Human Reproduction Vol.20, No.6 pp. 1573–1577, 2005 doi:10.1093/humrep/deh827
Advance Access publication March 3, 2005

Ovarian response to standard gonadotrophin stimulation for IVF is decreased not only in older but also in younger women in couples with idiopathic and male subfertility

A.J.Goverde[1,2,3], J.McDonnell[1], R.Schats[1], J.P.W.Vermeiden[1], R.Homburg[1] and C.B.Lambalk[1]

[1]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Vrije Universiteit Medical Centre, Amsterdam, The Netherlands
[2]Current address: Department of Reproductive Medicine, Division of Perinatology and Gynaecology, University Medical Centre Utrecht, P.O.Box 85500, 3508 GA Utrecht, The Netherlands

[3]To whom correspondence should be addressed. E-mail: A.J.Goverde@dog.azu.nl

BACKGROUND: With the occasional reports of unexpectedly poor ovarian response to controlled ovarian hyperstimulation (COH) for IVF in young normally cyclic women in mind, we studied age-related ovarian response to COH in a group of women who underwent standard IVF. METHODS: Ovarian response to COH was defined as the number of follicles ≥14 mm on the day of hCG administration. Ovarian response to COH was analysed by multiple regression analysis with woman's age and basal FSH concentration as explanatory variables in a prospective cohort of patients with idiopathic and mild male factor subfertility ($n = 85$), and additionally in a large retrospective cohort of women with unexplained, mild male and tubal subfertility ($n = 1155$), with age as explanatory variable. RESULTS: Ovarian response to COH was associated significantly with age ($P < 0.001$) and basal FSH concentration ($P = 0.002$). However, in women with idiopathic or mild male subfertility, in both cohorts the relationship took the form of an inverted U-shape with both older and—surprisingly—young women having a reduced ovarian response ($P < 0.001$). Maximum ovarian response was around the age of 28 years. In women with tubal infertility, there was only a linear decline of ovarian response with age. CONCLUSION: It is hypothesized that diminished ovarian response to COH in IVF is the very first sign of ovarian ageing in young women diagnosed with idiopathic and mild male subfertility.

Key words: controlled ovarian hyperstimulation/idiopathic subfertility/IVF/ovarian ageing/ovarian response

Introduction

Recently it has been proposed that subfertility may be the earliest sign of ovarian ageing (Nikolaou and Templeton, 2003). It has also been suggested that the interval between menopause and the onset of subfertility is fixed (te Velde *et al.*, 1998; van Zonneveld *et al.*, 2001; te Velde and Pearson, 2002). From data from assisted reproduction, it has been established that women who respond poorly to controlled ovarian hyperstimulation (COS) with gonadotrophins become menopausal earlier (Farhi *et al.*, 1997; de Boer *et al.*, 2003) and in this respect IVF could be viewed as a dynamic test of ovarian reserve. However, as yet there is no generally accepted definition of low or poor response to COH for IVF. It has been proposed that a number of follicles less than five on the day of hCG administration could be regarded as a low response (Lashen *et al.*, 1999), although others suggest that the gonadotrophin dosage should also be taken into account and that the definition of a poor response in IVF can only be met when the daily dosage of FSH is ≥300 IU/day (Kailasam *et al.*, 2004).

The central issue in ovarian ageing is the decline in the number of follicles (Faddy *et al.*, 1992). It is assumed that the ovarian follicle cohort available for recruitment by gonadotrophin stimulation for IVF is essentially constant until a certain age (Faddy and Gosden, 1995). A recent paper from Beckers *et al.* (2002) reported on the investigation of 11 women who showed an unexpectedly poor response to COH for IVF. Before starting the COH for their IVF treatment, these women were not expected to be at risk for poor response as they had been diagnosed with idiopathic subfertility after thorough infertility investigation and had regular cycles, with the majority of them having FSH levels in the normal range at the beginning of the cycle. These unexpected poor responders differed from normal controls in a number of aspects: although their basal FSH levels were within the normal range, they had higher median basal FSH concentrations,

and significantly lower antral follicle counts. The antral follicle count has been proposed as a reliable predictor for low ovarian response (Bancsi *et al.*, 2002).

We conducted a prospective study in which couples diagnosed with idiopathic or mild to moderate male subfertility underwent IVF as a first line treatment. These prospective data allowed us to evaluate the ovarian response to a standardized stimulation protocol in relation to patient factors in women with normal cycles. The outcome of this analysis was subsequently tested in another cohort of patients attending our tertiary IVF clinic at a later period to validate the original findings.

Materials and methods

Study population

Study one

This study was part of a prospective randomized study on cost-effectiveness of treatment with intrauterine insemination (IUI) or IVF (Goverde *et al.*, 2000). For this report, we focus on IVF data only. Couples were diagnosed as having idiopathic subfertility if no abnormality was found during an extensive investigation of infertility including a basal body temperature chart, a late luteal phase endometrial biopsy, a post-coital test, a hysterosalpingogram, a diagnostic laparoscopy, and at least two semen analyses. Male subfertility was diagnosed if at least three out of five semen analyses showed a total motile sperm count of $<20 \times 10^6$ progressively motile sperm in the ejaculate and if the remainder of the infertility investigation revealed no additional abnormalities. In both groups of patients, semen processing by Percoll 40/80% gradient centrifugation yielded a minimum of 1×10^6 progressively motile sperm at least once.

In this study, couples who had been affected by idiopathic subfertility for ≥ 3 years or by mild or moderate male subfertility for ≥ 1 year participated. Couples were excluded if the woman had cycle disorders, or untreated endometriosis (American Society of Reproductive Medicine criteria grade 2–4), or bilateral occluded tubes or if a semen sample yielded $<1 \times 10^6$ progressively motile sperm after processing by Percoll 40/80% gradient centrifugation, if $>20\%$ of sperm carried antibodies as tested with an immunobead test after Percoll processing, or if $>50\%$ of sperm had no acrosome.

The study was done according to the rules of the Declaration of Helsinki, and was approved by the Committee for the Ethics of Research Involving Human Subjects of the Vrije Universiteit Medical Centre, Amsterdam, The Netherlands.

The couples underwent standard IVF as described by Roseboom *et al.* (1995). Woman's age determined the stimulation protocol to be applied in the first IVF cycle. In women aged ≤ 38 years, the stimulation protocol stipulated 150 IU of hMG (Pergonal; Ares Serono, Switzerland) or FSH (Metrodin; Ares Serono) daily after a long GnRH agonist (100 μg triptorelin s.c. daily; Decapeptyl; Ferring, Denmark) down-regulation protocol. In women aged >38 years, a 'short' stimulation protocol was applied with GnRH agonist (100 μg triptorelin s.c. daily) starting on the second day of the cycle and COH with gonadotrophins in a dosage of 225 IU/day starting at day 3 of the cycle. The dosage of gonadotrophins was increased in subsequent cycles if fewer than four follicles >14 mm were present at the day of hCG administration. In both protocols, GnRH agonists and gonadotrophins were discontinued when transvaginal ultrasonography showed the presence of at least one follicle with a diameter of ≥ 18 mm, and a minimum of three follicles of ≥ 16 mm in diameter. Thirty-five hours before follicle aspiration, 10 000 IU hCG (Pregnyl; Ares Serono) was given unless the serum estradiol (E_2) concentration was $>20\,000$ nmol/l. Follicular aspiration guided by transvaginal ultrasonography was done under systemic analgesia (7.5 mg diazepam orally and 50 mg pethidine hydrochloride intramuscularly), and all follicles present were aspirated. All women had a basal serum FSH concentration assessed at cycle day 3, either in the cycle that they had started late luteal GnRH-agonist down-regulation, or in the cycle used for IVF treatment in the case of a short stimulation protocol.

Serum E_2 and FSH levels were determined by commercially available immunometric assays (Amerlite; Amersham, UK). For E_2, the inter-assay coefficient for variation (CV) was 11% at 250 pmol/l and 8% at 8000 pmol/l, the intra-assay CV was 13% at 250 pmol/l, 9% at 1100 pmol/l and 9% at 5000 pmol/l. The lower limit of detection was 90 pmol/l. For FSH, the inter-assay CV was 9% at 3 IU/l and 5% at 35 IU/l, the intra-assay CV was 9% at 5 IU/l, 8% at 15 IU/l and 6% at 40 IU/l. The lower limit of detection was 0.5 IU/l.

Study two

After we received the results from the first analysis, we decided to test these results retrospectively in a larger population. From the database of our tertiary IVF clinic, we selected all women aged <40 years who underwent their first IVF cycle in the period of January 1, 2000 until January 1, 2004 with standard COH as described previously. During this period, recombinant FSH was introduced as an alternative preparation for COH, but the same stimulation dosages were maintained. All 679 first IVF cycles of women who underwent conventional IVF for idiopathic or mild male subfertility, as well as all 476 first IVF cycles in women with tubal infertility, were analysed in the same way in order to examine the effects of age and infertility diagnosis on ovarian response.

Statistical analysis

Ovarian response was defined as the number of follicles with diameter ≥ 14 mm on the day of hCG administration. Two analyses were conducted on the original study population. The first analysis was performed on the first treatment cycle to remove dependence between cycles. The second analysis was done on all cycles, with the patient as the unit of analysis. For the second study, a first cycle analysis was done in a different patient cohort, categorized to the indication for IVF treatment, in order to validate the findings of the analysis of our original study group.

Ovarian response was analysed using logistic regression with woman's age as explanatory variable in both studies, and basal FSH concentration as an additional explanatory variable in the prospective study. Ovarian response was modelled as a function of age and basal FSH concentration using Poisson regression models. To incorporate possible non-linear effects of patient age, age was also included as a quadratic variable. To prevent possible co-linearity between the age variables, age was centred at 30 years (which approximates the mean age) before carrying out the regressions. Additionally, age was used as a linear and quadratic variable to evaluate the effect on basal FSH concentration as well. All calculations were carried out using Stata 8.0.

Results

Study one

In the prospective study, 85 couples (59 couples with idiopathic subfertility and 26 couples with mild male subfertility) underwent 85 first IVF cycles and 270 IVF cycles in total.

Womens' ages ranged from 22 to 39 years in the first IVF cycle, and from 22 to 41 years in all IVF cycles. The age cohort of 22–30 years contained 30 women, and 55 women were aged >30 years.

Ovarian response to COH was significantly associated with woman's age ($P < 0.001$) but there was also a significant non-linear effect of woman's age on ovarian response ($P < 0.001$). Ovarian response according to age is plotted in Figure 1. In the first IVF cycle, ovarian response to COH took the form of an inverted U-shape (i.e. quadratic) with maximum response of ~9.1 follicles at 28.4 years. Not only women at the end of the age scale but younger women also had a significantly decreased ovarian response: ~7.8 follicles at the age of 22.5 years and ~5.2 follicles at the age of 39.9 years. In subsequent IVF cycles, where a higher dosage of gonadotrophins was applied if needed in the case of poor response, we found the same non-linear effect of age on ovarian response to stimulation (data not shown).

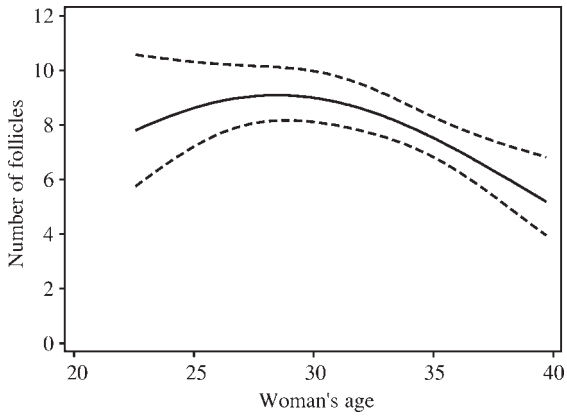

Figure 1. Modelled average ovarian response (total number of follicles ≥14 mm) and 95% confidence interval to standard controlled ovarian hyperstimulation on the day of hCG administration as a function of woman's age. Data from prospective standard stimulation study.

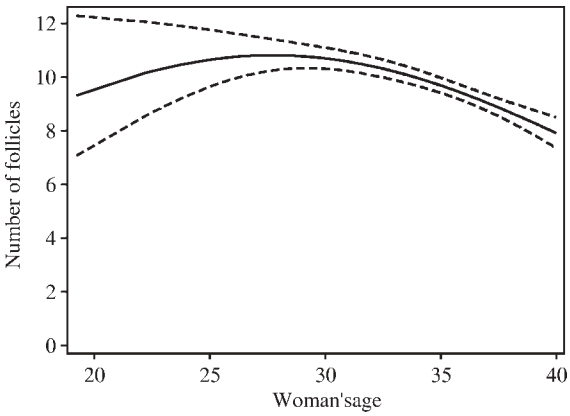

Figure 2. Modelled average ovarian response (total number of follicles ≥14 mm) and 95% confidence interval to controlled ovarian hyperstimulation on the day of hCG administration in women with idiopathic subfertility as a function of woman's age. Data from the routine tertiary IVF clinic database (years 2000–2004).

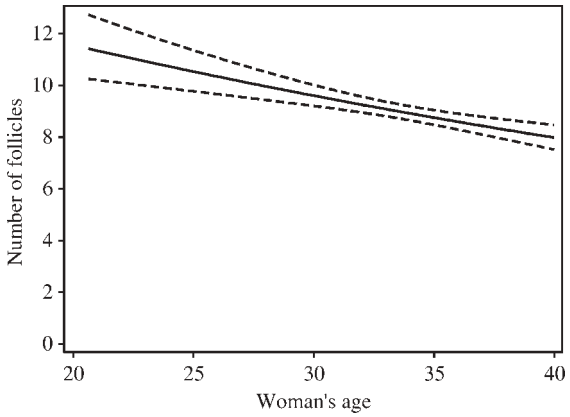

Figure 3. Modelled average ovarian response (total number of follicles ≥14 mm) and 95% confidence interval to controlled ovarian hyperstimulation on the day of hCG administration in women with tubal subfertility as a function of woman's age. Data from the routine tertiary IVF clinic database (years 2000–2004).

We found a negative association of basal FSH concentration with ovarian response ($P = 0.002$). With increasing basal FSH concentration, the ovarian response decreases. We also found an association of age with basal FSH level ($P = 0.008$), which is weakly quadratic.

Study two

In the large retrospective patient cohort, we analysed all 679 first IVF cycles of women who underwent conventional IVF for idiopathic or mild male subfertility, as well as all 476 first IVF cycles in women with tubal infertility. Again we found a similar age effect with an inverted U-shaped curve of ovarian response to age in the groups with idiopathic and male factor infertility as in the prospective study group ($P = 0.004$, Figure 2). In contrast, we did not find the association of lower age with diminished ovarian response in women with tubal infertility from this cohort. However, there was a linear decline in follicle cohort size with advancing age ($P < 0.001$, Figure 3).

Discussion

The nature of our prospective study allowed us to analyse the effect of age on ovarian response to standard COH. We found the expected decrease in ovarian response with increasing woman's age. To our surprise, however, we also found a reduced quantitative ovarian response to standardized COH in young, normally cycling women who underwent IVF for idiopathic and male subfertility. In this study the sample size was limited. Therefore in order to verify our observation we performed the same analysis retrospectively in a second, much larger cohort. Again we found a similar age effect with an inverted U-shaped curve of ovarian response to age in the group with idiopathic and mild male factor subfertility, but not in the group with tubal subfertility. These similar findings substantiate our earlier observation that there is a somewhat diminished quantitative ovarian response in normally cycling women aged <30 years. To the best of our knowledge, this

phenomenon has not been described elsewhere in the literature.

We can only speculate on the mechanisms behind our observation of a diminished ovarian response to COH in IVF in normally cyclic women. Theoretically, a diminished ovarian response to COH results from limited pharmacokinetic availability, less sensitive receptors, or limited ovarian reserve. We observed multifollicular growth in all women irrespective of age, indicating that sufficient amounts of FSH reached the target organ such that it enabled overriding the ovarian threshold. Therefore, it is unlikely that differences of availability of FSH could be responsible. Similarly, we are not aware of studies that indicate that diminished bioactivity and improper receptivity could be a cause, especially in younger patients. On the contrary, from a report on the bioactivity of urinary FSH it was concluded that although there was a moderate variability in clearance rate and thus in exposure, the bioactivity was higher in a relatively young population than in older women (Karlsson *et al.*, 1998). The possibility of a defective receptor, especially present in younger and not middle-aged women, is not very likely either: our study group consisted of normally cycling women and there is no logical explanation why they would have defective receptor activity in a stimulated cycle whereas they show normal receptivity in the spontaneous cycle. So far, studies with regard to FSH receptor gene polymorphisms that code for a less sensitive receptor do not show uneven distribution in age (Perez Mayorga *et al.*, 2000). Moreover, in the patients attending our tertiary outpatient fertility department, we did not establish a difference in the distribution of FSH receptor variation comparing women aged <30 years with women aged >30 years (unpublished data).

That leaves the possibility of failure of the target organ as the last possible explanation for our observation. It is generally known that higher woman's age has a detrimental effect on the outcome of COH (Commenges-Ducos *et al.*, 1998). But it has also been reported that younger women with idiopathic subfertility may unexpectedly perform poorly on COH (Beckers *et al.*, 2002). From their study in poor responders in IVF, it was concluded that there is a subgroup of young women with idiopathic infertility in whom the ovarian cohort is similar to that of older women and that the smaller cohort size may be the first sign of early ovarian ageing. We believe we have shown here that even before performing poorly on COH, there are signs that in young women with idiopathic subfertility the cohort size may be decreased significantly. Further, albeit circumstantial support for our hypothesis of target organ failure may come from the fact that these women were already having problems in conceiving naturally in the absence of overt abnormalities.

Apparently, within the group of women diagnosed with idiopathic or mild male subfertility is a subgroup in whom follicle cohort size is already relatively small at a young age. If we regard this as the earliest sign of early ovarian ageing, the next step will be that we will have to search actively for this phenomenon in young women who have difficulty conceiving, since the consequences are that with a smaller follicle cohort all reproductive problems will advance (Akande *et al.*, 2002). This was reported by Farhi *et al.* (1997) and de Boer *et al.* (2003), who showed that absence of, or even poor ovarian response to, gonadotrophin stimulation could be the prelude to early menopause. The antral follicle cohort, i.e. the total number of small antral follicles present before starting gonadotrophin administration, may be considered as a diagnostic tool for this active search as it was recently presented as a good predictor of follicle cohort size (Bancsi *et al.*, 2002; Popovic-Todorovic *et al.*, 2003). Unfortunately, at the time of our study, the antral follicle count had not yet been introduced and these data were not collected in our patients. As far as we know, no reports on the antral follicle count in a large group of women with idiopathic infertility have been reported.

We hypothesize that a diminished quantitative but not yet full-blown 'poor response' to standardized COH for IVF may be a first sign of early ovarian ageing. More especially, when standard infertility work-up does not reveal any abnormality in young subfertile women, their ovarian reserve should be investigated.

Acknowledgements

This study was financially supported by the Health Insurance Executive Board, Amstelveen, The Netherlands. The authors are grateful to Ms F.Wegener Sleeswijk for her contribution in the care for the patients participating in this study.

References

Akande VA, Fleming CF, Hunt LP, Keay SD and Jenkins JM (2002) Biological versus chronological ageing of oocytes, distinguishable by raised FSH levels in relation to the success of IVF treatment. Hum Reprod 17,2003–2008.

Bancsi LFJMM, Broekmans FJM, Eijkemans MJC, de Jong FH, Habbema JDF and te Velde ER (2002) Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril 77,328–336.

Beckers NGM, Macklon NS, Eijkemans MJC and Fauser BCJM (2002) Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for in vitro fertilization exhibit follicular phase characteristics suggestive of ovarian aging. Fertil Steril 78,291–297.

de Boer EJ, den Tonkelaar I, te Velde ER, Burger CW and van Leeuwen FE on behalf of the OMEGA-project group (2003) Increased risk of early menopausal transition and natural menopause after poor response at first IVF treatment. Hum Reprod 18,1544–1552.

Commenges-Ducos M, Tricaud S, Papaxanthos-Roche A, Dallay D, Horovitz J and Commenges D (1998) Modelling of the probability of success of the stages of in-vitro fertilization and embryo transfer: stimulation, fertilization and implantation. Hum Reprod 13,78–83.

Faddy MJ and Gosden RJ (1995) A mathematical model of follicle dynamics in the human ovary. Hum Reprod 10,770–775.

Faddy MJ, Gosden RG, Gougeon A, Richardson SJ and Nelson JF (1992) Accelerated disappearance of ovarian follicles in midlife: implications for forecasting menopause. Hum Reprod 7,1342–1346.

Farhi J, Homburg R, Ferber A, Orvieto R and Ben Rafael Z (1997) Non-response to ovarian stimulation in normogonadotrophic, normogonadal women: a clinical sign of impending onset of ovarian failure pre-empting the rise in basal follicle stimulating hormone levels. Hum Reprod 12, 241–243.

Goverde AJ, McDonnell J, Vermeiden JPW, Schats R, Rutten FFH and Schoemaker J (2000) Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet 335,13–18.

Kailasam C, Keay SD, Wislon P, Ford WCL and Jenkins JM (2004) Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. Hum Reprod 19,1544–1547.

Karlsson MO, Wade JR, Loumaye E and Munafo A (1998) The population pharmocokinetics of recombinant- and urinary-human follicle stimulating hormone in women. Br J Clin Pharmacol 45,13–20.

Lashen H, Ledger W, Lopez-Bernal A and Barlow D (1999) Poor responders to ovulation induction: is proceeding to in-vitro fertilization worthwhile? Hum Reprod 14,964–969.

Nikolaou D and Templeton A (2003) Early ovarian ageing: a hypothesis. Detection and clinical relevance. Hum Reprod 18,1137–1139.

Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E and Simoni M (2000) Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab 85,3365–3369.

Popovic-Todorovic B, Loft A, Lindhard A, Bangsbøll S, Andersson AM and Nyboe Andersen A (2003) A prospective study of predictive factors of ovarian response in 'standard' IVF/ICSI patients treated recombinant FSH. A suggestion for a recombinant FSH dosage normogram. Hum Reprod 18,781–787.

Roseboom TJ, Vermeiden JPW, Schoute E, Lens JW and Schats R (1995) The probability of pregnancy after embryo transfer is affected by the age of the patient, cause of infertility, number of embryos transferred and the average morphology score, as revealed by multiple logistic regression analysis. Hum Reprod 10,3035–3041.

te Velde ER and Pearson PL (2002) The variability of female reproductive ageing. Hum Reprod Update 8,141–154.

te Velde ER, Dorland M and Broekmans FJM (1998) Age at menopause as a marker of reproductive ageing. Maturitas 30,119–125.

van Zonneveld P, Scheffer GJ, Broekmans FJM and te Velde ER (2001) Hormones and reproductive ageing. Maturitas 38,83–94.

Submitted on July 29, 2004; resubmitted on January 3, 2005; accepted on February 1, 2005