Increased risk of early menopausal transition and natural menopause after poor response at first IVF treatment

Evelien J.de Boer, Isolde den Tonkelaar, Egbert R.te Velde, Curt W.Burger, Flora E.van Leeuwen on behalf of the OMEGA-project group

Department of Epidemiology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam; International Health Foundation, Europalaan 506, 3526 KS Utrecht; Department of Reproductive Medicine, Division of Obstetrics, Neonatology and Gynaecology, University Medical Center Utrecht, Postbus 85500, 3508 GA Utrecht and Department of Gynaecology and Obstetrics, Erasmus Medical Center, Postbus 2040, 3000 CA Rotterdam, The Netherlands

To whom correspondence should be addressed. E-mail: f.v.leeuwen@nki.nl

BACKGROUND: Our aim was to examine whether women who had a low number of retrieved oocytes at their first IVF attempt reach the menopausal transition and/or the natural menopause earlier than women of similar ages with a high number of retrieved oocytes. METHODS: We conducted a retrospective cohort study among women in The Netherlands who received IVF treatment between 1983 and 1995. For the present study, we selected all cohort members who had a regular menstrual cycle at the time of the first visit to the gynaecologist (n = 4601). After a median follow-up of 5.5 years, 3871 (84%) women still had a regular menstrual cycle pattern, 547 (12%) women had entered the menopausal transition (i.e. no menses for 3–11 months, use of HRT or irregular menstrual cycles) and 27 (1%) women had reached natural menopause. We examined whether the quantity and the quality of the retrieved oocytes were related to an early menopausal transition and early menopause. The live birth rate per embryo transfer was used as indicative of the quality of the oocytes. RESULTS: The age-adjusted odds ratio (OR) for having entered the menopausal transition/natural menopause for women with a poor response (0–3 oocytes) at their first IVF attempt was 3.1 [95% confidence interval (CI) 2.4–3.8] compared with women with a normal response (>3 oocytes). Women who were stimulated with gonadotrophins during IVF treatment but did not undergo an IVF puncture because of an anticipated poor response (cancelled IVF cycle) had an age-adjusted OR of 3.2 (95% CI 2.3–4.3). There was no significant difference in the odds of reaching the menopausal transition/natural menopause, after adjustment for age and the number of retrieved oocytes, between women who did and did not have a live birth following their first embryo transfer (OR = 1.3; 95% CI 0.95–1.7). CONCLUSIONS: These results indicate that a low remaining quantity of oocytes, as reflected by a low number of retrieved oocytes at first IVF treatment, is an important predictor of the risk of an early menopausal transition/natural menopause. The quality of the oocytes did not affect the risk of an early menopausal transition/natural menopause once the number of retrieved oocytes had been taken into account. Our findings support the concept that the number of remaining follicles in the ovaries is one of the main aspects of reproductive ageing.

Key words: epidemiology/IVF treatment/menopausal transition/natural menopause/retrieved oocytes

Introduction

The ovarian concept of reproductive ageing assumes that the age-related loss in female fertility is dictated by the decline of both the quantity and the quality of the follicles (Te Velde and Pearson, 2002). The years before the final menstrual period, when variability in the menstrual cycle is increased, are known as the menopausal transition (WHO Scientific Group, 1996). However, the definition of the menopausal transition remains vague since variability of the cycle was not defined. Recently, Mitchell et al. (2000), Harlow et al. (2000a) and Soules et al. (2001) defined the menopausal transition based upon increasing variability and developed a staging system (Soules et al., 2001) within the time period of the menopausal transition (Soules et al., 2001). Richardson et al. (1987) showed that in women with regular menstrual cycles (premenopausal), follicle counts were 10 times greater than in perimenopausal women of similar ages, while follicles were virtually absent in post-menopausal women.

Early ovarian depletion is likely to be reflected by a low number of retrieved oocytes (poor oocyte response) after gonadotrophin stimulation in IVF treatment. This is supported by the fact that in older women, fewer oocytes are retrieved after stimulation with gonadotrophins during IVF treatment...
than in younger women (Navot et al., 1991; Cahill et al., 1994; Seifer et al., 1999; Check et al., 2000). Thus, a low number of retrieved oocytes after gonadotrophin stimulation for IVF would be expected to be associated with an earlier start of the menopausal transition. Indeed, in a small case–control study, we recently observed that the retrieval of a low number of oocytes at first IVF treatment was associated with greater risk of early menopause (<46 years of age) (de Boer et al., 2002). In the present, much larger, study, we examine whether women who had a poor oocyte response at their first IVF attempt enter the different menopausal transition stages earlier, or reach natural menopause earlier, than women of similar ages with a normal oocyte response. We also investigated whether poor quality of the retrieved oocytes is predictive of early menopausal transition or early natural menopause.

Materials and methods

Study population and study procedures

We conducted a retrospective cohort study of women who underwent IVF treatment in The Netherlands. The study populations, study procedures and data collection methods have been described in detail previously (Klip et al., 2001; de Boer et al., 2002). Briefly, subjects are participants in a nationwide cohort study (the OMEGA-project) of 19 840 women treated with IVF in one of the 12 IVF clinics in The Netherlands between January 1, 1983 and January 1, 1995. The main purpose of the OMEGA study is to examine the risk of hormone-related cancers in women who received hormone stimulation for IVF. All institutional ethics committees of the participating clinics approved the study procedures.

Between 1997 and 2000, all women received a questionnaire, an information letter and a study brochure. A total of 19 242 women received the 23-page study questionnaire, asking each participating subject for their written informed consent for data abstraction from the medical records. Seventy-three percent of the women returned the questionnaire. The upper part of Figure 1 displays a graphical presentation of the OMEGA study population.

Data collection

From the questionnaire, the following data were obtained: menopausal status, age at menopause, menstrual cycle characteristics, use of HRT, use of oral contraceptives (OCs), parity and smoking habits. In the questionnaire, women were asked whether they used HRT for menopausal complaints, whether their menstrual cycle had ceased for 3–11 months prior to completion of the questionnaire and whether this was due to pregnancy/lactation or other reasons. In addition, women...
were also asked whether or not their last vaginal bleeding had occurred at least 12 months before completion of the questionnaire. Menopause was considered natural if it was not the result of (or possibly related to) surgery (hysterectomy, oophorectomy) or due to chemotherapy/radiotherapy for cancer treatment.

Trained research assistants abstracted data from the medical files on: gynaecological history, subfertility diagnosis, fertility hormones used prior to IVF treatment, and detailed information about each subsequent IVF treatment (all fertility drugs and the corresponding dosages used during IVF treatment, the number of retrieved oocytes, occurrence of complications and whether or not the treatment resulted in a pregnancy).

Analytic cohort
To be eligible for the cohort study presented here, women had to have a regular menstrual cycle pattern at the time of their first visit to the gynaecologist. Women were defined as having a regular menstrual cycle pattern when they were able to predict the next menstrual cycle always or almost always within 4 days and when their mean menstrual cycle length was between 21 and 35 days. Of all initial 10 215 IVF-treated women who returned the questionnaire and for whom medical record data were available, 6872 women reported to have had a regular menstrual cycle pattern at the time of the first visit to the gynaecologist. The lower part of Figure 1 presents the distribution of the source population (n = 10 215) according to menstrual cycle characteristics and various exclusion criteria. Finally, 4601 women were eligible for the present study.

Subsequently, we examined whether, at the end of the follow-up period (at the completion of the questionnaire, follow-up time ranged from 0.5 to 16.2 years), women had kept their regular menstrual cycle pattern (premenopausal) or whether they had reached the menopausal transition or the natural menopause.

Definitions of variables and methods of analysis
In the analysis, we compared the odds of having entered the menopausal transition or having reached natural menopause between women with a normal response and women with a poor response or a cycle cancellation due to insufficient follicle growth (anticipated poor response) at their first IVF attempt. Type of response was taken as a proxy for oocyte quantity. By comparing only the number of retrieved oocytes, we had to exclude those women whose next menstrual cycle was not predictable within 4 days and who had a mean cycle length of <21 or >35 days (‘middle’ menopausal transition) (group b). Women whose menstrual cycle had ceased 3–11 months prior to the completion of the questionnaire (unless this was due to lactation/pregnancy or surgery such as hysterectomy) were categorized into the ‘late’ menopausal transition (group c). Women who (had) used HRT for menopausal complaints were considered as a separate category because they could not be assigned to one of the stages of the menopausal transition (group d). Women whose last vaginal bleeding had occurred at least 12 months prior to the completion of the questionnaire (unless this was due to lactation/pregnancy or surgery such as hysterectomy, or due to chemotherapy/radiotherapy for cancer treatment) were considered as having reached the natural menopause. Women who considered themselves as post-menopausal but used HRT at that same time were included in the HRT subgroup. Women whose menstrual cycle pattern could not be categorized into either the premenopausal group, the various menopausal transition stages, natural menopause or HRT subgroup were categorized into the so-called ‘other’ group.

Women were defined as smokers when they had smoked more than one cigarette a day for at least 1 year at the time of the first oocyte retrieval.

The following three oocyte quality measures were used for the analyses of the results. Live birth rate (LBR) per started cycle, defined as any birth event in which at least one baby is born alive divided by the number of started IVF cycles (as a percentage), relates to the overall clinical effectiveness of the entire treatment cycle, including the ovarian response to stimulation, the ovum pick-up procedure, whether or not fertilization takes place, early embryonic development in vitro and the technique of the embryo transfer. The LBR per embryo transfer, which has the number of embryo transfers as denominator, is only related to the last phases of the treatment cycle which depend more directly on the oocyte quality. The ability of the embryo to implant into the uterus can also be regarded as a reflection of oocyte quality. It is defined by the number of gestational sacs visualized with ultrasound divided by the number of embryos replaced (Van Kooij et al., 1996). Since this information was not available, we used the LBR per transferred embryo as a proxy of the implantation rate. The LBR per transferred embryo was defined as the number of babies born alive divided by the number of embryos replaced. For example, if the birth of a set of twins was the result of the transfer of two embryos, the LBR per embryo was 100%. If one child was born after four embryos had been transferred, the LBR per embryo was 25%. These examples demonstrate that several implantations may take place in one woman. These implantation successes cannot be taken as independent observations, a phenomenon known as overdispersion (Cox, 1970). The Williams procedure (Williams, 1982) for dependency was used to correct for this phenomenon (Van Kooij et al., 1996).
All descriptive analyses were processed with SPSS® (version 10.0, SPSS Inc., Chicago, IL, USA). Because no normal distribution could be assumed, the comparison of the median number of retrieved oocytes between women who entered the menopausal transition or had reached natural menopause and women with a regular menstrual cycle pattern was analysed and tested by the Mann-Whitney U-test. Differences between group means and distributions over categories were tested by Student’s t-test or χ² test. A two-sided P-value <0.05 was considered statistically significant.

We also estimated the age-specific prevalences of having reached the menopausal transition or natural menopause, for normal and (anticipated) poor responders separately. Three year age-specific moving averages were used for graphical presentation. The age-specific prevalence curve for the normal responders was based on the estimates between the ages of 29 and 46 years. For the poor and anticipated poor responders, the age-specific prevalence curve was based on the estimates between the ages of 35 and 48 years. All other age-specific prevalences were based on fewer than 50 women and were therefore omitted in the graphical presentation.

Multinomial logistic regression analysis was used to determine the odds ratios for having entered various stages of the menopausal transition or having reached natural menopause according to the number of retrieved oocytes at first IVF treatment. Since we did not have information as to when women had entered the menopausal transition, the outcome used in the analysis was whether or not women had entered the various stages of the menopausal transition at the end of follow-up. Although we had information as to when women had reached the natural menopause, for reasons of comparability we used a uniform outcome measure and also determined whether or not women had reached the natural menopause according to the number of retrieved oocytes at first IVF treatment. It is important to emphasize that our measure of disease occurrence is actually a prevalence measure instead of an incidence measure. Odds ratios, two-sided P-values, and 95% confidence intervals (CIs) were calculated with a normal response at first IVF treatment as the reference category. In a multivariate multinomial logistic regression model, it was determined whether or not it was necessary to adjust for potential confounders, such as the woman’s age at time of filling out the questionnaire (continuous), the age at which the first IVF treatment took place (3 years age groups), calendar year of IVF treatment (continuous), subfertility diagnosis (four categories), live birth after IVF (yes/no), smoking habits (yes/no and one category containing ‘unknown’ smoking status), the number of IVF cycles per woman (continuous) and the number of ampoules of HMG/FSH used for ovarian stimulation during the first IVF attempt (four categories). Potential confounders were evaluated by adding each confounder separately into the logistic model. If this resulted in a >10% change in the odds ratio (compared with the crude odds ratio), the confounder was included in the final multinomial logistic model (Rothman and Greenland, 1998).

Results

Table I presents cohort characteristics according to the type of response at the women’s first oocyte retrieval. Of all women, 80% had >3 oocytes at their first oocyte retrieval (normal response), 14% had a poor response (≤3 oocytes) and in 6% the first IVF treatment was cancelled due to anticipated poor

---

**Table I.** Cohort characteristics according to type of oocyte response at first IVF treatment (n = 4601)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal response (n = 3675)c</th>
<th>Poor response (n = 636)c</th>
<th>P-valuea</th>
<th>Anticipated poor response (n = 290)c</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at first IVF treatment, years (range)</td>
<td>33.0 (20.5–44.1)</td>
<td>34.9 (23.2–47.1)</td>
<td>&lt;0.0001d</td>
<td>34.3 (24.8–44.9)</td>
<td>&lt;0.0001d</td>
</tr>
<tr>
<td>Median follow-up time in years (range)</td>
<td>5.4 (0.5–16.2)</td>
<td>6.2 (1.2–14.2)</td>
<td>&lt;0.0001d</td>
<td>5.4 (1.2–13.5)</td>
<td>0.07d</td>
</tr>
<tr>
<td>Median age at completion of the questionnaire, years (range)</td>
<td>38.6 (24.1–55.2)</td>
<td>41.4 (27.4–55.3)</td>
<td>&lt;0.0001d</td>
<td>40.3 (27.0–51.4)</td>
<td>&lt;0.0001d</td>
</tr>
<tr>
<td>Median number of retrieved oocytes, no. (range)</td>
<td>9 (4–66)</td>
<td>2 (0–3)</td>
<td>&lt;0.0001d</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LBR per started cycle (%)</td>
<td>18 (647/3675)</td>
<td>6 (39/636)</td>
<td>&lt;0.0001f</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LBR per embryo transfer (%)</td>
<td>21 (647/3107)</td>
<td>9 (39/433)</td>
<td>&lt;0.0001f</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LBR per transferred embryo (%)</td>
<td>10 (849/8405)</td>
<td>6 (45/778)</td>
<td>&lt;0.0001d</td>
<td>0.01f</td>
<td>&lt;0.0001f</td>
</tr>
<tr>
<td>Smoking (at time of first oocyte retrieval), no. (%)</td>
<td>2125 (58)</td>
<td>344 (54)</td>
<td>138 (48)</td>
<td>1513 (41)</td>
<td>278 (44)</td>
</tr>
<tr>
<td>Yes</td>
<td>1513 (41)</td>
<td>278 (44)</td>
<td>144 (50)</td>
<td>37 (1)</td>
<td>14 (2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>37 (1)</td>
<td>14 (2)</td>
<td>8 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfertility diagnosis, no. (%)</td>
<td></td>
<td></td>
<td>&lt; 0.0001f</td>
<td>104 (36)</td>
<td>0.5f</td>
</tr>
<tr>
<td>Tubal</td>
<td>1290 (35)</td>
<td>257 (40)</td>
<td>81 (28)</td>
<td>1172 (32)</td>
<td>156 (25)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td>81 (28)</td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other or unknown</td>
<td>394 (11)</td>
<td>91 (14)</td>
<td>31 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean duration of subfertility (years ± SD)k</td>
<td>3.7 ± 2.6</td>
<td>4.2 ± 3.2</td>
<td>0.001d</td>
<td>4.1 ± 3.3</td>
<td>0.05d</td>
</tr>
</tbody>
</table>

aP-value (normal response versus poor response).
bP-value (normal response versus anticipated poor response).
cNormal response, >3 oocytes retrieved at first IVF treatment or cycle cancellation due to OHSS; poor response, 0–3 oocytes retrieved at first IVF treatment; anticipated poor response, cycle cancellation due to anticipated poor response.
dDetermined by Student’s-t-test.
eDetermined by Mann-Whitney U-test.
fDetermined by χ² test.
gDetermined by means of logistic regression with Williams correction.
hAny birth event with at least one baby born alive/started cycle.
iAny birth event with at least one baby born alive/embryo transfer.
jAny birth event with at least one baby born alive/transferred embryos.
kKnown for n = 3794.
response. Women with a(n) (anticipated) poor response more often had their first IVF treatment at an older age than women with a normal response. Consequently, both women who had a poor response and women with an anticipated poor response had a higher median age at the time of the completion of the questionnaire compared with normal responders. The LBR per started cycle, LBR per embryo transfer and the LBR per transferred embryo were significantly higher among the normal responders than among the poor responders. Women with a(n) (anticipated) poor response less often had a live birth at or after their IVF attempt(s) compared with normal responders (39 and 34% versus 52%, data not shown). Poor responders and anticipated poor responders were more often smokers at the time of the first oocyte retrieval than normal responders.

After a median follow-up period of 5.5 years, 3871 (84%) women out of the cohort of 4601 women reported to have kept a regular menstrual cycle pattern, 547 (12%) had entered the menopausal transition and 27 (1%) had reached natural menopause. In addition, 156 (3%) women were categorized into the so-called ‘other’ group. The latter group consisted of women who reported the predictability of their next menstrual cycle and/or their mean menstrual cycle length as ‘unknown’ (n = 78), women who did not report their current menstrual cycle characteristics at all (n = 44) or women who reported a mean cycle length <21 or >35 days and yet reported being able to predict the next menstrual cycle within 4 days (n = 34). For women who had reached the menopausal transition/natural menopause, the median follow-up time was 6.6 years. The median number of retrieved oocytes at first IVF treatment was significantly lower for women who had entered the menopausal transition or had reached natural menopause compared with the women who still menstruated regularly (five and two versus eight, respectively).

The menopausal transition group was divided into the following four tentative subgroups: (a) 209 (5%) were classified in the ‘early’ menopausal transition group; (b) 182 (4%) in the ‘middle’ menopausal transition group; (c) 40 (1%) in the ‘late’ menopausal transition; and (d) 116 (3%) women were categorized into the HRT subgroup. Apparently, few women had entered the ‘late’ menopausal transition group or had reached natural menopause compared with the women who still menstruated regularly (five and two versus eight, respectively).

Figure 2 presents the menstrual cycle characteristics at the end of follow-up according to type of oocyte response at first IVF treatment.

Figure 3 presents the age-specific prevalence rates for having reached any stage of the menopausal transition or natural menopause, for normal and (anticipated) poor responders. A comparison of the curves (i.e. the start of the slopes) suggests that poor and anticipated poor responders reach the menopausal transition/natural menopause ~6–7 years earlier than normal responders.

Table II presents the odds ratios for having entered one of the four different stages of the menopausal transition or natural menopause, according to the type of oocyte response at first IVF treatment. All analyses were adjusted for age at completion of the questionnaire (continuous together with age squared) which was the only confounder according to our criteria. For women with a poor response and for women with an anticipated poor response, the odds ratios for having entered the ‘late’ menopausal transition after a median of 5.5 years of follow-up were 5.0 and 7.4, respectively, compared with women with a normal response. The association between the number of retrieved oocytes at first IVF treatment and the ‘early’ menopausal transition was less strong, albeit significant. The risk of having entered any of the stages of the menopausal transition was ~3-fold elevated for women both with a poor and an anticipated poor response at their first IVF attempt, as compared with women with a normal response. Poor and anticipated poor response were most strongly associated with the risk of reaching natural menopause in the 5–10 year period.
after the first IVF treatment, with odds ratios of 14.6 and 17.5, respectively. When all menopausal transition categories and natural menopause were considered together, the odds ratios for reaching the menopausal transition/natural menopause for poor and anticipated poor responders were 3.1 (95% CI 2.4–3.8) and 3.2 (95% CI 2.3–4.3) respectively (data not shown). When we excluded from the analyses all women whose first cycle was cancelled due to an anticipated poor response or due to an OHSS (n = 319) and included the actual number of retrieved oocytes in the multinomial regression model, the age-adjusted odds ratio for having entered the menopausal transition/natural menopause was 0.94 per additional oocyte retrieved (95% CI 0.92–0.96). This implies that for each additional oocyte retrieved at first IVF treatment, the risk for entering the menopausal transition or reaching natural menopause in the subsequent 5–10 year period was 6% lower.

Table III presents the overall odds ratios for having entered the menopausal transition (all three stages and HRT subgroup) combined with having reached natural menopause according to several characteristics. Women who smoked at the time of the first oocyte retrieval were more likely to have entered the menopausal transition/ menopause. Furthermore, we used the number of retrieved oocytes at first IVF treatment as indicative of the quality of the oocytes. Women who had no live birth following their first embryo transfer had an increased risk, although not statistically significant, to have reached the menopausal transition/natural menopause. However, when we also adjusted for the number of retrieved oocytes at first IVF treatment (as a continuous variable), the odds ratio of reaching the menopausal transition/natural menopause for women who had no live birth following their first embryo transfer decreased from 1.4 to 1.3 (95% CI 0.95–1.7; data not shown). When we studied the separate and joint effects of oocyte response (proxy of quantity) and the occurrence of a live birth after embryo transfer (proxy for quality) on the risk of having entered the menopausal transition/natural menopause, no interaction effects between quantity and quality of the oocytes emerged (data not shown).

**Discussion**

Our findings support the prevailing concept of female reproductive ageing, which assumes that the age-dependent loss of female fertility is dictated by the decline in both the quantity and quality of the oocyte/follicle pool. Women with a poor

---

### Table II. Odds ratios for having entered various stages of the menopausal transition (early, middle, late and HRT use)a or having reached natural menopause according to the type of response at first IVF treatment (n = 4601)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Early menopausal transitionb</th>
<th>Middle menopausal transitionb</th>
<th>Late menopausal transitionb</th>
<th>HRT usec</th>
<th>Any stage of the menopausal transitiond</th>
<th>Natural menopausee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multivariate OR</td>
<td>Multivariate OR</td>
<td>Multivariate OR</td>
<td>Multivariate OR</td>
<td>Multivariate OR</td>
<td>Multivariate OR</td>
</tr>
<tr>
<td>Normal response</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Poor response</td>
<td>1.8 (1.3–2.6)</td>
<td>4.7 (3.3–6.6)</td>
<td>5.0 (2.4–10.4)</td>
<td>2.5 (1.6–4.0)</td>
<td>2.9 (2.3–3.6)</td>
<td>14.6 (4.6–46.7)</td>
</tr>
<tr>
<td>Anticipated poor response</td>
<td>1.6 (0.95–2.8)</td>
<td>3.7 (2.3–6.0)</td>
<td>7.4 (3.2–17.1)</td>
<td>4.0 (2.4–6.9)</td>
<td>3.0 (2.2–4.1)</td>
<td>17.5 (4.8–64.6)</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval, from multinomial logistic regression.

*Early* menopausal transition, mean menstrual cycle length 21–35 days and next menstrual cycle not predictable within 4 days; *middle* menopausal transition, mean menstrual cycle length <21 or >35 days and next menstrual cycle length not predictable within 4 days; *late* menopausal transition, no menses for 3–11 months prior to the completion of the questionnaire; HRT use: women who (had) used HRT for menopausal complaints; natural menopause, no menses for at least 12 months prior to completion of the questionnaire.

*All four subgroups of the menopausal transition.

*Adjusted for age at completion of the questionnaire (continuous and age squared).

---

### Table III. Odds ratios for having reached the menopausal transition or natural menopause according to potential confounding factors (n = 4601)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of premenopausal women (n = 3871)</th>
<th>No. of women having reached the menopausal transition or natural menopause (n = 574)a</th>
<th>Multivariate OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking (at time of first oocyte retrieval)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2234</td>
<td>289</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>1591</td>
<td>278</td>
<td>1.5 (1.2–1.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>46</td>
<td>7</td>
<td>1.4 (0.6–3.0)</td>
</tr>
<tr>
<td>Subfertility diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>1330</td>
<td>251</td>
<td>1.0</td>
</tr>
<tr>
<td>Male</td>
<td>1220</td>
<td>141</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>886</td>
<td>111</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>435</td>
<td>71</td>
<td>0.9 (0.6–1.2)</td>
</tr>
<tr>
<td>LBR per embryo transfer (at first IVF treatment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women who had at least one live birth</td>
<td>600</td>
<td>58</td>
<td>1.0</td>
</tr>
<tr>
<td>Women who had no live birth</td>
<td>2421</td>
<td>339</td>
<td>1.4 (1.1–1.9)</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval, from multinomial logistic regression.

*All four subgroups of the menopausal transition and natural menopause combined.

*Adjusted for age at the completion of the questionnaire (continuous and age squared).
response, or an anticipated poor response, at their first IVF treatment are more likely to enter the menopausal transition or reach natural menopause in the next decade as compared with normal responders. The association between poor oocyte response and the risk of reaching the menopausal transition was stronger for the later stages of the menopausal transition and the natural menopause. These findings are in line with previous studies in which we observed that (anticipated) poor responders are at greater risk of becoming post-menopausal at the age of 46 years or before, compared with normal responders (de Boer et al., 2002). Women with a poor response were also found to have a lower LBR per transferred embryo, as indicative of poor oocyte quality (Table 1), compared with normal responders. There was no significant difference in the odds ratio of entering the menopausal transition/natural menopause between women who did and did not have a live birth following their first embryo transfer. Our findings provide convincing evidence for the quantitative aspect of the ovarian ageing.

We also found that smokers at the time of the first oocyte retrieval were more likely to have entered the menopausal transition or to have reached natural menopause. These findings are in line with the literature, which consistently shows that smoking decreases menopausal age (Jick and Porter, 1977; McKinlay et al., 1992; Torgerson et al., 1994; van Noord et al., 1997; Harlow and Signorello, 2000). In addition, women with a(n) (anticipated) poor response during their first IVF attempt were more often smokers than women with a normal response. This observation is in line with studies which showed that cigarette smoke components might influence oocyte quantity and quality directly or indirectly (Zenzes et al., 1995; Zenzes, 2000).

Defining the menopausal status of women is a difficult task for researchers and depends greatly on the source and completeness of the data. Many definitions of the menopausal status are based upon the work of Treloar (1981) and Vollman (1977). Terms such as premenopause, perimenopause, climacteric, menopause and menopausal transition are often used in different ways and regularly create confusion. The World Health Organization (WHO Scientific Group, 1996) recommended the use of the terms premenopause, perimenopause and the menopausal transition. According to the WHO, ‘the menopausal transition is the time before the final menstrual period when variability in the menstrual cycle is usually increased’. However, this definition remains vague since variability was not defined.

The criterion of ‘variability’ or ‘predictability’ is used in many studies defining menopausal status (Cooper and Baird, 1995; McKinlay, 1996; Harlow et al., 2000b; Mitchell et al., 2000; Gold et al., 2001). Except for the study of Mitchell et al. (2000) and the STRAW workshop (Soules et al., 2001), most of these studies lack any specific description of these terms. Brambilla et al. (1994) and Dudley et al. (1998) suggested that difficulties in predicting the next menstrual cycle could be one of the early signs of approaching menopause. Furthermore, in several studies, the occurrence of amenorrhoea for 3–11 months is used as a criterion for the menopausal transition or late perimenopause (McKinlay et al., 1992; Brambilla et al., 1994; Guthrie et al., 1996; Dennerstein et al., 1997; Dudley et al., 1998; Harlow et al., 2000b; Gold et al., 2001). In addition to the predictability of the next menstrual cycle and the mean cycle length, all participating women in the OMEGA-project were asked to report whether they used HRT. Since HRT in The Netherlands is nearly always prescribed to treat menopausal complaints, and not just to prevent osteoporosis during premenopause, we decided to include these women in the menopausal transition group. Since no questions were included in the questionnaire about the reasons for OC use, we were not able to distinguish women who used OCs for treating menopausal complaints. Therefore, women who used OCs 1 year prior the completion of the questionnaire (n = 978) and those with missing data on OC use (n = 181) were excluded from the analyses. To investigate the possibility of selection bias, we analysed the distribution of the oocyte response at first IVF treatment in these excluded women. Of all the excluded women due to reasons of OC use (n = 1159), 78% had more than three oocytes at their first oocyte retrieval (normal response), 16% had a poor response (three or fewer oocytes) and in 5% the first IVF treatment was cancelled due to anticipated poor response. The percentages of (a)n (anticipated) poor and normal response are similar to those observed for the eligible women in our study, rendering selection bias unlikely.

One hundred and fifty-six (3%) women were categorized into the so-called ‘other’ group (i.e. menstrual cycle characteristics unknown). Women who had a poor response or anticipated poor response were more likely to have entered this group. We cannot exclude the possibility that the women in this subgroup who reported their menstrual cycle as unknown or did not report their menstrual cycle at all might actually have reached the menopausal transition or the natural menopause. Any resulting bias is likely to be small due to the small proportion of women concerned.

For the present study, we only selected cohort members who had a regular menstrual cycle at the time of the first visit to the gynaecologist. Since our main interest was to study the women’s reproductive ageing from cycle regularity to irregularity, in relation to the number of retrieved oocytes at the first IVF attempt, we excluded women who had an irregular menstrual cycle at the first visit to the gynaecologist. Furthermore, we used both the mean menstrual cycle length and the predictability to define the regularity of the menstrual cycle at the time of the first visit to the gynaecologist. We did so to avoid possible misclassification, assuming that the reported mean menstrual cycle length would be less reliable if women were not able to predict their next menstrual cycle.

According to our results, the association between oocyte response and risk of entering the menopausal transition after a median follow-up of 5.5 years was stronger for the later stages of the menopausal transition and for the natural menopause. While poor responders at first IVF treatment had a 15-fold increased risk of natural menopause, their risk of entering the early menopausal menopausal transition was only 2-fold increased. When we considered women in the ‘early’ menopausal transition as premenopausal, the age-adjusted odds ratios for having entered the menopausal transition/natural...
menopause for poor and anticipated poor responders increased to 3.9 (95% CI 3.0–5.1) and 4.3 (95% CI 3.1–6.1) respectively, as compared with normal responders at their first IVF treatment. Misclassification of menopausal transition according to the current menstrual cycle pattern is less likely to have occurred for women who were categorized into the ‘middle’ and ‘late’ menopausal transition. Many aspects of the female reproductive ageing process are not well understood, and it remains difficult to design a classification system which is adequate and widely applicable. It is indeed not certain whether less predictability of the menstrual pattern is the first sign of the menopausal transition, as we assumed in the definition of early menopausal transition.

By comparing only the number of retrieved oocytes at first IVF treatment, and not at subsequent IVF attempts, most of the effects of previous ovarian stimulation and adaptation of stimulation regimens based on previous response experiences were avoided. However, in The Netherlands, stimulation regimes for IVF treatment have changed over the past years and women might, before they underwent IVF treatment, already have received gonadotrophin stimulation followed by intrauterine insemination, after which the stimulation protocols for IVF treatment might have been adapted. Therefore, we added the number of ampoules of HMG/FSH used during IVF treatment and year of IVF treatment as possible confounders in the multinomial logistic regression analyses. Both possible confounders did not change our risk estimates by more than 10% and were therefore not included in the final multinomial logistic regression analysis.

When interpreting our results, the strengths and limitations of the study design need to be considered. For 3227 IVF-treated women (Figure 1) participating in the OMEGA-project who returned the questionnaire, data from the medical files could not yet be obtained. Since this was due to limited project funding resulting in a random sample of records not yet completed, it is highly unlikely that this has led to selection bias. With respect to selection bias due to non-response to the mailed questionnaire, the following information is useful. From an anonymous sample of the non-responding women (n = 1672), we abstracted data from the medical files. Of the non-responding women to the OMEGA questionnaire, 72% had a normal oocyte response at their first IVF attempt, 19% had a poor response and 9% had an anticipated poor response. The percentages of (anticipated) poor responders among the non-responding women were somewhat higher than those found in the present study. These results suggest that women with a low response or an anticipated poor response at their first IVF attempt (and therefore a lower probability of having a live birth) were less inclined to participate. However, non-response bias could only have occurred if non-responding women who had a(n) (anticipated) poor response would also have entered the menopausal transition more or less frequently than responding women. We investigated this in a group of 213 women who initially did not respond to the OMEGA questionnaire, but who eventually did respond after a phone call. In this group of women, the percentages across oocyte response categories (75% normal response, 16% poor response and 10% anticipated poor response) were very similar to those of women who persisted in non-response also after a telephone call. Reassuringly, the age-adjusted odds ratios for reaching the menopausal transition/natural menopause were comparable with those for the total group of responders, 1.8 (95% CI 0.6–5.0) for a poor oocyte response and 2.9 (95% CI 0.9–9.0) for anticipated poor response. This finding renders selection bias unlikely.

Furthermore, since in the OMEGA questionnaire information on menstrual cycle characteristics was collected retrospectively, women might have been misclassified according to their menstrual cycle pattern. However, we would expect the proportion of misclassified women to be the same among those with a normal response and a(n) (anticipated) poor response. This so-called non-differential bias would have attenuated the observed risk estimates.

In conclusion, our results indicate that women with a low number of retrieved oocytes at first IVF treatment have an increased risk of reaching the menopausal transition or natural menopause in the decade after the first IVF treatment.

Acknowledgements

We are greatly indebted to the participants of the OMEGA-project. This study would not have been possible without the efforts of all women who participated. We owe a special thanks to H.Klip PhD, without whose effort the OMEGA-project would never have been so successful. We are especially grateful to the research assistants M.Schippers, I.M.Versteegden, S.Braak, A.H.W.van den Belt-Dusebout, G.M.Plas, I.Van Gils and I.Verburg for abstracting data from the medical files in the participating hospitals. Furthermore, we would like to thank the medical registries of the participating clinics for making patient selection possible; and all attending physicians for providing access to their patients’ medical files. The OMEGA-project group include the following persons: M.Kortman MD and E.R.te Velde MD PhD (University Medical Center Utrecht), N.Macklon MD PhD (Erasmus Medical Center–Rotterdam), C.A.M.Jansen MD PhD (Diaconessenhuis–Voorburg), R.A.Leerentveld MD PhD (Isala clinics, Zwolle), W.N.P.Willemsen MD PhD (Academic Hospital Nijmegen, St Radboud), R.Schats MD PhD (Academic Hospital Free University–Amsterdam), N.Naaktgeboren PhD and F.M.Helmerhorst MD PhD (Leiden University Medical Center), R.S.G.M.Bots MD PhD (St Elisabeth Hospital–Tilburg), A.H.M.Simons MD (Academic Hospital Groningen), H.V.Hogerzeil MD PhD MD PhD (Academic Medical Center–Amsterdam), J.J.H.Evers MD PhD (Academic Hospital Maastricht), and P.A.van Dop MD PhD (Catharina Hospital–Eindhoven). Finally, we would like to thank C.W.N.Looman (MSc), Department of Public Health IMGZ, Erasmus University Rotterdam, for the statistical analyses of the live birth rates.

References


Submitted on May 23, 2002; resubmitted on February 17; 2003; accepted on March 19, 2003