Supplementary Methods

ART and hormonal stimulation regimens 1983-1994 (OMEGA-I)

During the first years of ART treatment, regimens included gonadotropins (human menopausal gonadotropin [hMG] and/or follicle stimulating hormone [FSH]) with or without clomiphene (a drug with anti-estrogenic effects causing endogenous gonadotropins to rise). From 1988-1989 on, the stimulation protocol of hMG and/or FSH in combination with gonadotrophin releasing hormone (GnRH) agonists was introduced nationally. The use of clomiphene in ART treatment disappeared almost completely after 1989, while gonadotropin stimulations without agonists (in the first ART cycle) decreased from 80% in 1986 to around 10% after 1990. Along with the increasing use of agonists with gonadotropins, the number of ampoules administered and the number of oocytes obtained also increased. The average number of oocytes obtained during a first ART cycle increased from 5.4 in 1986 to 10.7 in 1994[1].

ART and stimulation regimens 1995-2000 (OMEGA-II)

Between 1995 and 2001 the stimulation protocol of hMG and/or FSH in combination with agonists was the most common stimulation scheme. From cycle day 21 onwards GnRH agonists were injected for desensitization of the natural cycle and prevention of premature ovulation. Stimulation with hMG and/or FSH started shortly after the first day of the menstruation and continued for about 10 days. Thirty-six hours before oocyte puncture hCG was injected.

Additional information non-ART group:

Women seeking subfertility treatment in 1980-2001 who were not treated with ART were eligible for the non-ART group. We selected women for the non-ART group from the

registries of eight of the 12 IVF clinics and two regional hospitals. Other IVF clinics did not have a complete registry of subfertile women not treated with ART. In order to yield sufficient numbers of non-ART subjects, women seeking subfertility treatment in the years before ART became a routine procedure in The Netherlands were included. Most women in the non-ART group who registered for their first consultation in the 1980s underwent tubal surgery and/or hormonal treatments. The majority of those who registered after 1990 withdrew from the waiting list for ART because they pursued other treatment options, reached the age of 40 years (the upper age limit for ART at the time), became pregnant or decided to refrain from ART for various reasons, such as divorce.

In total, 1,144 women originally selected into the non-ART group subsequently received ART. These women contributed person time to the non-ART group until the date of first ART treatment, and switched to the ART group from this date (according to standard cohort methodology regarding time dependent allocation of person-years in case of changing exposure).

Additional information OMEGA-II:

While in the OMEGA-I cohort only a paper version of the risk factor questionnaire was available, in OMEGA-II a web-based version was also available. The majority of Dutch women who underwent ovarian stimulation for ART in the years 1995 until 2001 received one or two ART cycles. As women with more ART cycles may have a greater risk of adverse events, the OMEGA-II study included all women who received three or more ART cycles and a hospital-stratified sample of women with 1-2 cycles. In seven IVF clinics 50% of women with one or two ART treatment cycles were (randomly) selected and in five (larger) hospitals all women treated with one or two ART treatment cycles between 1995 and 2000 were

invited; to allow for future studies with other research questions, requiring the entire population of ART-treated women..

Additional information borderline tumors:

For 14 women with discordant diagnoses according to NCR and PALGA, consensus on classification was reached through independent review of the pathology reports by a gynecopathologist (HHvB) and a gynecologic oncologist (CWB). In addition, if paraffin-embedded tissue blocks were available, pathology slides were examined by a gynecopathologist (HHvB).

References

1. De Boer EJ, van Leeuwen FE, Den Tonkelaar I, *et al.* [Methods and results of in-vitro fertilisation in the Netherlands in the years 1983-1994]. Ned Tijdschr Geneeskd 2004;148(29):1448-1455.

Supplementary Table 1. Incidence of Invasive Ovarian Cancer and Borderline Ovarian Tumor in non-ART women compared with the General Population, excluding the first year of follow-up.

Fertility treatment characteristics	Invasive ovarian cancer[a]				Borderline ovarian tumor[b]			
	Person-years	Observed/ Expected	SIR (95% CI)	P-value[c]	Person-years	Observed/ Expected	SIR (95% CI)	P-value[c]
Non-ART	236,907	37/32.1	1.15 (0.81-1.59)		236,721	16/8.7	1.84 (1.05-2.99)	
Age at 1st visit gynecologist (years)								
<30	99,121	10/9.3	1.10 (0.53-2.01)		99,080	4/3.1	1.30 (0.35-3.33)	
30-32	54,224	10/7.1	1.41 (0.68-2.59)		54,153	6/2.0	3.00 (1.10-6.54)	
33-35	44,422	8/7.3	1.10 (0.48-2.17)		44,382	3/1.8	1.65 (0.34-4.83)	
≥36	39,139	9/8.6	1.05 (0.48-1.99)	0.92	39,106	3/1.8	1.67 (0.35-4.89)	0.61
Time since 1st visit to the gynecologist (years)[d]								
1-9	82,625	8/6.0	1.34 (0.58-2.63)		82,614	3/1.4	2.12 (0.44-6.19)	
10-19	92,757	14/12.0	1.17 (0.64-1.96)		92,692	8/4.7	1.71 (0.74-3.38)	
≥20	61,213	15/14.1	1.07 (0.60-1.76)	0.88	61,104	5/2.6	1.93 (0.63-4.49)	0.95
Parity								
Non-ART and nulliparous	60,131	18/9.22	1.95 (1.16-3.09)		60,121	1/2.3	0.44 (0.01-2.45)	
Non-ART and parous	173,321	18/22.3	0.81 (0.48-1.27)	0.01	173,145	15/6.3	2.39 (1.34-3.94)	0.69
Main subfertility diagnosis								
Male factor	52,304	6/6.0	0.99 (0.37-2.16)		52,305	0/1.9	0.00 (0.00-1.99)	
Tubal factor	92,662	14/14.3	0.98 (0.53-1.64)		92,539	11/3.5	3.14 (1.57-5.63)	
Unexplained or other factor	91,940	17/11.7	1.45 (0.85-2.33)	0.02	91,878	5/3.3	1.50 (0.49-3.51)	0.01

ART = assisted reproductive technology; CI = confidence interval; SIR = standardized incidence ratio.

[a] Women with a first invasive ovarian cancer or ovarian cancer diagnosed within 3 months after an invasive cancer in the abdominal area (n=6) or women who developed invasive ovarian cancer following borderline ovarian cancer (n=1) are included in the analyses. Time at risk ends at date of diagnosis of invasive ovarian cancer.

[b] Only first borderline ovarian tumors are included in the analyses, subsequent invasive ovarian cancers after a borderline ovarian tumor (n=1) are ignored as events and in calculating the follow-up duration.

[c] P-value of Likelihood ratio test.

[d] The follow-up category to which women were allocated was calculated from start of first ART treatment/visit gynecologist till censoring date; also for women whose observation time started after 1989.

Supplementary Table 2. Risk of Invasive Ovarian Cancer and Borderline Ovarian Tumor Risk according to Histologic Type.

Fertility treatment characteristics	Serous ovarian cancer			Non-Serous ovarian cancer[a]		
	No. of ovarian cancers	No. of women	Adj. HR (95% CI)[b]	No. of ovarian cancers	No. of women	Adj. HR (95% CI)[b]
ART exposure						
Non-ART	16	9,972	1 [Reference]	21	9,972	1 [Reference]
ART	66	30,565	1.52 (0.87-2.68)	49	30,565	0.68 (0.40-1.15)
	Serous borderline ovarian tumor			Non-Serous borderline ovarian tumor[a]		
	No. of borderline ovarian tumors	No. of women	Adj. HR (95% CI)[c]	No. of borderline ovarian tumors	No. of women	Adj. HR (95% CI)[c]
ART exposure						
Non-ART	5	9,972	1 [Reference]	12	9,972	1 [Reference]
ART	43	30,565	3.44 (1.35-8.77)	36	30,565	1.17 (0.60-2.29)

Adj. = adjusted; ART = assisted reproductive technology; CI = confidence interval; HR = hazard ratio; No. = number.

[a] Non-serous includes endometrioid, clear cell, mucinous, not other specified and other histologic types.

[b] Analyses are adjusted for age at start treatment/visit gynecologist and parity.

[c] Analyses are adjusted for age at start treatment/visit gynecologist, parity, and tubal subfertility.

Supplementary Table 3. Invasive Ovarian Cancer and Borderline Ovarian Tumor Risk according to Fertility Treatment Characteristics.						
	including year 1			excluding year 1		
Fertility treatment characteristics	No. of ovarian tumors[a]	No. of women[a]	Adj. HR (95% CI)[b]	No. of ovarian tumors[a]	No. of women[a]	Adj. HR (95% CI)[b]
ART exposure						
Non-ART	57	9,988	1 [Reference]	54	9,972	1 [Reference]
ART	200	30,625	1.16 (0.86-1.57)	193	30,565	1.18 (0.87-1.61)
Total no. of ART cycles						
0	57	9,988	1 [Reference]	54	9,972	1 [Reference]
1-2	81	12,505	1.25 (0.88-1.77)	79	12,474	1.28 (0.90-1.83)
3-4	73	11,605	1.08 (0.75-1.54)	71	11,586	1.11 (0.77-1.59)
≥5	46	6,515	1.15 (0.78-1.71)	43	6,505	1.13 (0.76-1.70)
Response at 1st ART cycle[cd]						
Normal response	102	14,973	1 [Reference]	97	14,943	1 [Reference]
Poor response	28	4,345	0.76 (0.50-1.16)	28	4,340	0.79 (0.52-1.21)
Missing	70	11,307	0.94 (0.69-1.27)	68	11,282	0.96 (0.71-1.31)
OHSS[de]						
Never	195	29,660	1 [Reference]	188	29,602	1 [Reference]
Ever	5	965	0.92 (0.38-2.24)	5	963	0.95 (0.39-2.32)
Clomiphene use[d]						
Never	62	9,726	1 [Reference]	60	9,712	1 [Reference]
Ever	39	6,527	0.98 (0.67-1.45)	38	6,515	0.78 (0.52-1.17)
Missing	99	14,372	1.06 (0.76-1.47)	95	14,338	0.92 (0.66-1.27)
Main subfertility diagnosis						
Male factor	60	11,591	1 [Reference]	56	11,572	1 [Reference]
Tubal factor	104	12,981	1.36 (0.99-1.87)	101	12,952	1.40 (1.01-1.94)
Unexplained or other factor	93	16,041	1.05 (0.76-1.45)	90	16,013	1.08 (0.77-1.51)

Adj. = adjusted; ART = assisted reproductive technology; CI = confidence interval; HR = hazard ratio; No. = number; OHSS=ovarian hyperstimulation syndrome.
[a] Not all numbers add up to 100%, because of missing values.
[b] Each variable was analyzed in a separate model. Analyses are adjusted for age at start treatment/visit gynecologist, parity, and tubal subfertility.
[c] Poor response includes canceled first cycles because of anticipated poor response and less than four oocytes; normal response includes four or more oocytes collected in first cycle.
[d] Among ART treated women only.
[e] OHSS Includes women who had had no ovum pick-up because of (anticipated) OHSS.