The ovarian response to controlled stimulation in IVF cycles may be predictive of the age at menopause

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STUDY QUESTION: Can the number of oocytes retrieved in IVF cycles be predictive of the age at menopause?

SUMMARY ANSWER: The number of retrieved oocytes can be used as an indirect assessment of the extent of ovarian reserve to provide information on the duration of the reproductive life span in women of different ages.

WHAT IS KNOWN ALREADY: Menopause is determined by the exhaustion of the ovarian follicular pool. Ovarian reserve is the main factor influencing ovarian response in IVF cycles. As a consequence the response to ovarian stimulation with the administration of gonadotrophins in IVF treatment may be informative about the age at menopause.

STUDY DESIGN, SIZE, DURATION: In the present cross-sectional study, participants were 1585 infertile women from an IVF clinic and 2635 menopausal women from a more general population.

PARTICIPANTS/MATERIALS, SETTING, METHODS: For all infertile women, the response to ovarian stimulation with gonadotrophins was recorded. For menopausal women, relevant demographic characteristics were available for the analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: A cubic function described the relationship between mean numbers of oocytes and age, with all terms being statistically significant. From the estimated residual distribution of the actual number of oocytes about this mean, a distribution of the age when there would be no oocytes retrieved following ovarian stimulation was derived. This was compared with the distribution of the age at menopause from the menopausal women, showing that menopause occurred about a year later.

LIMITATIONS, REASONS FOR CAUTION: The retrieved oocyte data were from infertile women, while the menopausal ages were from a more general population.

WIDER IMPLICATIONS OF THE FINDINGS: In the present study, we have shown some similarity between the distributions of the age when no retrieved oocytes can be expected after ovarian stimulation and the age at menopause. For a given age, the lower the ovarian reserve, the lower the number of retrieved oocytes would be and the earlier the age that menopause would occur.

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Key words: ovarian reserve / oocytes / menopause prediction

Introduction

Menopause, which is characterized by the cessation of menstrual cycles, can be only recognized retrospectively a year or more after the beginning of amenorrhoea. The few years preceding the final menstrual period are characterized by high variability and irregularity in the menstrual cycle and are commonly known as the menopausal transition.

Age at menopause has relevant implications for female health since late menopause is associated with increased risk of breast cancer and early menopause is associated with increased risk of osteoporosis.
cardiovascular disease, early cognitive decline, ovarian cancer, colorectal cancer, respiratory and urogenital disease (Broekmans et al., 2009; Parsa and Parsa, 2009; De Vos et al., 2010; Dossus et al., 2010; Francucci et al., 2010; Oktay et al., 2010; Rocca et al., 2010; Shuster et al., 2010). Therefore the possibility of predicting the age of menopause could represent desirable and real progress in medicine. Indeed this could permit preventive and therapeutic strategies against several pathological conditions. More importantly, as women increasingly postpone childbirth, prediction of early menopause in young women could be of increasing clinical value.

Several models for the decline of the ovarian reserve, including the Faddy-Gosden (Faddy and Gosden, 1996), Hansen (Hansen et al., 2008) and Wallace (Wallace and Kelsey, 2010) models, show that the mean number of primordial follicles declines with increasing age and when this number is reduced below a certain threshold, ovulation ceases and menopause occurs. The median age at menopause in the female population is about 51 years, and it has been calculated that it occurs when the number of resting follicles in the ovary falls to ~1000 and consequently the number of antral follicles is zero or one (Faddy and Gosden, 1996; Gougeon, 1998; te Velde and Pearson 2002; Wallace and Kelsey, 2010).

The huge variation in the age at menopause can be logically explained by the high variability in the extent of the primordial follicular pool among the female population. For example, the estimated range of resting follicles in the ovary at the age of 35 years is between 19,000 and 135,000 (Wallace and Kelsey, 2010). Such high variability is then reflected in a high variability of the age at which the primordial follicles will be exhausted and women enter menopause. A larger pool of resting follicles is associated with a later age at menopause, while a smaller pool predicts the occurrence of an early menopause (Gougeon, 1998; Hansen et al., 2008; Wallace and Kelsey, 2010; Fleming et al., 2012).

The number of antral follicles is strongly related to the number of primordial follicles. For this reason it is possible to assess the ovarian reserve by measuring the number and the endocrine activity of antral follicles and this can be done by measuring hormonal (AMH, FSH or inhibin B) and/or ultrasound (antral follicle count, AFC) markers which reflect the pool of antral follicles (also called functional ovarian reserve) (Fleming et al., 2012; La Marca et al., 2012; lliodromiti and Nelson, 2013; Nelson, 2013).

Previous studies have shown the existence of a significant correlation between AFC and age at menopause (Broekmans et al., 2004; Wellons et al., 2013); however, the strongest evidence for the predictability of the age at menopause has been obtained in research focused on the correlation with serum concentrations of AMH (Van Disseldorp et al., 2008; Dolleman et al., 2013, 2014; La Marca et al., 2013a; Tehrani et al., 2013; Ramezani Tehrani et al., 2014).

Other studies have investigated the correlation between the ovarian response to stimulation in IVF treatment and the age of menopause and have consistently shown that poor response to ovarian stimulation might be considered a risk factor for early menopause (De Boer et al., 2003; Lawson et al., 2003).

As a matter of fact, the number of oocytes retrieved after gonadotrophin stimulation in IVF treatment accurately reflects the ovarian reserve, with the prevalence of poor responders to gonadotrophin ovarian stimulation increasing with ageing (Ferraretti et al., 2011). As a consequence, the response to ovarian stimulation with gonadotrophins during IVF treatment may correlate with age at menopause, so that the number of retrieved oocytes may be predictive of age at menopause. In the present study, we estimated the relationship between age and the response to ovarian stimulation expressed in terms of the number of retrieved oocytes and showed how this could provide useful information about the menopausal transition for individual women.

Materials and Methods

Study design and subjects

This study was based on methodology developed in previous studies (Broekmans et al., 2004; Van Disseldorp et al., 2008, La Marca et al., 2013a) and involved two independent samples of women. All patients gave written informed consent for their clinical data to be used for research purposes. Institutional Review Board approval was obtained.

The first group was composed of 1585 women aged 19–44 years undergoing IVF treatment cycles. All patients had been trying to conceive for at least 12 months and attended a fertility workup. Data on these IVF cycles were collected and recorded in the database of the fertility centre at the Mother Infant Department of University Hospital Policlinico di Modena, Italy. Cycles were selected for analysis if the following inclusion criteria were satisfied: (i) first IVF/ICSI attempt; (ii) regular menstrual cycles (25–35 days); (iii) treatment with a long GnRH agonist protocol based on daily administration of leuprolin or triptorelin (Enantone die, Takeda, Italy; Decapeptyl, Ferring, Italy) on Day 21 of the previous luteal phase of the stimulation cycle; (iv) stimulation with recombinant FSH at a dose of 200–225 IU/day (Gonal F, Merck Serono, Italy, or Puregon, MSD, Italy); (v) oocyte pick up performed by a senior and experienced operator; and (vi) complete patient records on anamnestic, clinical and IVF cycle characteristics. Clinical exclusion criteria were: irregular cycles, evidence of PCO status, previous ovarian surgery, endometriosis, Day 3 FSH >15 IU/l, presence of ovarian cysts, history of PID, use of hormonal contraception in the previous 3 months, or any known metabolic or endocrine disease.

For ovarian stimulation, subcutaneous FSH was started when pituitary desensitization was achieved (after 14 days from the initiation of GnRH agonists) evidenced by the absence of ovarian follicles >10 mm and endometrial thickness <4 mm on transvaginal ultrasound examination, and then the dose was adjusted on day 6–7 of stimulation according to ovarian response. When at least three follicles reached ≥18 mm, 10 000 IU of hCG (Gonasi, IBSA, Italy) was administered intramuscularly or subcutaneously and 34–36 h later, follicles were aspirated under patient sedation.

Treatment cycle cancellation occurred if there was no response with no follicle growth evident following at least 1 week of FSH administration, or if there was an excessive response with the growth of more than 20 follicles ≥11 mm in mean diameter and/or E2 level ≥4000 pg/ml on the day scheduled for the final oocyte maturation induction. Cycles cancelled for absent response were assigned a number of retrieved oocytes of zero, while cycles cancelled for excessive response were arbitrarily assigned a number of retrieved oocytes that was 75% of the number of follicles larger than 11 mm.

The second group was composed of 2635 women participating in the GOEROM study focused on clinical research on menopause for Italian women (Cagnacci et al., 2005). This GOEROM study was a retrospective study focused on clinical research on menopause for women living in the Italian region of Emilia Romagna and involving four university hospitals (Bologna, Ferrara, Parma and Modena). All women (whose ages at the time of inclusion ranged from 41 to 61 years) were menopausal, with physiological menopause defined as amenorrhoea for ≥12 months. The mean age of the women at the time of inclusion was 52.5 ± 0.1 (mean ± SEM) years and the time since menopause was 2.3 ± 0.02 (mean ± SEM) years.

Statistical analysis

The retrieved oocyte data were analysed using generalized linear modelling similar to the modelling of antral follicle counts and AMH (Broekmans
et al., 2004; van Disseldorp et al., 2008; La Marca et al., 2013a). As the data represented counts (ranging from 0 to 20+), these had to be treated as a discrete variable, and a negative binomial probability distribution was used to describe their substantial variation (cf. Broekmans et al., 2004). The logarithm of the mean was described by a polynomial function of age (i.e. $\alpha_0 + \alpha_1 \times \text{age} + \alpha_2 \times \text{age}^2 + \ldots$), and the variance given by mean $\times (1 + b \times \text{mean})$ where $b$ is the dispersion parameter of the negative binomial distribution (Hilbe, 2011, Chapters 7 and 8). All parameters ($\alpha_0$, $\alpha_1$, $\alpha_2$, etc. and $b$) describing this model were estimated by maximum likelihood.

Having estimated such a model for changing numbers of retrieved oocytes with increasing age, a distribution of the age at which zero oocytes could be retrieved from the ovaries (because of ovarian exhaustion) was derived from the relationship:

$$
\text{probability of zero oocytes at or before age } y = \left(1 + b \times \text{(mean number of retrieved oocytes at age } y)\right)^{-1/b}
$$

This distribution was then compared with the empirical distribution of menopausal ages from the GOERM data.

A nomogram of age-specific percentiles of numbers of retrieved oocytes was constructed from the fitted negative binomial model, along with the corresponding percentiles of the derived distribution of age at which no oocytes could be retrieved.

### Results

Numbers of retrieved oocytes were obtained from 1585 women ranging in age from 19 to 44 years; the number of retrieved oocytes ranged from 0 to 26 and showed a correlation with age of $-0.35$ ($P < 0.01$). Menopausal age was gathered from 2635 women and ranged from 30 to 61 years, with a mean of 49.2 years and a median of 50 years.

Table I shows parameter estimates of the generalized linear model for numbers of retrieved oocytes. A cubic polynomial function of age adequately described the decline in the logarithm of the mean number of retrieved oocytes with increasing age (goodness of fit statistic 1575 on 1580 degrees of freedom). All terms (linear, quadratic and cubic) were significant ($P$-values $<0.002$) while additional (quartic and quintic) terms were not at all significant ($P$-values $>0.25$). The dispersion parameter of the negative binomial distribution was significantly greater than 0 ($P$-value $<0.001$), confirming the substantial residual variation in the data. Figure 1 shows a plot of the retrieved oocyte data along with the fitted mean and 95% confidence limits; a non-parametric (smoothed) estimate of the mean is shown for comparison. Reliable estimation of the mean number of retrieved oocytes is apparent for ages over 25 years (covering 99% of the women’s ages). Figure 2 shows a comparison of this estimated mean number of retrieved oocytes with the estimated mean AMH levels from La Marca et al. (2013a), where it can be seen that the shapes of the two curves are rather similar, particularly after age 30.

Shown in Fig. 3 is the empirical distribution of menopausal ages together with the distribution of the age at which no oocytes would be retrieved after ovarian stimulation. It is clear that this latter age lags the age at menopause by about 1 year, as exemplified by the distribution of the age 1 year after which no oocytes would be retrieved after ovarian stimulation, also shown in Fig. 3; this is quite similar to the distribution of age at menopause.

Figure 4 shows a nomogram of age-specific percentiles of numbers of retrieved oocytes. As the numbers of retrieved oocytes are discrete, this nomogram is essentially a series of discrete points (dots) showing the number of retrieved oocytes and age corresponding to the percentages indicated (for example, the column of dots at number of retrieved oocytes $=5$ gives the ages at which the probabilities that the number of retrieved oocytes is less than or equal to five would be 0.25, 0.50, 0.75 and 0.90). These points have been joined together for each probability for clarity. The point corresponding to a woman of age about 31 having four retrieved oocytes would indicate that she was at the 25% quantile. Table II shows the corresponding percentiles of the distribution of the age at which the number of retrieved oocytes would be zero; so a woman of age about 31 having four retrieved oocytes could have ovarian exhaustion just before age 46 years. Notice that the percentile estimates in Table II are more precise at lower percentages, so that any predictions here would be more useful for women with numbers of retrieved oocytes below the median.

### Discussion

The concordance between the distributions of menopausal age and the age 1 year after which no oocytes would be retrieved after ovarian stimulation shown in Fig. 3 is quite remarkable given that these two distributions are from completely independent data sets; the first was estimated from infertile women undergoing IVF treatment and the other was an empirical distribution from a quite different population. This demonstrates some association between the number of oocytes retrieved in IVF treatment cycles and the age at which menopause may occur. However, the menopausal ages were from a more general population than the infertile women undergoing IVF treatment who provided the data on numbers of retrieved oocytes.

Even if clear evidence has not yet been published in the medical literature regarding the age at menopause for infertile women, our current understanding is suggestive of a possible reduction in ovarian reserve for a relevant percentage of infertile women. Indeed, several authors have reported significant reductions in mean numbers of antral follicles or serum AMH levels in infertile compared with fertile women.

### Table I Parameter estimates (with standard errors in brackets) of the generalized linear model for numbers of retrieved oocytes.

<table>
<thead>
<tr>
<th>Mean number of retrieved oocytes:</th>
<th>$\exp\left{15.22(4.21) - 1.21(0.38) \times \text{age} + 0.038(0.011) \times \text{age}^2 - 0.00040(0.00011) \times \text{age}^3\right}$</th>
</tr>
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<tbody>
<tr>
<td>Residual (negative binomial) variance of number of retrieved oocytes:</td>
<td>$\text{mean} \times (1 + 0.26(0.016) \times \text{mean})$</td>
</tr>
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(Gleicher et al., 2010; Rosen et al., 2011; La Marca et al., 2012; Dolleman et al., 2013) which in turn may indicate in some way an expected early entry into the menopausal period for infertile women. Moreover, large epidemiological studies have clearly shown parity as a positive independent indicator of late menopause (Rodstrom et al., 2003; Parazzini, 2007) and, since infertile women are candidates for low parity, a relationship between infertility and a somewhat earlier age at menopause appears reasonable. We have also made the implicit assumption, in deriving the probability distribution of the age at which no oocytes would be retrieved, that once this happens it remains the case thereafter. All this does suggest that the association we have found between numbers of retrieved oocytes and menopause may well be stronger than that demonstrated here.

The results of this study are consistent with the well-known correlations between ovarian reserve, defined as the total number of resting follicles in the ovary at a certain point in a woman’s life, and the length of reproductive life and ovarian response in IVF. The number of oocytes retrieved in IVF treatment cycles is mainly determined by the number of FSH-responsive antral follicles present in the ovaries at the beginning of stimulation. A reduction in the follicle pool with ageing is associated with a similar decline in the number of retrievable oocytes (Gougeon, 1986; La Marca et al., 2011).
Currently, there are two well-established biomarkers used to measure ovarian reserve: serum AMH concentration and AFC that are thought to be strongly and directly related to the pool of resting ovarian follicles (Hansen et al., 2011). AMH and AFC are also reliable predictors of ovarian response to gonadotrophin administration in IVF treatment cycles that mainly depend on ovarian reserve (La Marca et al., 2013b). For this reason, by measuring AMH and AFC, it is possible to predict the potential response to ovarian stimulation and the reproductive outcome of an IVF treatment cycle (La Marca et al., 2010a, b, 2013b; Broer et al., 2011, 2013). Moreover, several groups have shown that AFC or AMH can be used as predictors for age at menopause (Broekmans et al., 2004; Van Desselorp et al., 2008; Freeman et al., 2012a, b; Dolleman et al., 2013, 2014; La Marca et al., 2013a; Tehrani et al., 2013; Wellons et al., 2013; Ramezani Tehrani et al., 2014), and these studies all suggested that in women of similar age a lower serum AMH level or AFC may be indicative of an earlier age at menopause.

In clinical IVF practice, ovarian response to gonadotrophin stimulation is considered as the best ‘in vivo test’ for the assessment ovarian reserve, with hyperresponse and poor response suggesting enhanced and diminished ovarian reserve, respectively. Lawson et al. (2003) conducted a retrospective study including poor responders and controls, who underwent IVF treatment. All women were < 40 years of age at the time of ovarian stimulation and were sent a questionnaire > 10 years after their IVF cycle, seeking information on menstrual cycles. Independently of age, women who had poor response to gonadotrophins were more likely to refer premenopausal symptoms. Patients with a poor response in IVF cycles were 23 times more likely to enter menopause within 10 years of treatment than controls, hence demonstrating that poor response may be predictive of the age at menopause.

In a large cohort study (De Boer et al., 2003) of 4601 women treated for infertility, it was shown that after a mean follow-up of 5.5 years, 3871 (84%) women still had a regular menstrual cycle, 547 (12%) had entered the menopausal transition and 27 (1%) were menopausal. Women who had a poor response to ovarian stimulation were more likely to have menopause or menopausal transition than women with a normal ovarian response, hence confirming that a poor response in IVF in terms of retrieved oocytes is a reliable indicator of reduced ovarian reserve which is the main factor determining the age at menopause.

In our study, we found a strong negative correlation between numbers of retrieved oocytes and female age, and constructed a nomogram of age-specific percentiles of numbers of retrieved oocytes (Fig. 4) and the corresponding percentiles of the age at which ovarian exhaustion could be expected (Table II). Interestingly and expectedly, the decline in the estimated mean number of retrieved oocytes had a pattern rather similar to that previously reported for serum AMH levels.

We have used the number of retrieved oocytes as an indirect assessment of the extent of ovarian reserve (number of primordial follicles) to provide information on the duration of the reproductive life span of women of different ages. We have demonstrated some conformity between the distribution of observed age at menopause and predictions based on falling numbers of retrieved oocytes (Fig. 3). For example, for a woman just under 25 years of age the 5% quantile is 2 retrieved oocytes, corresponding to a severely reduced ovarian reserve (Fig. 4). According to our model, the age at which such a woman would not have any oocytes retrieved after ovarian stimulation would be 41 years (Table II), with menopause occurring up to a year or so later.

This model should thus be of interest and utility for clinicians when counselling women treated in IVF clinics for infertility problems, particularly those with low numbers of retrieved oocytes for their age, but would naturally benefit from follow-up studies assessing its reliability.

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Authors’ roles

The authors were responsible for the following roles: A.L.M.: study design, execution and analysis and manuscript drafting; E.P.: study design and critical discussion; G.D.: manuscript drafting; G.S.: bibliographic research and manuscript editing; S.G.: critical discussion; A.C.: critical discussion; and M.J.F.: study design, execution and analysis and manuscript drafting.

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Conflict of interest

None declared.

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