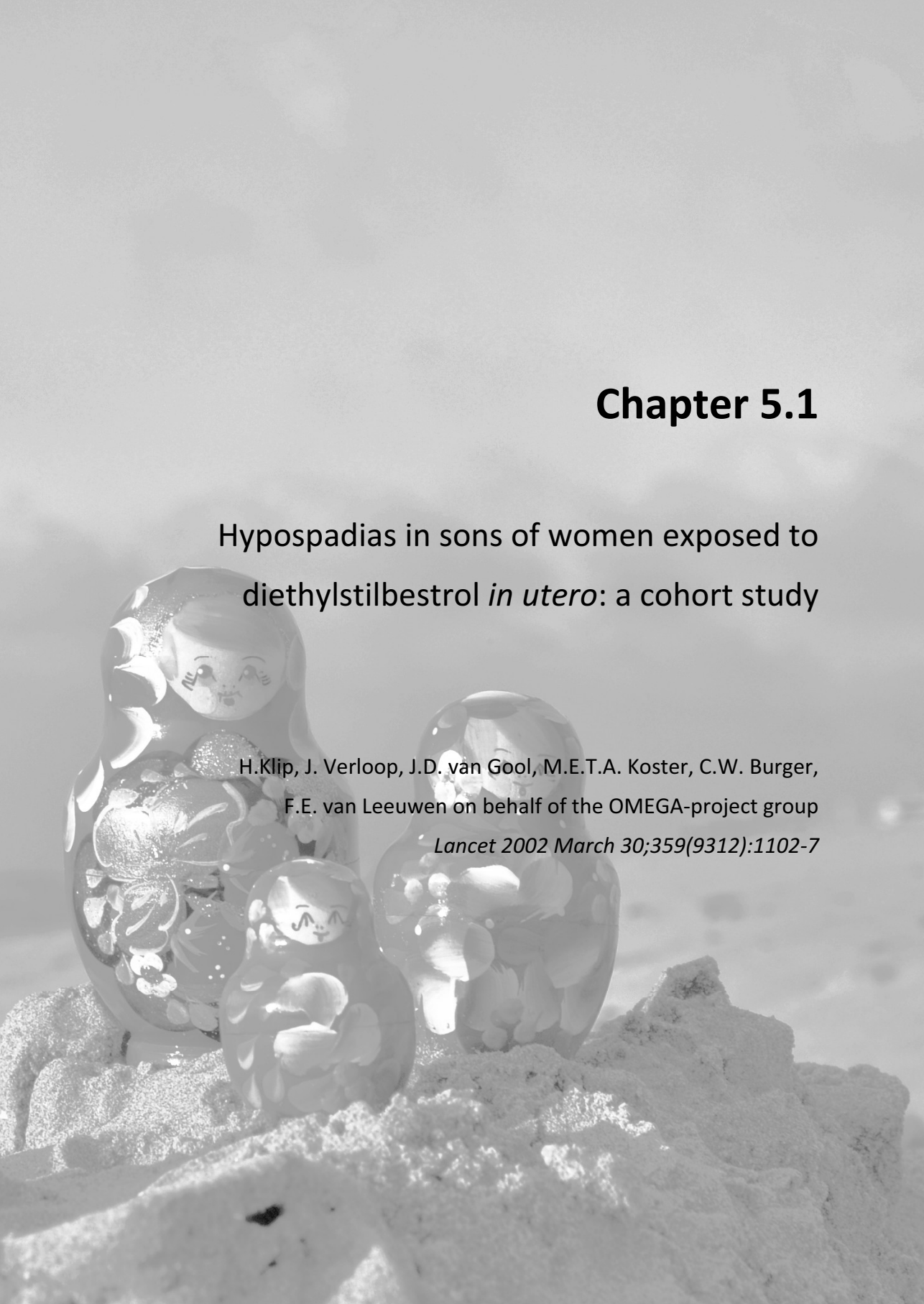

Chapter 5.1

Hypospadias in sons of women exposed to diethylstilbestrol *in utero*: a cohort study

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5.1.1 Summary

Background

Transgenerational effects of diethylstilbestrol (DES) have been reported in animals, but effects in human beings are unknown. Alerted by two case reports, we aimed to establish the risk of hypospadias in the sons of women who were exposed to DES *in utero*.

Methods

We did a cohort study of all sons of a Dutch cohort of 16,284 women with a diagnosis of fertility problems. We used a mailed questionnaire assessing late effects of fertility treatment to identify boys with hypospadias. We compared the prevalence rate of hypospadias between boys with and without maternal DES exposure *in utero*.

Findings

16,284 mothers (response rate 67%) reported 8,934 sons. The mothers of 205 boys reported DES exposure *in utero.* Four of these children were reported to have hypospadias. In the remaining 8,729 children, only eight cases of hypospadias were reported (prevalence ratio=21·3 [95%CI=6·5-70·1]). All cases of hypospadias were medically confirmed. Maternal age or fertility treatment did not affect the risk of hypospadias. Children conceived after assisted reproductive techniques such as in-vitro fertilisation were not at increased risk of hypospadias compared with children conceived naturally (PR=1·8, 95%CI=0·6-5·7).

Interpretation

Our findings suggest an increased risk of hypospadias in the sons of women exposed to DES *in utero*. Although the absolute risk of this anomaly is small, this transgenerational effect of DES warrants additional studies.

5.1.2 Introduction

Exposure to diethylstilbestrol (DES) could have adverse effects in the third generation from exposure[1;2]. DES, a potent synthetic oestrogen, was widely prescribed to pregnant women between 1938 and 1975 for treatment of imminent and habitual abortions. In the early 1970s, results of several epidemiological studies showed that use of DES during pregnancy was associated with a strongly increased risk of vaginal and cervical clear-cell adenocarcinoma in women exposed to DES *in utero*[3;4]. Furthermore, these daughters had a high frequency of reproductive tract abnormalities that were linked with fertility problems[4;5]. Although the consequences of DES exposure *in utero* seem less severe for boys than girls, some concerns have been raised. The association between in-utero exposure to DES and development of testicular cancer is controversial, but a link with cryptorchidism has been noted[4;6;7]. Some investigators have reported other abnormalities of the urogenital system in the sons of women exposed to DES, including epididymal cysts, hypotrophic testes, and testicular varicoceles[8-10]. The occurrence of hypospadias after exposure to DES *in utero* has been noted only in case reports[10]. Hypospadias is a congenital defect of the urogenital system in which the urethral opening is located on the ventral surface of the penis or on the scrotum[11]. The prevalence of congenital hypospadias in the USA is about 2·0-6·0 per 1,000 live male births, and has been rising during the past few decades[12-15]. Hypospadias can cause poor control of the urinary stream, infertility related to misdirected ejaculation during coitus, and psychosocial stress related to body image. Although hypospadias can be surgically corrected, urological complications can occur[11].

The causes of hypospadias are largely unknown. Sexual differentiation is determined by testosterone and its metabolites. Genetic defects in androgen receptors or the enzymes involved in testosterone metabolism are known to result in hypospadias[11;16]. However, these genetic defects are rare and account for only a small subset of cases[11]. It has been postulated that changes in concentrations of sex hormones during the critical period of penile and urethral development (weeks 8-14), caused by endogenous or exogenous factors, may play a part in the development of hypospadias[11;15-17]. Little is known about health problems in children born to women who were exposed to DES before birth[18]. Work in animals has raised the possibility that adverse effects of DES may be transmitted to subsequent generations[1;2;19-21]. For example, Newbold and colleagues[1;2] reported an increased incidence of reproductive tract tumours in

male and female descendants of mice developmentally exposed to DES. Between 1994 and 1997, one of us (JDvG) noted hypospadias in two boys born to women with a history of prenatal exposure to DES. When this observation was reported to the Netherlands DES Information Centre, the prevalence of hypospadias was assessed in an existing database from an anonymous survey of women exposed to DES before birth. These women (1,153) gave birth to 24 boys with hypospadias: a five times higher prevalence than expected[12-14]. However, no conclusions could be drawn since no appropriate control group had been included, selection bias in the sample was likely, and the severity of hypospadias had not been assessed. Therefore, we aimed to assess the association between maternal exposure to DES *in utero* and hypospadias in a cohort of women with diagnosed fertility problems.

5.1.3 Methods

Participants

OMEGA is a nationwide cohort study of 26,428 women with fertility problems diagnosed in 12 in-vitro fertilization (IVF) clinics in the Netherlands between 1980 and 1995. The study was designed mainly to assess delayed effects of hormone stimulation in IVF, in particular the risk of ovarian cancer and other hormone-related cancers, and includes data on the health of children conceived by IVF. Women eligible for OMEGA had to have been unable to conceive after 1 year or more of frequent unprotected intercourse, and been older than 18 years at the time of their first visit to a fertility clinic. Initially, we recruited 19,840 women in the exposed (IVF) group and 6,588 controls (no IVF). When selecting the control group, we attempted to match women for frequency of diagnoses of fertility problems. Participating clinics provided names, birth dates, and addresses of women for our study. Women who were alive on Jan 1, 1997, and who we knew that we could trace (24,335) received a letter containing information about the study and a 23-page questionnaire on gynaecological disorders before and after fertility treatment, reproductive risk factors for hormonerelated cancers, and other characteristics. An informed consent form was attached to the questionnaire asking each woman for written permission for data abstraction from the medical records and linkage with the Netherlands Cancer Registry. The information letter was signed by the gynaecologist who had originally attended the woman or, if the gynaecologist was no longer working at the clinic,

the current head of the IVF department. After 4-6 weeks, non-responders were sent a reminder letter. Nonresponders to the second letter were approached once by telephone. 29,148 pregnancies were reported in the 16,284 questionnaires that were returned (response rate 67%). We excluded miscarriages (10,291), infants born dead (524), and children who had not yet been born at the time of the questionnaire (404). Infants were eligible for inclusion if the duration of gestation was at least 26 weeks. If gestation was 24 or 25 weeks, we checked for information on the vital status of the child at birth. If the mother had not indicated whether or not her child was born alive, the child had to weigh at least 1 kg to be included. We excluded 78 pregnancies because lack of information on duration of gestation and birthweight made it unclear whether the child had been born alive. We excluded 298 children whose sex was unknown, 78 of unknown birth date, 128 who died within 3 weeks of birth, and 8,413 girls. Thus, our cohort consisted of 8,934 boys, 205 of whom were born to women who had been exposed to DES *in utero.*

Procedures

In each clinic, parents' fertility problems were ascertained from medical records and classified as being tubal factor, male factor, ovarian disorder (including ovulation disorder, polycystic ovary syndrome, and premature menopause), cervical factor, uterine abnormality, endometriosis, or unexplained. Only medically verified causes of fertility problems were noted. Details of fertility treatment were also obtained from the medical records. Completed maternal questionnaires provided detailed information on the method of conception, duration of gestation in weeks, date of birth, sex, birthweight, and number of siblings of the child. Self-reported serious health problems of children were ascertained from the open-ended question "were there, at any time, serious health problems diagnosed in one or more of your children? If so, which health problems and in which child?" All answers were entered into the database exactly as they were written in the questionnaires. We searched the database for the term hypospadias and similar related terms. If a mother had reported the occurrence of hypospadias in her child as an answer to the question on health problems we contacted her again by letter to obtain permission to obtain more specific information from the physician treating her child. All mothers responded to our request for more information. Paediatricians were asked to provide information on date of diagnosis of hypospadias, surgical procedures, and severity of the disorder. The classification of hypospadias was based on the anatomical location of the

urethral meatus. We established maternal exposure to DES *in utero* from a question in the risk factor questionnaire. For 127 (62%) women of the cohort exposed to DES before birth (DESexposed group) we were able to validate the self-reported exposure against the data abstracted from their fertility treatment records. Additionally, we telephoned the subgroup of women who reported a son with hypospadias to ascertain the trimester of maternal prenatal exposure to DES and whether or not there was medical confirmation of DES exposure.

Table 1 Prevalence ratio of hypospadias among 8,934 male offspring, by maternal DES exposure *in utero*, and several pregnancy characteristics

	No.	No. of Hypospadias	PR	95%CI
DES exposure *in utero* of mother				
No	8,729	8	1·0 (reference)	
Yes	205	4	21·3	6·5-70·1
Assisted Reproductive Technology (ART)*				
No	3,819	5	1·0 (reference)	
Yes	4,960	7	1·8	0·6-5·7
Use of ART in non-DES daughters*				
No	3,730	4	1·0 (reference)	
Yes	4,846	4	0·8	0·2-3·1
Maternal age*				
≤35	6,783	8	1·0 (reference)	
≥36	2,077	4	1·6	0·5-5·4
Duration of gestation (weeks)*				
≤37	2,417	6	2·6	0·8-7·9
≥38	6,161	6	1·0 (reference)	
Birth weight (g)*				
≤2,499	1,608	4	2·1	0·6-7·1
≥2,500	6,847	8	1·0 (reference)	

* Due to missing variables, numbers of males don't add up to 8,934 for all the factors other than DES exposure

Statistical analysis

We used χ^2 analysis to compare the distribution of maternal and child characteristics (categorical variables) between the DES-exposed and unexposed groups (no maternal prenatal

exposure to DES). All p values were two-sided. We compared the risk of hypospadias between the sons of DES-exposed and unexposed mothers by calculating prevalence ratios and corresponding 95%CIs using either the sons or the mothers as the unit of analysis to account for non-independence. We analysed the effect of covariates (maternal in-utero exposure to DES, gestational age, and number of siblings) on the association between DES exposure and risk of hypospadias, using logistic regression.

5.1.4 Results

Twelve cases of hypospadias were reported. Four of 205 boys born to DES-exposed women had hypospadias compared with eight of 8,729 boys born to unexposed women - a prevalence ratio (PR) of 21·3 (95%CI=6·5-70·1), table 1. Siblings might have caused non-independence of results. Because no boys with hypospadias had brothers with the same disorder, we recalculated the PR for hypospadias using mothers (rather than boys) as the denominator. Accounting for non-independence, the PR of hypospadias was 20·7 (95%CI=6·3-68·0). We accounted for potentially fewer sons in the exposed group (Table 2) by calculating the PR for hypospadias using only the oldest son born to each mother (PR=23·6, 95%CI=7·0-79·9) or the youngest (PR=17·7, 95%CI=4·6-67·9). Table 3 shows characteristics of the 12 boys with hypospadias. Seven children were conceived by IVF, including one child conceived through intracytoplasmic sperm injection (ICSI) and one through frozen embryo transfer. Duration of pregnancy (32-43 weeks) and birthweight (995-4450 g) varied widely. All boys with hypospadias had one or more operations. Hypospadias was classified as coronal or subcoronal (two), distal shaft (four), penile shaft (two), and penoscrotal (four). More severe cases occurred in the sons of the exposed group than the unexposed group. One boy with hypospadias had hydrocephalus. Two women from the exposed group had obtained their mothers' original medical records, which confirmed prenatal exposure to DES. The medical records of the mothers of the other two women could not be traced. One of these women from the exposed group had abnormalities of the reproductive system, characteristic of prenatal exposure to DES. She also had oral confirmation from her mother that DES was used during all months of the pregnancy. The other woman had past letters from her mother (who had been a pharmacist) in which DES exposure was confirmed. We analysed potential confounding factors[11;15;16;22] for the association between

Table 2 Distribution of maternal and child characteristics by maternal exposure to DES *in utero*

	Sons				
	Exposed (N=205)		Unexposed (N=8,729)		
Characteristics	**No.**	**%**	**No.**	**%**	**p value***
Method of conception					
Natural	89	43·4%	3,730	42·7%	0·58
Fertility drugs (no ART)	6	2·9%	212	2·4%	
Insemination	16	7·8%	500	5·7%	
IVF	92	44·9%	4,134	47·4%	
Maternal age at birth (yr.)					
≤24	16	7·8%	958	11·0%	
25-29	37	18·0%	1,616	18·5%	0·81
30-34	84	41·0%	3,389	38·8%	
35-39	61	29·8%	2,340	26·8%	
≥40	7	3·4%	352	4·0%	
Duration of gestation (weeks)					
≤32	17	8·3%	417	4·8%	0·004
33-36	34	16·6%	1,238	14·2%	
37-38	56	27·3%	1,763	20·2%	
39-40	59	28·8%	3,318	38·0%	
≥41	34	16·6%	1,642	18·8%	
Birth weight (g)					
≤1,499	13	6·3%	256	2·9%	0·16
1,500-2,499	30	14·6%	1,309	15·0%	
2,500-3,499	80	39·0%	3,770	43·2%	
3,500-3,999	51	24·9%	2,073	23·7%	
≥4,000	22	10·7%	851	9·7%	
Index child part of multiple pregnancy					
No	156	76·1%	6,358	72·8%	0·07
Yes, twin	27	13·2%	1,613	18·5%	
Yes, triplet of more	4	2·0%	351	4·0%	
Unknown	18	8·8%	407	4·7%	
	Mothers				
	Exposed (N=157)		Unexposed (N=6,494)		
	No.	**%**	**No.**	**%**	**p value***
Origin of subfertility					
Male factor	23	14.6%	1,200	20·0%	0·32
Female factor	74	47.1%	2,970	45·4%	
Both	30	19.1%	961	10·7%	
Unexplained subfertility	27	17.2%	1,246	23·1%	
Number of sons					
1	113	72.0%	4,606	70.9%	0·45
2	40	25.5%	1,584	24.4%	
≥3	4	2.5%	304	4.7%	

* Two-tailed Chi square test

prenatal maternal DES exposure and risk of hypospadias (Table 2). Exposed and unexposed groups did not differ with respect to methods of conception, which parent had the fertility problem, maternal age at pregnancy, and number of twin pregnancies. More women in the exposed than unexposed group had cervical (p<0·0001) or uterine causes (p<0·0001) for their fertility problem. Sons of women in the exposed group compared with those of the unexposed group had a shorter duration of gestation, and a higher proportion had an extremely low birthweight. More than 70% of the mothers had only one son in the cohort. A slightly lower proportion of women from the exposed group had more than one son than women in the unexposed group (p=0·45). The risk of hypospadias in the exposed group was 20·1 times higher (95%CI=5·9-68) than in the unexposed group after adjustment for maternal age, duration of gestation, and birthweight.

Since 4,960 (56%) boys in our cohort had been conceived by assisted reproductive techniques, we also analysed the association between this treatment and hypospadias (Table 1). Children conceived through assisted reproductive techniques did not have an increased risk of hypospadias compared with children conceived naturally (PR=1·8, 95%CI=0·6-5·7). Results were similar when we restricted the analysis to sons of women in the unexposed group (PR=0·8, 95%CI=0·2-3·1). Older maternal age, short duration of pregnancy (≤37 weeks), and low birthweight (≤2,499 g) were all positively, though not significantly, associated with risk of hypospadias (Table 1).

5.1.5 Discussion

Our results suggest that sons of women who were exposed to DES *in utero* have a strongly increased risk of hypospadias. The risk of hypospadias has been analysed in epidemiological studies in relation to factors associated with increased oestrogen concentrations during pregnancy such as fertility problems, twins, maternal age, and multiparity[17;22;23]. The roles of exogenous hormones[16;24], assisted reproductive techniques[16;25], birthweight, and duration of gestation have also been assessed. Only low birthweight and retarded fetal growth, assessed as birthweight adjusted for gestational age, have been consistently associated with risk of hypospadias[26]. This finding suggests that hypospadias could be associated with early malfunction of the placenta, resulting in decreased secretion of placental and fetal hormones

Table 3 Characteristics of the twelve cases with hypospadias

	DES exposure *in utero*		Offspring							Mother		
	DES confirmation	Trimester of exposure	Method of conception	Duration gestation (wk)	Birth weight (g)	Age (yr)	Siblings	Twin pregnancy	Severity of hypospadias	Maternal age (yr)	Previous miscarriages	No. of previous IVF cycle
	Women exposed to DES *in utero*											
1	Original medical file†	Up to 7th week	IVF§	33	1,960	7·0	Brother	No	Penoscrotal	30·3	0	0
2	Oral and written confirmation‡	1st trimester	IVF§	39	2,450	2·5	None	No	Penoscrotal	39·8	0	0
3	Original medical file†	1st trimester	IVF§	39	2,980	5·6	None	No	Penoscrotal	35·4	1	5
4	Oral confirmation‡; DES-related abnormalities	All trimesters	Natural	34	2,860	8·6	None	No	Distal shaft	34·3	7	0
	Women unexposed to DES *in utero*											
5	-	-	IVF§	32	995	9·3	Brother	Yes	Penoscrotal	37·6	0	1
6	-	-	IVF§	33	2,170	6·3	Sister	No	Distal shaft	28·6	0	2
7	-	-	ICSI§	37	2,845	1·4	Brother	Yes	Subcoronal	28·8	0	2
8	-	-	IVF(cryo)	37	2,900	3·2	Sister	Yes	Penile	33·3	0	2
9	-	-	Natural	39	3,300	12·0	None	No	Coronal	22·5	0	0
10	-	-	Natural	41	3,575	2·9	None	No	Distal shaft	36·9	2	0
11	-	-	Natural	40	4,000	11·7	None	No	Penile	24·8	2	0
12	-	-	Natural	43	4,450	2·8	None	No	Distal shaft	40·7	4	0

† Original medical file of grandmother

‡ Oral and/or written confirmation by grandmother

§ All the mothers of children conceived by IVF and ICSI received Human Menopausal Gonadotrophin and/or Follicle Stimulating Hormone (range 20-33 ampoules) and 10,000 IU Human Chorionic Gonadotrophin. Furthermore, mothers received various protocols to support the luteal phase of the menstrual cycle. In addition, two mothers used gonadotrophin-releasing hormone agonists (GnRH-AG) to down-regulate the responsiveness of the pituitary to GnRH

that could in turn disturb fetal development[23]. In our study, all participants were contacted individually, whereas in most other studies, data on risk factors for hypospadias were taken from registries that did not contain information on maternal exposure to DES[17;26]. The OMEGA cohort, however, was not designed for specific disorders in children. In the questionnaire, only one question was included in which mothers were asked to report serious health problems affecting their child(ren). Because we could identify cases of hypospadias only through this open-ended question, underascertainment is likely. In the European Registration Of Congenital Anomalies (EUROCAT) in northern Netherlands, 20 000 births are monitored every year[27]. During 1981-98, in the three northern provinces of the Netherlands, there were 1·98 cases of hypospadias per 1,000 live male births. Based on this prevalence, about 17 boys would be expected to have hypospadias in our unexposed group. Since our study was nationwide and 73% of our population were born after Jan 1, 1990, we also assessed the prevalence of hypospadias according to EUROCAT registration in southern Netherlands (available from Jan 1, 1990, onwards). 1·74 cases of hypospadias occurred per 1,000 live male births during 1990-98. According to this rate, 11 cases of hypospadias would have been expected in the unexposed group. In our study, only eight cases of hypospadias were reported in the unexposed group, all of which required surgery. More than three-quarters of hypospadias are cases of mild (glandular or coronal) to moderate (distal, middle, and proximal shaft), and 5-15% are severe (penoscrotal, scrotal, or perineal)[28]. In our cohort, four of the 12 cases of hypospadias were severe, and three of these occurred in the sons of women in the exposed group. Participants in both groups probably did not report all mild or moderate cases of hypospadias because they did not consider these to be a serious health problem. It is noteworthy that the prevalence of hypospadias in sons of women exposed to DES *in utero* who were registered at the Netherlands DES Information Centre (20·8 per 1,000 live male births) is similar to the rate we found (19·5 per 1,000 live male births).

We obtained data retrospectively, thus selection or reporting bias could have occurred. Women exposed to DES who had children with congenital abnormalities might have been more inclined to respond to the questionnaire or report hypospadias in their sons. However, because our study focused on the late effects of IVF treatment, this bias also applied to mothers who had undergone IVF treatment. We did not find an increased risk of hypospadias in boys conceived by IVF, rendering selection or reporting bias unlikely. To assess the potential for selection bias further, we obtained data from the medical records of 2,515

random non-responders from the original OMEGA cohort. In the non-responders group, the proportion of women with DES-related subfertility problems stated in the records was 0·88% versus 1·84% in the responders groups. Thus, women exposed to DES might have been slightly overrepresented in our study population. This factor would result in selection bias only if women in the nonresponders' group were less likely than those in the responders' group to have a son with hypospadias. An estimated 4,417 boys were born to non-responders and about 100 of these (2·3%) were sons of DES-exposed women. Even if none of these 100 had hypospadias, four of 305 is still a higher proportion than expected from previous published work. Women who are aware of in-utero exposure to DES tend to be knowledgeable and concerned about potential health effects, and the women in our exposed group already had a health effect (fertility problem) that was possibly attributable to their exposure. Thus, they might be more apt to report health problems than non-exposed women, even though hypospadias in their sons is not a known consequence of DES exposure. However, the prevalence rate of hypospadias in sons of DES daughters is not only strongly increased compared with our control group without maternal exposure to DES *in utero*, but also in comparison with published prevalence rates of hypospadias. We further assessed the effect of reporting bias using the prevalence of other congenital anomalies in our cohort. We intended to assess the prevalence of cryptorchidism, but no cases were reported in sons of women in the exposed group. Therefore, we assessed the prevalence of heart and circulatory anomalies. We noted 29 self-reports of congenital heart defects: one in the exposed group and 28 in the unexposed group (PR=1·5, 95%CI=0·2-11·1). About 22 cases in our cohort would have been expected[27], which renders reporting bias unlikely. Children could have been misclassified if information on maternal in-utero exposure to DES was unreliable. Classification was based on answers to a question in the study questionnaire. Because we did not have medical records for the full cohort, verification of this information was possible for only 127 of 205 exposed women. We recorded exposure to DES from a woman's medical files only when DES-related disorders contributed to her fertility problem. Exposure to DES *in utero* was mentioned as (one of) the reason(s) for fertility problems in only 73 (57%) of the 127 women. Ideally, a report of DES exposure *in utero* should be confirmed by information from the mother's pregnancy record. For practical reasons, we attempted to confirm exposure status only if women reported a son with hypospadias. Written confirmation of the grandmother's use of DES during pregnancy was obtained for three of four children. The records of the fourth child had been

destroyed, but his mother had very specific DES-related disorders. Although we did not confirm the exposure status of the other 201 women who reported in-utero exposure to DES, misclassification in this group would have led to an underestimation of the risk of hypospadias. Women exposed to DES *in utero* are more likely to have a premature infant[4], lower parity[29], or older age at first livebirth[29], and to undergo assisted reproductive techniques more frequently[4] than unexposed women. In our study, there were borderline significant differences between exposure groups with respect to duration of gestation, proportion of children with a very low birthweight, number of siblings, and number of multiple pregnancies. Of these factors, only short duration of gestation and very low birthweight were positively (but not significantly) associated with risk of hypospadias, and adjustment for these factors did not substantially affect the association between DES and hypospadias. Furthermore, a short gestation and low birthweight could not cause hypospadias because they postdate the development of the disorder. A possibility is that, in some cases, maternal exposure to DES *in utero* might result in malfunction of the placenta, leading to both low birthweight for gestational age and hypospadias. More than half our cohort of live male births was conceived through an assisted form of conception. We found no association between IVF and hypospadias, in contrast with others[16;25;30]. However, the two studies in which the greatest risk of hypospadias was reported were based on small numbers of participants[16;30]. In a study that included 9,111 infants born after IVF, 28 received a diagnosis of hypospadias, of whom 18 were conceived by standard IVF and ten after ICSI. The expected number of cases was 15·7, resulting in a relative risk of 1·1 (95%CI=0·7-1·8)[16]. Children born after use of assisted reproductive techniques did not have an increased risk of hypospadias in our study, but this could have resulted from interaction between DES and assisted reproductive techniques. To separate the effects of DES from IVF and ICSI treatment, the risk of hypospadias should be assessed in naturally conceived sons. We can only speculate about the biological mechanisms that may lead to a higher risk of hypospadias in DESgrandchildren. One hypothesis is that DES exposure *in utero* might lead to genetic or epigenetic changes in the primordial oocytes of the DES daughter that were being formed in the first trimester of gestation. These changes might subsequently be transmitted to the next generation[1;2]. Another (less likely) hypothesis could be that DES-related genetic or epigenetic changes in somatic cells of the fetus might have induced a disturbed hormonal balance in DES daughters in adult life. Our results are based on only a few cases of hypospadias and thus require confirmation by other epidemiological studies. Cohort studies with large

populations of women with medically confirmed exposure to DES would enable assessment of the possible multigenerational effects of this drug. Unfortunately, few such cohorts with appropriate control groups[29] are available. However, despite the high prevalence ratio, the absolute risk of hypospadias for the sons of women exposed to DES *in utero* is low.

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