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ORIGINAL ARTICLE *Reproductive epidemiology*

Risk of borderline and invasive ovarian tumours after ovarian stimulation for *in vitro* fertilization in a large Dutch cohort

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BACKGROUND: Long-term effects of ovarian stimulation for IVF on the risk of ovarian malignancies are unknown.

METHODS: We identified a nationwide historic cohort of 19 146 women who received IVF treatment in the Netherlands between 1983 and 1995, and a comparison group of 6006 subfertile women not treated with IVF. In 1997–1999, data on reproductive risk factors were obtained from 65% of women and data on subfertility (treatment) were obtained from the medical records. The incidence of ovarian malignancies (including borderline ovarian tumours) through 2007 was assessed through linkage with disease registries. The risk of ovarian malignancies in the IVF group was compared with risks in the general population and the subfertile comparison group.

RESULTS: After a median follow-up of 14.7 years, the risk of borderline ovarian tumours was increased in the IVF group compared with the general population [standardized incidence ratio (SIR) = 1.76; 95% confidence interval (CI) = 1.16–2.56]. The overall SIR for invasive ovarian cancer was not significantly elevated, but increased with longer follow-up after first IVF ($P = 0.02$); the SIR was 3.54 (95% CI = 1.62–6.72) after 15 years. The risks of borderline ovarian tumours and of all ovarian malignancies combined in the IVF group were significantly increased compared with risks in the subfertile comparison group (hazard ratios = 4.23; 95% CI = 1.25–14.33 and 2.14; 95% CI = 1.07–4.25, respectively, adjusted for age, parity and subfertility cause).

CONCLUSIONS: Ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. More large cohort studies are needed to confirm these findings and to examine the effect of IVF treatment characteristics.

Key words: ovarian stimulation / ovarian malignancies / fertility drugs / infertility / *in vitro* fertilization

Introduction

Currently, 1.2–2.3% of children born in the Western world are conceived by assisted reproductive technologies (Kremer *et al.*, 2008; Wright *et al.*, 2008). In the Netherlands, it has been estimated that the number of treatment cycles increased by 40% from 1996 till 2005 (Kremer *et al.*, 2008). Fertility drugs (FDs) used in IVF treatment temporarily raise serum levels of exogenous gonadotrophins and gonadal hormones, and consequently increase the chances of multiple folliculogenesis and ovulations. The long-term effects of ovarian stimulation are unknown. In view of the assumed role of 'incessant ovulation' (Fathalla, 1972) and increased gonadotrophin levels in ovarian cancer pathogenesis (Cramer and Welch, 1983; Risch, 1998; Vlahos *et al.*, 2010) concerns have been raised that ovarian stimulation and multiple ovarian punctures as used in IVF may increase the risk of ovarian malignancies (Fishel and Jackson, 1989). Invasive ovarian cancer accounts for 6% of female cancer deaths in the USA (Jemal *et al.*, 2008).

Over the past decades, several studies reported a significant increase of ovarian cancer risk after FD use (Whittemore *et al.*, 1992; Rossing *et al.*, 1994; Brinton *et al.*, 2005; Sanner *et al.*, 2009; Källén *et al.*, 2011), but others did not observe such an elevated risk (Franceschi *et al.*, 1994; Bristow and Karlan, 1996; Mosgaard *et al.*, 1997; Modan *et al.*, 1998; Venn *et al.*, 1999; Parazzini *et al.*, 2001; Dor *et al.*, 2002; Doyle *et al.*, 2002; Ness *et al.*, 2002; Rossing *et al.*, 2004; Dos Santos Silva *et al.*, 2009; Jensen *et al.*, 2009), or reported non-significant risk increases for subgroups (Shushan *et al.*, 1996; Ness *et al.*, 2002; Brinton *et al.*, 2004). Some studies noted an elevated risk of borderline ovarian tumours following the use of FDs (Harris *et al.*, 1992; Rossing *et al.*, 1994; Shushan *et al.*, 1996; Parazzini *et al.*, 1998; Ness *et al.*, 2002; Sanner *et al.*, 2009). Borderline ovarian tumours are low-grade ovarian malignancies with far less aggressive behaviour than invasive ovarian cancer (Bell, 2005; Hart, 2005).

Short follow-up, low statistical power and lack of control for important confounders, such as cause of subfertility and parity, have limited the conclusions from previous studies. We report here on a large nationwide cohort study in the Netherlands (the OMEGA study) that was designed to examine long-term risk of ovarian malignancies (both invasive ovarian cancer and borderline ovarian tumours) after ovarian stimulation for IVF. A unique feature of our study is that data on reproductive factors were obtained from the participating women, whereas detailed information on subfertility cause and treatment was abstracted from the medical files.

Patients and Methods

Study population

In 1995–1996, we identified a nationwide historical cohort of 19 861 subfertile women who received at least one IVF cycle with ovarian stimulation between 1983 and 1995 in 1 of the 12 IVF hospitals with legal permission to provide IVF treatment in the Netherlands. Since the registration of IVF treatment was obligatory by law, all IVF clinics in the Netherlands could provide a minimal data set with names, birth dates and addresses of eligible women. The institutional ethics committees of all IVF clinics approved the study procedures, which have been described previously (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003).

To obtain a large enough comparison group of subfertile women not treated with IVF, we identified women who were diagnosed with fertility problems shortly before IVF became a routine procedure for subfertile patients. The non-IVF comparison group consisted of 6604 women whose subfertility was diagnosed in the four participating clinics that had a computerized registry of all subfertile women evaluated during 1980–1995. We attempted to frequency match the non-IVF comparison group according to the distribution of subfertility diagnoses in the IVF group. Most women in the non-IVF group registered for their first consultation in the 1980s (before IVF became a routine procedure) and underwent tubal surgery and/or hormonal treatments. The majority of those who registered after 1990 withdrew from the waiting list for IVF because they pursued other treatment options, reached the age of 40 years (the upper age limit for IVF at the time), became pregnant or decided to refrain from IVF for various reasons, such as divorce. When the non-IVF group was compared with the IVF group, it turned out that 911 women selected into the non-IVF comparison group subsequently received IVF. These women had subfertility treatments other than IVF in one centre and subsequently received IVF in a second centre. In the description of the cohort, these women are included in the IVF group (Table I) (see also section 'Statistical analysis').

Based on names, birth dates and addresses at the time of subfertility treatment all cohort members were traced. Given that the subjects' last visit to the fertility clinic could date back to 1980, extensive tracing techniques were required to obtain current addresses of all women (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003), using the municipal population offices that fully cover the Netherlands. From the initial 26 465 women, 4.2% was not approached (the OMEGA cohort study, Fig. 1).

Risk factor questionnaire

Between 1997 and 1999, 25 353 women received a risk factor questionnaire, a study information letter, and a brochure. Each participant was asked written informed consent for medical record data abstraction and future linkage with disease registries. The study information letter was signed by the treating gynaecologist or, if he/she had left, the current head of the IVF department. In the study information letter as well as in the brochure, women were informed about the purpose, the design and the privacy aspects of the study. The purpose of the study was stated as follows: 'to examine whether women who underwent an IVF treatment more frequently report gynaecological health problems compared with women who did not have an IVF treatment'. After 4–6 weeks, non-responders were sent a reminder. Non-responders to the second letter were approached by telephone. The 23 page questionnaire ascertained information on the women's reproductive histories, subfertility treatment, use of exogenous hormones, lifestyle factors and family history of cancer.

A total of 16 343 women returned the questionnaire (response rate 65.2%). The response rate was substantially lower in the non-IVF group (48.7%) than in the IVF group (71.1%).

Medical records

Trained abstractors collected information on cause of subfertility and all fertility treatments. Cause of subfertility was classified as tubal, male factor, endometriosis, ovarian disorders, cervical factor, uterine abnormalities or unexplained. Multiple causes of subfertility were registered if applicable.

For each IVF and insemination cycle, we recorded date, dosage and type of FDs used in each phase of the menstrual cycle (hMG, FSH, clomiphene, hCG, GnRH and progesterone), number of oocytes collected and outcome. For FDs used prior to inseminations/IVF, we also coded date, dosage and type of FDs used per cycle. We made special attempts to collect information on subfertility treatments provided outside the participating IVF

Table I Population characteristics of the OMEGA cohort by exposure status.

	IVF group (n = 19 146)		Non-IVF group (n = 6006)		Total (n = 25 152)	
	n	%	*n*	%	*n*	%
Year of birth						
≤1953	2527	13.2	1711	28.5	4238	16.8
1954–1957	4991	26.1	1440	24.0	6431	25.6
1958–1960	5995	31.3	1506	25.1	7501	29.8
≥1961	5633	29.4	1349	22.5	6982	27.8
Age at first IVF treatment or visit (years)						
≤26	1425	7.4	1159	19.3	2584	10.3
27–29	3015	15.7	1233	20.5	4248	16.9
30–32	4929	25.7	1339	22.3	6268	24.9
33–35	4711	24.6	1152	19.2	5863	23.3
≥36	5066	26.5	1123	18.7	6189	24.6
Subfertility diagnosis[a,b]						
Tubal	6025	31.5	1938	32.3	7963	31.7
Endometriosis	1970	10.3	349	5.8	2319	9.2
Male factor	5492	28.7	809	13.5	6301	25.1
Hormonal factor[c]	1287	6.7	409	6.8	1696	6.7
Unexplained	3412	17.8	537	8.9	3949	15.7
Other factors	912	4.8	360	6.0	1272	5.1
Missing	3309	17.3	2388	39.8	5697	22.7
Number of IVF treatments[b]						
1–2 cycles	6304	32.9				
3–4 cycles	6271	32.8				
5 or more cycles	3352	17.5				
Missing	3219	16.8				
Time since first treatment or visit (years)						
≤5 years	493	2.6	31	0.5	524	2.1
5–9 years	689	3.6	147	2.4	836	3.3
10–14 years	10 343	54.0	1526	25.4	11 869	47.2
≥15 years	7621	39.8	4302	71.6	11 923	47.4
Median years of follow-up	14.3		16.4			

[a]Women could have more than one cause of subfertilly, except for unexplained and missing, which were unique classifications.
[b]Information based on medical records; for women without medical record data, information was added from health questionnaire survey.
[c]Included ovulation disorders, polycystic ovary syndrome and premature menopause.

clinics, by screening intake forms and letters from other treating physicians. Due to limited funding, we could only complete medical record abstraction for 9 out of 12 centres, i.e. 13 807 women (76% of women in the IVF group) (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003).

Incidence of ovarian malignancies

Cancer incidence in the period 1989–2003 was ascertained through linkage with the population-based Netherlands Cancer Registry (NCR) (International Agency for Research on Cancer, 2003), and incidence of ovarian malignancies (including borderline ovarian tumours) through June 2007 was ascertained through linkage with the Dutch nationwide network and registry of histo- and cytopathology (PALGA). PALGA contains records of all histological diagnoses made in the Netherlands, with computerized data submission by all pathology laboratories, and nationwide coverage since 1989 (Casparie *et al.*, 2007). We linked with PALGA since the NCR had incomplete data on borderline ovarian tumours; in addition PALGA case ascertainment is complete till 2 weeks prior to linkage, while the NCR lags a few years behind. We used a record linkage protocol developed previously (van den Brandt *et al.*, 1990), which was based on the first four characters of the family name, gender and date of birth. All positive matches were checked for administrative twins by place of birth, postal code at cancer diagnosis and first initial. The NCR and PALGA granted us permission to not only link responders who gave permission, but also non-responders and deceased women, under additional privacy regulations. Only women who explicitly refused future linkage with disease registries (n = 1017; 4.0% of all women) were excluded from linkage. For each ovarian malignancy, we received information on date of diagnosis and morphology. Vital status as of June 2007 was obtained by linkage with the Central Bureau for Genealogy, which keeps computerized records of all deceased persons in the Netherlands since 1994.

Statistical analysis

The analytic study cohort consisted of 25 152 women; 19 146 women in the IVF group and 6006 women in the non-IVF group (Fig. 1). Because the NCR and PALGA did not fully cover the Netherlands before 1989, the observation time for each participant started on 1 January 1989 or the date of first IVF treatment (IVF group), or clinic visit for subfertility evaluation (non-IVF group), whichever came last. Person-years of observation were calculated to the date PALGA follow-up ended (June 2007), date of ovarian cancer diagnosis or date of death, whichever came first. Women selected into the non-IVF comparison group who subsequently received IVF contributed person-time to the non-IVF group until the date of first IVF treatment, and switched to the IVF group after this date, according to standard cohort methodology regarding time-dependant allocation of person-years in case of changing exposure (Breslow and Day, 1987). Women diagnosed with ovarian cancer before entering the cohort (n = 14) or before 1989 (n = 13), were excluded from the analysis.

First, we compared ovarian cancer incidence in the IVF group and non-IVF group with incidence in the general population. We determined the standardized incidence ratio (SIR) as the ratio of the observed (O) and expected (E) number of cancers in the cohort. Expected numbers were based on age- and calendar period-specific reference rates for invasive ovarian cancer and borderline ovarian tumours from the NCR and PALGA, respectively (International Agency for Research on Cancer, 2003). Incidence rates for borderline ovarian tumours were calculated by the authors (T.M.M. and F.E.v L.), based on annual numbers of borderline ovarian tumour diagnoses obtained from PALGA. In all analyses, the subfertility cause(s) and treatments were preferably based on the medical records, and only derived from the woman's questionnaire if the records had not been abstracted. Information on reproductive factors was derived from the women's questionnaires, since these variables could change after IVF treatment. For non-responding women information from hospital databases was added when available. Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF, and was based on information from the medical records combined with the risk factor questionnaire.

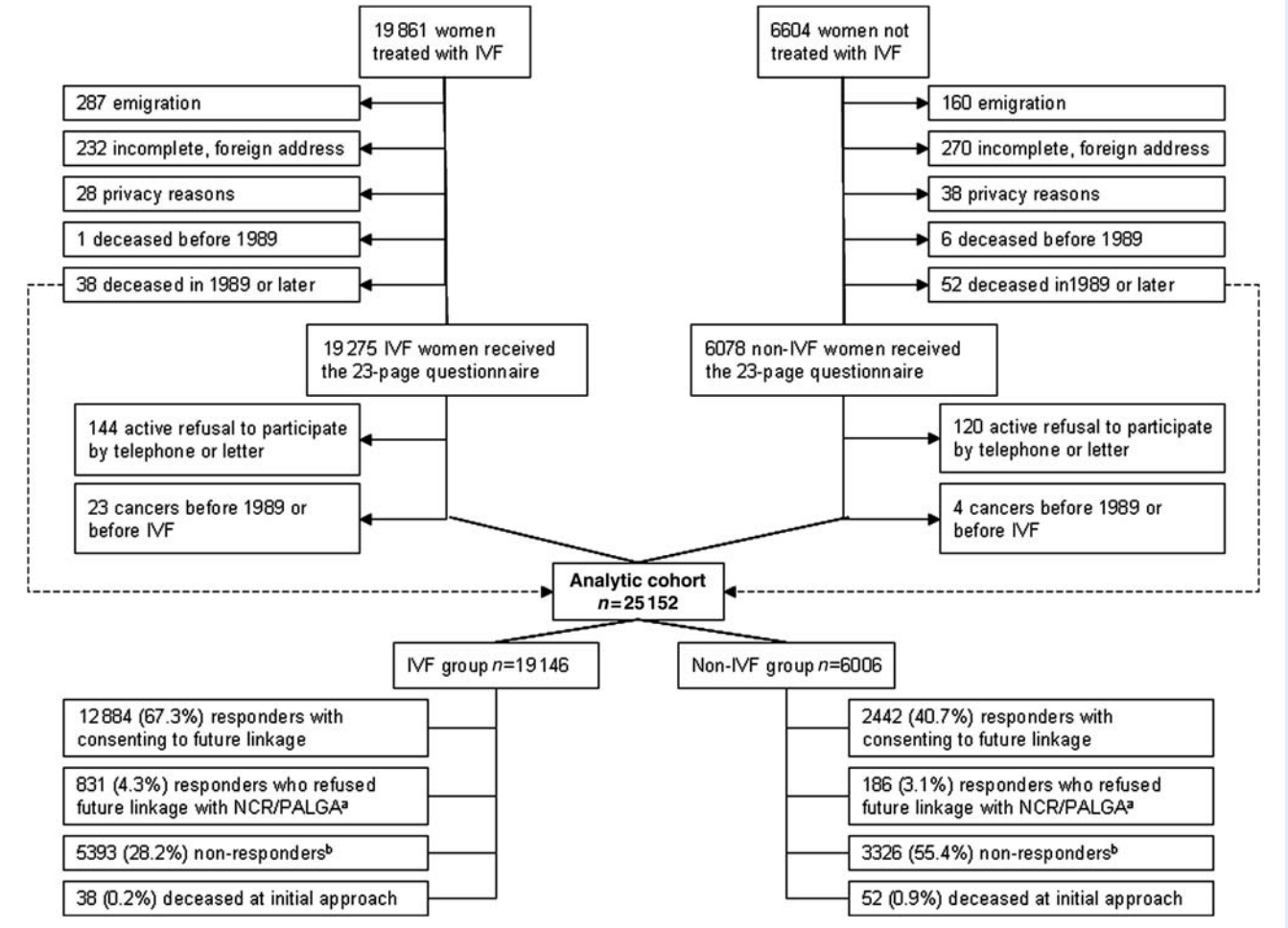

Figure 1 Identification of the OMEGA study cohort. [a]Women in this category contributed person time till date of questionnaire completion. [b]Including women who returned an empty questionnaire ($n = 66$) and questionnaires that were returned to sender ($n = 656$).

Cox proportional hazards models were used to compare cancer risk between the IVF group and the non-IVF group, adjusting for age and potential confounders such as parity and subfertility cause. Forward stepwise confounder selection, in which the effect of adding one confounder at a time was evaluated, was based on a >10% change in the risk estimate of the exposure variable of interest, irrespective of significance values.

In all analyses missing values were included as a separate category. Data were analysed with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Population characteristics

Characteristics of 19 146 IVF-treated women and 6006 women not treated with IVF are presented in Table I. Women in the non-IVF group had a slightly longer median duration of follow-up than women in the IVF group (16.4 versus 14.3 years) and they were also older at the end of follow-up (mean age 49.4 versus 47.5 years). These differences reflect the initial inclusion criteria for the IVF and the non-IVF groups, with an over-representation of women in the non-IVF group seeking subfertility treatment in the years before IVF treatment became a routine procedure. Cause of subfertility was related to tubal problems in 32% of women, 25% had male-factor subfertility, 9% endometriosis, 7% hormonal subfertility, 16% unexplained subfertility and 23% was missing (percentage add up to >100% due to multiple causes of subfertility). A total of 42% of the cohort was nulliparous at questionnaire completion. In the IVF group, 40% of women had one to two stimulated IVF cycles, 39% had three to four cycles and 21% received five or more cycles. IVF stimulation regimens used in the cohort have been described in detail previously (de Boer *et al.*, 2004). In brief, clomiphene/hMG or FSH/hMG stimulation protocols were used till 1988–1989, whereas stimulation with GnRH agonists became common after 1990 (from 20% in 1986 to about 90% after 1990). Furthermore, from 1984 to 1994, the number of ampoules of gonadotrophins strongly increased, as did the number of retrieved oocytes at the first IVF cycle (from 5.4 in 1986 to 10.7 in 1994) (de Boer *et al.*, 2004).

Comparisons with external reference rates

After a median follow-up time of 14.7 years, 77 ovarian malignancies were observed in the full cohort [SIR = 1.43; 95% confidence interval

(CI) = 1.12–1.78]; 42 invasive ovarian cancers and 35 borderline ovarian tumours (Table II). Sixty-one ovarian malignancies were observed in the IVF group (SIR = 1.59; 95% CI = 1.21–2.04) and 16 in the non-IVF group (SIR = 1.02; 95% CI = 0.59–1.66). Compared with the general population rates, we observed a significantly increased risk for borderline ovarian tumours in the IVF group (SIR = 1.93; 95% CI = 1.31–2.73) and no increase in the non-IVF group (SIR = 0.67; 95% CI = 0.18–1.71). The SIRs for invasive ovarian cancer were not significantly raised in either IVF-treated women (1.35; 95% CI = 0.91–1.92) or non-IVF women (1.24; 95% CI = 0.64–2.17). The morphologies of the invasive ovarian cancers were serous (60%), mucinous (7%), clear-cell (7%), endometrioid (21%) and other (5%). Of the borderline ovarian tumours, 63% were serous and 37% were mucinous. Serous borderline ovarian tumours and invasive ovarian cancers occurred more frequently in the IVF group than in the non-IVF group ($P = 0.04$).

The SIRs in both the IVF group and non-IVF group were strongly increased in the first year of follow-up (3- to 18-fold), possibly related to work-up for subfertility diagnosis and treatment. When we excluded the first year of follow-up, the SIR for all ovarian malignancies was 1.49 (95% CI = 1.12–1.94) in the IVF group and 0.85 (95% CI = 0.45–1.45) in the non-IVF group. After 15 or more years, the SIR for invasive ovarian cancer in the IVF group was 3.54 (95% CI = 1.62–6.72, P for trend = 0.02), whereas the SIR in the non-IVF group was close to unity (Table II). No clear increase with longer follow-up was seen for borderline ovarian tumours (P for trend = 0.49).

Within the IVF group, SIRs of ovarian malignancy did not increase with a greater number of IVF cycles or ampoules of gonadotrophins (Table III). The mean number of oocytes harvested per stimulated cycle and the maximum number over all treatment cycles were used as a proxy for a woman's responsiveness to ovarian stimulation; the total number of oocytes collected over all cycles was used as a proxy for the amount of damage to the ovarian epithelium. The SIRs did not appear to be associated with any of these variables. FD use prior to IVF treatment was not associated with an increased SIR for all ovarian malignancies combined; for invasive ovarian cancer the SIR was non-significantly increased (SIR = 1.69; 95% CI = 0.95–2.79), while for borderline ovarian tumours the SIR was increased for women who did not use FDs prior to IVF treatment (SIR = 2.93; 95% CI = 1.71–4.69). These observations must be interpreted with caution since information on previous FD use was missing for 27% of women.

Table II Incidence of ovarian malignancies by years of follow up and exposure status.

Follow-up	IVF group				Non-IVF group				Total			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All ovarian malignancies												
<1 years	6	1.52	3.94	1.44–8.57	3	0.31	9.55	1.97–27.91	9	1.84	4.90	2.24–9.30
1–4 years	9	7.52	1.20	0.55–2.27	1	1.74	0.57	0.01–3.20	10	9.27	1.08	0.52–1.98
5–9 years	16	12.41	1.29	0.74–2.09	3	3.58	0.84	0.17–2.45	19	15.99	1.19	0.72–1.86
10–14 years	18	13.22	1.36	0.81–2.15	4	4.63	0.86	0.23–2.21	22	17.85	1.23	0.77–1.87
≥15 years	12	3.73	3.22	1.66–5.62	5	5.36	0.93	0.30–2.18	17	9.08	1.87	1.09–3.00
All intervals	61	38.41	1.59	1.21–2.04	16	15.63	1.02	0.59–1.66	77	54.03	1.43	1.12–1.78
All intervals excl. first year	55	36.88	1.49	1.12–1.94	13	15.31	0.85	0.45–1.45	68	52.20	1.30	1.01–1.65
Invasive ovarian cancer												
<1 years	2	0.78	2.57	0.31–9.26	3	0.16	18.35	3.79–53.60	5	0.94	5.30	1.72–12.37
1–4 years	5	3.94	1.27	0.41–2.96	1	0.93	1.07	0.03–5.97	6	4.88	1.23	0.45–2.68
5–9 years	4	6.90	0.58	0.16–1.48	2	2.03	0.99	0.12–3.56	6	8.93	0.67	0.25–1.46
10–14 years	10	8.13	1.23	0.59–2.26	2	2.85	0.70	0.09–2.54	12	10.98	1.09	0.56–1.91
≥15 years	9	2.54	3.54	1.62–6.72	4	3.68	1.09	0.30–2.79	13	6.22	2.09	1.11–3.57
All intervals	30	22.30	1.35	0.91–1.92	12	9.65	1.24	0.64–2.17	42	31.95	1.31	0.95–1.78
All intervals excl. first year	28	21.52	1.30	0.86–1.88	9	9.48	0.95	0.43–1.80	37	31.01	1.19	0.84–1.64
Borderline ovarian tumours												
<1 years	4	0.74	5.38	1.46–13.77	0	0.15	0	0.00–24.59	4	0.89	4.47	1.21–11.45
1–4 years	4	3.58	1.12	0.03–2.86	0	0.81	0	0.00–4.55	4	4.39	0.91	0.25–2.33
5–9 years	12	5.51	2.18	1.13–3.81	1	1.55	0.64	0.02–3.59	13	7.06	1.84	0.98–3.15
10–14 years	8	5.09	1.57	0.68–3.10	2	1.79	1.12	0.14–4.04	10	6.87	1.45	0.70–2.68
≥15 years	3	1.18	2.53	0.52–7.40	1	1.68	0.60	0.02–3.32	4	2.86	1.40	0.38–3.58
All intervals	31	16.10	1.93	1.31–2.73	4	5.98	0.67	0.18–1.71	35	22.08	1.59	1.10–2.20
All intervals excl. first year	27	15.36	1.76	1.16–2.56	4	5.83	0.69	0.19–1.76	31	21.19	1.46	0.99–2.08

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

Table III Incidence of ovarian malignancies in IVF-treated women, according to IVF treatment characteristics, subfertility and parity.

IVF group	Person years	All ovarian malignancies				Invasive ovarian cancer				Borderline ovarian tumours			
		Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
Total number of IVF cycles[a,b]													
1–2 cycle(s)	82 599	21	13.99	1.50	0.93–2.29	11	8.12	1.35	0.68–2.42	10	5.87	1.70	0.97–3.74
3–4 cycles	84 025	22	14.46	1.52	0.95–2.30	10	8.43	1.19	0.57–2.18	12	6.04	1.99	1.22–4.14
≥5 cycles	47 661	12	8.43	1.42	0.74–2.49	7	4.97	1.41	0.57–2.90	5	3.45	1.45	0.47–3.38
Subfertility diagnosis[b,c,d]													
Tubal	84 822	35	14.96	2.34	1.63–3.25	15	8.90	1.69	0.94–2.78	20	6.06	3.30	2.02–5.10
Endometriosis	26 853	14	4.59	3.05	1.67–5.12	10	2.68	3.73	1.79–6.86	4	1.90	2.10	0.57–5.38
Male factor	70 793	16	11.53	1.39	0.79–2.25	11	6.58	1.67	0.83–2.99	5	4.95	1.01	0.33–2.36
Hormonal factor[e]	16 873	3	2.64	1.14	0.23–3.32	2	1.49	1.34	0.16–4.84	1	1.15	0.87	0.02–4.86
Unexplained	45 846	5	7.97	0.63	0.20–1.46	3	4.67	0.64	0.13–1.88	2	3.30	0.61	0.07–2.19
Other factors	12 005	4	2.02	1.98	0.54–5.07	2	1.17	1.71	0.21–6.19	2	0.85	2.35	0.28–8.48
Previous FD use[c,f]													
No	95 782	26	14.15	1.84	1.20–2.69	9	8.35	1.08	0.49–2.05	17	5.8	2.93	1.71–4.69
Yes	109 149	20	15.41	1.30	0.79–2.01	15	8.88	1.69	0.95–2.79	5	6.52	0.77	0.25–1.79
Missing	49 297	9	7.33	1.23	0.56–2.33	4	4.29	0.93	0.25–2.38	5	3.03	1.65	0.53–3.85
Parity[a]													
Nulliparous	86 058	24	12.82	1.87	1.20–2.79	9	7.58	1.19	0.54–2.25	15	5.24	2.86	1.60–4.72
Parous	123 242	21	17.38	1.21	0.75–1.85	14	10.03	1.40	0.76–2.34	7	7.35	0.95	0.38–1.96
Missing	44 928	10	6.68	1.50	0.72–2.75	5	3.91	1.28	0.41–2.98	5	2.77	1.81	0.59–4.22
Total no. of ampoules hMG/FSH[g]													
1–40 ampoules	48 033	10	6.85	1.46	0.70–2.69	5	3.99	1.25	0.41–2.93	5	2.86	1.75	0.57–4.08
41–80 ampoules	49 345	11	7.08	1.55	0.78–2.78	5	4.12	1.21	0.39–2.83	6	2.96	2.03	0.74–4.42
≥81 ampoules	57 749	14	8.60	1.63	0.89–2.73	8	5.06	1.58	0.68–3.11	6	3.54	1.69	0.62–3.69
Missing	99 101	20	14.35	1.39	0.85–2.15	10	8.35	1.20	0.57–2.20	10	6.00	1.67	0.80–3.07
Total no. of oocytes[g]													
0–19 oocytes	89 929	20	13.84	1.45	0.88–2.23	10	8.27	1.21	0.58–2.22	10	5.57	1.80	0.86–3.30
≥20 oocytes	79 186	16	10.63	1.50	0.86–2.44	7	6.01	1.16	0.47–2.40	9	4.62	1.95	0.89–3.70
Missing	85 113	19	12.42	1.53	0.92–2.39	11	7.24	1.52	0.76–2.72	8	5.18	1.55	0.67–3.05
Mean no. of oocytes[g]													
0–3 oocytes	21 468	6	3.81	1.57	0.58–3.43	3	2.40	1.25	0.26–3.65	3	1.41	2.12	0.44–6.20
4–6 oocytes	46 899	14	7.31	1.91	1.05–3.21	7	4.39	1.60	0.64–3.29	7	2.92	2.39	0.96–4.93
≥7 oocytes	100 747	15	13.35	1.12	0.63–1.85	6	7.50	0.80	0.29–1.74	9	5.85	1.54	0.70–2.92
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05
Maximum no. of oocytes[g]													
0–5 oocytes	33 819	9	5.75	1.56	0.72–2.97	5	3.57	1.40	0.45–3.27	4	2.19	1.83	0.50–4.69
6–10 oocytes	58 581	13	8.75	1.49	0.79–2.54	5	5.16	0.97	0.31–2.26	8	3.59	2.23	0.96–4.39
≥11 oocytes	76 714	13	9.97	1.30	0.69–2.23	6	5.56	1.08	0.40–2.35	7	4.41	1.59	0.64–3.27
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

[a]Information based on health questionnaire survey; for non-responding women information was added from the medical records.

[b]Missing values of this variable were retrospectively completed for all cases; among non-cases with missing values, we distributed person time according to the distribution of person-years over categories of this variable.

[c]Information based on medical records; for women without medical record data, information was added from health questionnaire survey.

[d]Women may contribute person-years to more than one type of subfertility except for the categories unexplained and missing, which were unique classifications.

[e]Hormonal factors included ovulation disorders, polycystic ovary syndrome and premature menopause.

[f]Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF.

[g]Information based solely on medical records; no data abstraction could be done for 24% of the cohort that did give informed consent to do so.

Table IV Adjusted HRs for cancer risk in IVF group versus non-IVF group.

Cancer site	Overall		≥1 year follow-up		≥10 years follow-up	
	HR	95% CI	HR	95% CI	HR	95% CI
All ovarian malignancies[a]	2.05	1.10–3.82	2.14	1.07–4.25	2.08	0.86–5.00
Invasive ovarian cancer[b]	1.14	0.54–2.41	1.51	0.65–3.54	2.26	0.78–6.55
Borderline ovarian tumours[c]	6.38	2.05–19.84	4.23	1.25–14.33	2.26	0.46–11.05

HR, hazard ratio; CI, confidence interval.
[a]Adjusted for age at end of follow-up, endometriosis, tubal problems.
[b]Adjusted for age at end of follow-up, endometriosis.
[c]Adjusted for age at end of follow-up, tubal problems, parity.

Endometriosis was associated with significantly increased risk of invasive ovarian cancer, whereas tubal problems significantly increased the SIR for borderline ovarian tumours.

Comparisons within the cohort

Direct comparison of the IVF group with the non-IVF group (Table IV) yielded an adjusted hazard ratio (HR) for all ovarian malignancies of 2.14 (95% CI = 1.07–4.25), excluding the first year of follow-up. The adjusted HRs for invasive ovarian cancer and borderline ovarian tumours were 1.51 (95% CI = 0.65–3.54) and 4.23 (95% CI = 1.25–14.33), respectively. No trends emerged with number of IVF cycles or other IVF treatment characteristics, but numbers in subcategories were small. Clomiphene use prior to IVF was not associated with increased risk of ovarian malignancies (HRs for all malignancies, invasive ovarian cancer and borderline ovarian tumours were 0.89 (95% CI = 0.45–1.77), 1.22 (95% CI = 0.50–2.99) and 0.62 (95% CI = 0.21–1.83), respectively). Finally, we compared the risk of all ovarian malignancies between the IVF group and women in the non-IVF group who never used FDs (HR = 1.83; 95% CI = 0.70–4.82, based on five cases in 2115 unexposed women).

Discussion

This large nationwide cohort study with a median follow-up of 15 years shows that women treated with ovarian stimulation for IVF have a 2-fold increased risk of ovarian malignancies compared with subfertile women not treated with IVF. The excess risk was mostly due to borderline ovarian tumours, but 15 or more years after IVF treatment we also observed a SIR of 3.5 for invasive ovarian cancer.

Surprisingly, we observed that a high proportion (46%) of all ovarian malignancies in the IVF group concerned borderline ovarian tumours, whereas in the general population (below the age of 50 years) borderline ovarian tumours account only for 15–30% (Hart, 2005) of epithelial ovarian malignancies. So far only few studies examined FD use in relation to risk of borderline ovarian tumours, related to the fact that most population-based cancer registries do not record borderline ovarian tumours. Our cohort study is the first one examining the risk of borderline ovarian tumours following IVF treatment. Strikingly, the few case–control studies that examined the risk of borderline ovarian tumours after FD use found 2- to 4-fold increased risks (Harris *et al.*, 1992; Rossing *et al.*, 1994; Shushan *et al.*, 1996; Parazzini *et al.*, 1998; Ness *et al.*, 2002), though based on small numbers. In a case–cohort study (Rossing *et al.*, 1994) reporting an 11-fold risk increase of ovarian malignancies after 12 or more cycles of clomiphene, 5 of the 11 ovarian tumours were borderline ovarian tumours. Although screening for ovarian tumours in IVF-treated women has never been recommended in the Netherlands, we considered whether the increased risk of borderline ovarian tumours in the IVF group might be due to increased medical surveillance. We sent a questionnaire about diagnostic procedures to the gynaecologists of all case subjects with a borderline ovarian tumour who had given permission to approach their physician ($n = 18$). We received information for 14 subjects; in all cases, the diagnosis was made subsequent to complaints for which the woman visited her gynaecologist, rendering surveillance bias an unlikely explanation of our findings. Remarkably, we observed a high proportion of serous borderline ovarian tumours (63%), which was also seen in one case–control study (Ness *et al.*, 2002). Mucinous borderline ovarian tumours are more frequent in the general population (Verbruggen *et al.*, 2009).

Risk of borderline ovarian tumours was particularly strongly elevated in the first year after IVF, which is in line with several case reports of borderline ovarian tumours developing during or shortly after ovarian stimulation treatments (Atlas and Menczer, 1982; Goldberg *et al.*, 1992; Nijman *et al.*, 1992), providing support for speculations that ovarian stimulation may induce growth in existing highly differentiated tumours (Brinton *et al.*, 2005). We excluded ovarian tumours occurring in the first year after IVF, because of concern that their diagnosis might be related to diagnostic and treatment procedures for infertility. The early increase in risk was followed by a SIR close to unity in the 1–4 year follow-up interval; subsequently, risk of borderline ovarian tumours remained elevated up to more than 15 years after first IVF treatment. Hence, our data suggest that IVF treatment may be causally related to a prolonged increase of the risk of highly differentiated tumours. The natural history of borderline ovarian tumours is unclear and it is unknown which part of borderline ovarian tumours, if undetected, would develop into invasive ovarian cancer (Singer *et al.*, 2003; Sherman *et al.*, 2004; Shih and Kurman, 2004).

A concerning finding of our study is the increased SIR of invasive ovarian cancer in the IVF group after more than 15 years of follow-up, which was not observed in the non-IVF group. We cannot compare this result with findings from others since our study is the first reporting on cancer risk more than 10 years after IVF treatment. However, Brinton *et al.* (2004) followed a large cohort of 12 193 women treated for infertility prior to the IVF era. After 15 or more years of follow-up they reported non-significantly elevated rate ratios of ovarian cancer, 1.48 (95% CI = 0.7–3.2) for clomiphene and 2.46 (95% CI = 0.7–8.3) for gonadotrophins (when compared with never use of these drugs). Sanner *et al.* (2009) reported on a Swedish cohort treated for infertility in the 1960s–1970s, with a median follow-up of 33 years. Gonadotrophins were associated with increased risk of invasive ovarian cancer (relative risk = 5.28, 95% CI = 1.70–16.47) but clomiphene was not (when compared with never use of these drugs) (Sanner *et al.*, 2009). Ovulation stimulating drugs such as clomiphene were introduced in the late 1960s and IVF treatment with gonadotrophins,

resulting in much stronger ovarian stimulation, did not become widely available until the late 1980s. Consequently, women exposed to clomiphene have just recently reached the age range at which ovarian cancer frequently occurs (>70 years), while the oldest IVF-treated women have only recently reached their 50s. Since the induction period of ovarian cancer with respect to established risk factors amounts to 25 years or more (Risch, 1998), much longer follow-up is needed to fully evaluate the effects of gonadotrophins.

If ovarian stimulation were causally related to the risk of ovarian malignancy, we would expect increasing risks with greater number of IVF cycles or number of oocytes harvested. No such dose–response trends emerged. However, numbers in relevant dose categories were small, and data were missing for 17% of subjects, which reduced power for these analyses. In addition, the number of IVF cycles and number of harvested oocytes are only proxies for the number of ovarian punctures, which may have reduced the power to detect a dose–response relationship.

Case–control studies of the association between ovarian cancer risk and FD use have shown inconsistent results, with some studies reporting increased risks for subgroups (e.g. nulliparous women) (Ness *et al.*, 2002; Rossing *et al.*, 2004) and some suggesting a dose–response effect for clomiphene (Ness *et al.*, 2002; Rossing *et al.*, 2004). Treatment with hMG or FSH, as in IVF, may increase the number of ovulations to approximately six to nine times that of untreated women (Fishel and Jackson, 1989), which is a much stronger increase than the doubling of ovulations with clomiphene (Glasier, 1990; Derman and Adashi, 1994).

Nationwide cohort studies of IVF-treated women have only been reported from Australia (Venn *et al.*, 1999), Israel (Lerner-Geva *et al.*, 2003) and Sweden (Källén *et al.*, 2011). The first two cohort studies did not show increased risk of ovarian cancer in the IVF group compared with the general population (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003), while the recent Swedish study reported for parous women increased risk of ovarian cancer after IVF, compared with all other Swedish women who gave birth in the study period (HR = 2.09; 95% CI = 1.39–3.12) (Källén *et al.*, 2011). However, this study had no information on subfertility cause; therefore it is not clear whether the risk increase is attributable to IVF or subfertility. Of all cohort studies including IVF-treated women, our study includes the largest number of ovarian malignancies ($n = 77$ versus 13, 3 and 26 cases in the cohort studies from Australia, Israel and Sweden) (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003; Källén *et al.*, 2011).

Our study design had several strengths and weaknesses. Advantages include the large size of our cohort and the long-term follow-up. Selection bias can be ruled out since we were able to link 96% of our cohort with the population-based cancer and pathology registries, enabling us to also evaluate the occurrence of borderline ovarian tumours. All ovarian malignancies were histologically confirmed. Furthermore, we collected reproductive variables after IVF directly from the participating women, whereas for the majority of women information on subfertility cause and treatment could be abstracted from the medical files. Our data also include information on FD use prior to IVF, although this was incomplete for 27% of women. A limitation of our study is, however, that the comparison group of women unexposed to IVF treatment was relatively small, and that a proportion of these women (40%), had used FDs (clomiphene) outside the IVF setting (as did 54% of women in the IVF group), thus restricting the power for comparisons with a truly unexposed reference group. However, if multiple ovarian punctures rather than hormonal stimulation would induce ovarian malignancy, potential differences in FD use outside the IVF setting are not relevant.

Unfortunately, the response rate to the questionnaire was lower in the non-IVF group (49 versus 71% in the IVF group). Since we were allowed to link non-responders with the NCR and PALGA, differential non-response could not affect our overall risk estimates. However, the larger proportion of missing values for potential confounders (reproductive factors, cause of subfertility) among controls complicated our multivariable analyses. Adjustment for potential confounders did not materially affect our risk estimates, however.

We wondered whether the increased SIR of invasive ovarian cancer observed in the IVF group after 15 years might be due to less oral contraceptive (OC) use and/or lower parity in IVF-treated women. However, in the non-IVF group no increased SIR after long-term follow-up was seen. The proportion of long-term (≥7 years) OC users was high in our cohort and very similar in the IVF group and the non-IVF group (39.2 and 38.1%, respectively). Dutch women start OC use early and have a late age at first birth (mean 1985–1995: 28 years (Statistics Netherlands; www.cbs.nl, 2011) and only 19.1% of the IVF group and 22.5% of the non-IVF group never used OC or used them <1 year. Consequently, OC use was not a confounder in our multivariable Cox analysis. IVF-treated women remained more often nulliparous then the non-IVF group (44 versus 35%), but adjustment for parity only affected our results for borderline ovarian tumours, not for invasive ovarian cancer.

Our study is the only IVF cohort including a comparison group of subfertile women not treated with IVF, in addition to a comparison with the general population. Such a comparison group is important since IVF-treated women differ from the general population with respect to several risk factors for ovarian malignancies, e.g. subfertility and nulliparity. We cannot exclude the possibility, however, that the severity of certain causes of subfertility in the IVF group was not the same as in the non-IVF group. Since adjustment for individual causes of subfertility only slightly affected our estimates of the risk associated with IVF (data not shown), residual confounding by severity of certain subfertility causes seems unlikely, however.

Another limitation of our study is that our results are based on IVF treatment protocols used until 1995, prior to the adoption of currently applied milder stimulation regimens.

In conclusion, our results suggest that ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. Knowledge about the magnitude of the risks associated with ovarian stimulation is important for women considering starting or continuing IVF treatment, as well as their treating physicians. Clearly, the outcome of weighing a wish to conceive against the potential risks associated with IVF may differ among couples considering fertility treatment. In the Netherlands the cumulative risk of ovarian malignancy (including borderline ovarian tumours) is small, i.e. 0.45% at the age of 55 years. If our results are true, we would estimate a 0.71% risk for women who underwent IVF. It should be explained to women opting for IVF treatment that a borderline ovarian tumour does not constitute a lethal disease, although it may require extensive surgery and cause substantial morbidity. Ovarian cancer, however, is a disease with a high case fatality rate, for which

effective screening methods are not available (Hermsen et al., 2007). Although our findings give reason for some concern, they are still based on rather small numbers, no dose–response relationship was found and the risk increase for invasive ovarian cancer was not statistically significant in multivariable analyses. Even larger prospective cohort studies of IVF-treated women, with prolonged follow-up and a subfertile comparison group not treated with IVF, are needed to confirm or refute our findings and to conduct dose–response analyses with more power.

Authors' roles

F.E.v L. and C.W.B. designed the OMEGA study and were principal investigators of the study. F.E.v L. also coordinated statistical analyses, contributed to interpretation of the data and drafted the paper. C.W.B. contributed to interpretation of the data and drafting of the manuscript. H.K. contributed to the design of the study, coordinated identification of the cohort and data collection, did statistical analyses and contributed to interpretation of data. T.M.M. coordinated data collection, did the statistical analyses, contributed to study design, interpretation of the data and drafting of the manuscript. A.M.G.vd S. contributed to data collection and statistical analysis. C.B.L., M.K., J.S.E.L., C.A.M.J., F.M.H., B.J.C., W.N.P., J.M.J.S., A.H.M.S., F.vd V., J.L.H.E., P.A.v D. and N.S.M. provided IVF patient data and contributed to interpretation of the data. All authors contributed to critical revisions of the draft manuscript. All authors saw and approved the final version of the report.

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Conflict of interest

J.L.H.E. declares that he works in a department that has received unrestricted research grants from MSD and Ferring.

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