Effects of age on hormonal and ultrasound markers of ovarian reserve in Chinese women with proven fertility

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BACKGROUND: A woman’s fertility is remarkably reduced with increasing age. Reproductive age can be assessed by a number of ovarian reserve tests. There are very few studies addressing the effects of age on these markers in fertile women, especially in Asia. METHODS: On the second to fourth day of the menstrual period, fertile Chinese women with regular monthly cycles and no history of ovarian surgery underwent a transvaginal scan with colour Doppler to determine ovarian volume, total antral follicle count (AFC) and mean peak systolic velocity (PSV) of ovarian stromal blood flow, and their serum FSH and inhibin B levels were checked. RESULTS: Out of 145 women scanned, 119 were included in the final analysis. AFC is the only ovarian reserve marker that was significantly different among four age groups (< 20, 21–30, 31–40 and >40 years). AFC had the best correlation with the age of the women, followed by FSH level and ovarian volume. The decline of AFC with age was 3.8% per year (95% confidence interval 2.7–4.9%) in the conventional linear regression model, which was not improved by the biphasic linear regression model. CONCLUSION: Of the parameters tested, AFC showed the best correlation with women’s age and declined linearly at a rate of 3.8% per year.

Key words: AFC/age/Chinese women/Doppler/ovarian reserve

Introduction

Fertility is remarkably reduced with increasing age of women in both spontaneous conceptions (Tietze, 1957) and assisted reproductive methods (Templeton et al., 1996). The decrease in fertility with ageing is probably due to a decreasing number of primordial follicles after birth. Block (1952) observed that >250 000 primordial follicles were present at menarche, whereas only a few hundred or thousands remained at the end of reproductive life. The cyclic development of follicles is finely controlled by a sequence of hypothalamic–pituitary–ovarian interactions, and angiogenesis is an important component of both the follicular and luteal phase of an ovarian cycle (Abulaafia and Sherer, 2000). Early follicular phase FSH level is increased with advancing age by a reduced inhibin-mediated feedback towards the pituitary gland.

A number of parameters known as ovarian reserve markers have been examined in many assisted reproduction technology (ART) programmes to predict ovarian responses prior to ovarian stimulation with gonadotrophin. Hormonal markers include basal FSH (Scott and Hofmann et al., 1995; Sharara et al., 1998) and inhibin B (Seifer et al., 1997, 1999; Tinkanen et al., 1999; Dzik et al., 2000) levels. Other less commonly used hormonal markers such as serum estradiol level (Licciardi et al., 1995), FSH/LH ratio (Mukherjee et al., 1996), GnRH agonist stimulation test (Padilla et al., 1990) and exogenous FSH ovarian reserve test (Fanchin et al., 1994) are of limited value in the prediction of ovarian response (Bukman and Heineman, 2001). Recently, ultrasound assessment of the ovarian volume (Syrop et al., 1995; Lass et al., 1997; Syrop et al., 1999), total antral follicle count (AFC) (Tomás et al., 1997); Chang et al., 1998a,b; Frattarelli et al., 2000; Ng et al., 2000; Hsieh et al., 2001; Nahum et al., 2001) and ovarian stromal blood flow (Zaidi et al., 1996; Engmann et al., 1999; Kupesic and Kurjak, 2002; Kupesic et al., 2003; Popovic-Todorovic et al., 2003) were also found to be useful in predicting ovarian responses.

Many women in developed countries delay childbearing to fulfil their personal commitments. An accurate assessment of reproductive age would be of help in counselling these women about their fertility potential and perhaps in scheduling pregnancies. The majority of ovarian reserve tests are performed in women presenting with infertility problems. To generalize data from infertile patients to fertile subjects seems inappropriate as infertile patients may have a more advanced reproductive age, which will contribute to their infertility problem. There are very few studies addressing the effects of
Materials and methods

Chinese women attending the Department of Obstetrics and Gynaecology, University of Hong Kong between December 2000 and April 2002 for termination of pregnancy or sterilization were recruited when the following criteria were met: (i) history of spontaneous conception; (ii) regular cycles with cycle length of 25–35 days with ≤4 days difference between cycles; and (iii) presence of both ovaries. Exclusion criteria were: (i) history of infertility and (ii) presence of gynaecological disorders such as menorrhagia or dysfunctional uterine bleeding; (iii) history of ovarian surgery; and (iv) history of taking a sex hormonal preparation such as menorrhagia or dysfunctional uterine bleeding. Every patient gave written informed consent prior to participating in the study, which was approved by the Ethics Committee, Faculty of Medicine, University of Hong Kong. They did not receive any monetary compensation for study participation.

Two to three months after termination of pregnancy or sterilization, they attended the Day Care Centre for a transvaginal ultrasound examination and blood test in the early follicular phase (days 2–4) of their period. All ultrasound examinations were carried out by E.H.Y.N. at ~8–10 a.m. using a 6.5 MHz vaginal probe (Aloka, Model SSD-5500, Aloka Co. Ltd, Tokyo, Japan) with the same ultrasound setting and without knowing the age of patients. The length, height and width of each ovary were measured in the sagittal and coronal planes during transvaginal scanning. Ovarian volume was then obtained using a formula for the volume of an ellipsoid, i.e. \( \pi/6 \times \text{length} \times \text{height} \times \text{width} \). The number of antral follicles <10 mm in each ovary was counted. The stromal blood flow of the ovary was assessed by colour Doppler ultrasound. Flow velocity waveforms were obtained from stromal blood vessels away from the ovarian capsule. The ‘gate’ of the Doppler was positioned when the vessel with good colour signals was identified on the screen. The peak systolic velocity (PSV) of stromal vessels was calculated electronically when three similar, consecutive waveforms of good quality were obtained. Ovarian stromal blood flow was evaluated at three positions at random, and the one with the highest PSV was chosen. As no significant difference in PSV between the left and right side of ovarian stromal vessels was obtained, the mean value was used. The intra-observer coefficient of variation was 7% for AFC, 7% for ovarian volume and 16% for PSV.

Blood was then taken for the measurement of FSH and inhibin B levels. Serum FSH was measured by a two-site sandwich immunoassay (Bayer Corporation, Tarrytown, NY), and the intra- and inter-assay coefficients of variation were 2.8 and 1.7%, respectively. Serum inhibin B was measured by a two-site enzyme-linked immunoassay (Serotec, Kidlington, Oxford, UK), and the intra- and inter-assay coefficients of variation were <7%.

Statistical analysis

Women aged <20, 21–30, 31–40 and >40 years were classified as groups I–IV, respectively. The outcome measures were FSH level, inhibin B level, mean ovarian volume (averaged volume of both ovaries) and total ovarian volume (volume of both ovaries), AFC (antral follicle number of both ovaries) and mean PSV of ovarian stromal blood vessels. Continuous variables were not normally distributed and are given as the median (2.5th–97.5th centiles), unless indicated. Statistical tests were carried out by Kruskal–Wallis and Mann–Whitney U-tests, where appropriate. Correlation was assessed by the Spearman rank method. A P-value (two-tailed) of <0.05 was taken as significant.

The effect of age on AFC was examined by both conventional linear regression and biphasic linear regression models, with and without a logarithmic transformation of AFC. A biphasic linear regression was fitted to the data with all parameters including the breakpoint estimated by the least squares estimates. The 95% confidence interval (CI) for the breakpoint was obtained by parametric bootstrap of size 1000 (Davison and Hinkley, 1997). The difference between these two...
models was examined by an F-test. The estimation was programmed in the Statistical Analysis System (SAS, SAS Institute Inc., Cary, NC) Version 8.2.

Results
A total of 253 eligible women were approached and agreed to participate in the study. Of these, 145 women attended the clinic for ultrasound examination and the others did not keep the appointment for personal reasons. After ultrasound examination, 26 women were excluded from the study: poor visualization of ovaries in 12 women, an ovarian cyst in 11 women and polycystic ovaries in three women. Therefore, only 119 women were included in the final analysis: 13 in group I; 17 in group II; 50 in group III; and 39 in group IV. Their demographic data and markers of ovarian reserve are summarized in Table I. Significant differences in AFC were found within the four age groups, but not between group I and group II (Table II).

The age of the women was significantly correlated with FSH level, mean/total ovarian volume and AFC (Table III). AFC was significantly correlated with age, FSH level and mean/total ovarian volume (Table III). FSH level was significantly correlated with age, AFC, mean/total ovarian volume (r = −0.382; P < 0.001) and inhibin B level (r = −0.187; P = 0.043), whereas the inhibin B level was correlated with serum FSH level only.

The overall decrease in the rate of AFC (after log transformation) with age was 3.8% per year (95% CI = 2.7–4.9%; P < 0.001) in the conventional linear regression model, which was not significantly improved by the biphasic linear regression model (P = 0.068). Using the biphasic linear regression model, the breakpoint was estimated as 29.1 years (95% CI = 17.5–41.9 years). For subjects younger than 29.1 years, AFC did not appear to decline significantly (1.6%; P = 0.361), while for subjects older than 29.1 years, AFC declined significantly by 5.7% per year (95% CI = 3.1–8.3%; P < 0.001). The decline of AFC (without log transformation) over age was 0.35 follicles per year (95% CI = 0.26–0.45%; P < 0.001) in the linear regression model (Figure 1).

Discussion
We recruited study subjects from women attending the hospital for termination of pregnancy or tubal sterilization. Women requesting pregnancy termination were in the younger age group and their normal fertility could be easily ascertained by the recent spontaneous pregnancy. Those requesting tubal sterilization were more likely to be in the older age group and their normal fertility could only be assumed by history of previous spontaneous pregnancies. Women with polycystic ovaries were excluded from the analysis to avoid bias due to exaggerated number of follicles and different follicular dynamics in their ovaries (Franks et al., 2000). Our results clearly showed a significant correlation of age with serum FSH level, mean/total ovarian volume and AFC in 119 fertile Chinese women. Their age did not have any effect on serum inhibin B levels and mean PSV of ovarian stromal blood flow. AFC achieved the best correlation with age, followed by serum FSH and mean/total ovarian volume. Indeed, AFC was the only ovarian reserve marker that was significantly reduced with advancing age (Table II). A strong negative correlation of age and AFC was found in fertile women (Reuss et al., 1996; Scheffer et al., 1999) and in infertile patients (Chang et al., 1998a,b; Frattarelli et al., 2000; Ng et al., 2000; Kupesic et al., 2003). No such correlation between age and AFC was present in the study of Tomás et al. (1997) involving women with polycystic ovaries.

The significance of AFC has been evaluated extensively in infertile patients undergoing ART treatment. Tomás et al. (1997) first concluded that AFC before ovarian stimulation was a better predictor of the ovarian response than the ovarian volume or age alone. Chang et al. (1998a) found a highly significant correlation between AFC and the number of oocytes, and a higher chance of cycle cancellation. Lower estradiol level and higher gonadotrophin dosage in cycles with antral follicle number ≤3. We demonstrated that AFC achieved the best predictive value, followed by basal FSH, body mass index and age of women, in a prospective study of 128 women undergoing the first IVF cycle using a standard regimen of ovarian stimulation (Ng et al., 2000). Other studies (Frattarelli et al., 2000; Hsieh et al., 2001; Nahum et al., 2001; Kupesic and Kurjak, 2002; Popovic-Todorovic et al., 2003)

Table III. Correlation coefficients of age of women and total antral follicle count with other ovarian reserve markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>Total AFC</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH level (IU/l)</td>
<td>0.215b</td>
<td>−0.573a</td>
</tr>
<tr>
<td>Inhibin B level (pg/ml)</td>
<td>−0.057</td>
<td>0.175</td>
</tr>
<tr>
<td>Mean ovarian volume (cm³)</td>
<td>−0.194c</td>
<td>0.382a</td>
</tr>
<tr>
<td>Total ovarian volume (cm³)</td>
<td>−0.194c</td>
<td>0.382a</td>
</tr>
<tr>
<td>Total AFC</td>
<td>−0.573a</td>
<td></td>
</tr>
<tr>
<td>Mean peak systolic velocity (cm/s)</td>
<td>0.066</td>
<td>−0.063</td>
</tr>
</tbody>
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*p < 0.0001; bP = 0.02; cP = 0.03.

Figure 1. Effect of the age of women on the total antral follicle count.
also confirm the importance of AFC in the prediction of ovarian response.

There is little information in the literature regarding the effect of age on AFC in fertile women. In 31 healthy Caucasian volunteers, AFC decreased by 0.95 follicles per year of age or ~60% between 22 and 42 years (Reuss et al., 1996). A more recent study (Scheffer et al., 1999) of 162 fertile women in The Netherlands demonstrated that AFC was best correlated with age and declined by 8.2% per year (95% CI 5.2–11.2%) in a linear regression model. Our study revealed that the decline of AFC in fertile Chinese women was only 3.8% per year (95% CI 2.7–4.9%) or 0.35 follicles per year (95% CI 0.26–0.45). Our decline rate was similar to 0.47 follicles per year in infertile Chinese women undergoing ovarian stimulation and artificial insemination in Taiwan (Chang et al., 1999b), but was much lower than those reported in Caucasians (Reuss et al., 1996; Scheffer et al., 1999). This may be a real difference, and further studies are warranted to investigate the reasons for the ethnic difference in AFC decline. Different AFC decline rates may explain variations in pregnancy rate between populations in studies of the efficacy of intrauterine contraceptive devices (UNDP/UNFPA/WHO, 1997) and of women with lactational amenorrhoea (World Health Organization Task Force on Methods for the Natural Regulation of Fertility, 1999).

Moreover, Scheffer et al. (1999) indicated a biphasic pattern of AFC decline in their population, as AFC declined by 4.8% per year before the age of 37 years compared with 11.7% thereafter. In this study, the conventional linear model gave the best fit to the data and was not improved by the biphasic linear regression model. A linear decline was also found by Reuss et al. (1996) and Chang et al. (1999b). When the biphasic linear regression model was applied, the breakpoint was at 29.1 years with a large 95% CI, reflecting a large variation of AFC in the same age group, especially in the older age group. The observation of Scheffer et al. (1999) is concordant with the widely accepted biphasic model of follicle disappearance from birth to menopause based on autopsy findings and histological analyses of ovaries (Faddy and Gosden, 1995). This bi-exponential model suggests that the total follicle number declines bi-exponentially with age and the loss of follicles accelerates around the age of 37–38 years. This model, however, has been questioned by re-examination of raw data with different statistical models, and there may not be any abrupt increase in the rate of follicular atresia after the age of 38 years (Leidy et al., 1998). Moreover, AFC shown on scanning may not truly reflect the follicle pool present in the ovaries, and the follicular dynamics from the primordial stage to the antral stage are poorly understood (McGee and Hsueh, 2000).

Ovarian volume in terms of total ovarian volume, volume of the smallest ovary and mean ovarian volume has also been evaluated to predict ovarian responses during ART treatment (Syrop et al., 1995, 1999; Lass et al., 1997) and was a better measure than basal FSH level (Syrop et al., 1999). There was a significant negative correlation between age of infertile women and ovarian volume shown by two-dimensional (Lass et al., 1997) and by three-dimensional (Kupesic et al., 2003) ultrasound, but Syrop et al. (1995) and Sharara and Mcclamrock (1999) could not demonstrate such a correlation. Christensen et al. (1997) revealed that the ovarian volume was not related to age in 428 healthy women aged 14–45 years attending a family planning clinic, but a linear relationship existed between menopause age and ovarian volume (Tepper et al., 1995). Our study found a moderate negative correlation of age with ovarian volume, similar to that of Scheffer et al. (1999). These data suggest that there are no major changes in ovarian volume during reproductive ages until the perimenopausal period.

Ovarian stromal blood flow can be evaluated by colour Doppler ultrasound and power Doppler. The ovarian response during ART was significantly correlated with mean PSV measured prior to pituitary downregulation (Zaidi et al., 1996) or after downregulation (Engmann et al., 1999). Those with normal ovarian responses had significantly higher velocity than poor responders (10.2 ± 5.8 versus 5.2 ± 4.2 cm/s). Other Doppler flow indices were not useful. Our study could not find any effect of age on mean PSV determined by colour Doppler, and this may be related to the method of detection. Power Doppler imaging is more sensitive than colour Doppler imaging at detecting low velocity flow, and hence improves the visualization of small vessels (Guerriero et al., 1999). Increasing age was associated with reduced ovarian stromal vascularity detected by three-dimensional power Doppler in general (Pan et al., 2002) and in infertile patients (Kupesic et al., 2003).

The early follicular phase FSH level taken prior to the treatment cycle is widely used in many ART programmes and is a better predictor of ovarian response than the age of women (Cahill et al., 1994; Sharif et al., 1998). Although there was a positive correlation of age with serum FSH level shown in this study, the majority of the women had a normal range of FSH levels (<10 IU/l), even in women >40 years. Inhibin B level may be an earlier marker for ovarian reserve than FSH level as decreased inhibin B levels were found in women with reduced ovarian reserve before a rise in FSH levels (Seifer et al., 1999). Seifer et al. (1997), Tinkanen et al. (1999) and Dzik et al. (2000) reported that women with inhibin B levels <45 pg/ml demonstrated a poorer ovarian response during ART than women with levels ≥45 pg/ml, while others (Corson et al., 1999; Hall et al., 1999; Creus et al., 2000) could not find any value in the prediction of ovarian response or pregnancy rate. Therefore, it is still a matter of controversy in the literature whether inhibin B is a useful marker for ovarian reserve. Our results could not show any significant correlation of age with serum inhibin B level. Inhibin B is secreted by granulosa cells of developing follicles and may reflect the number or quality of these developing follicles (Hall et al., 1999). There was no significant correlation between inhibin B level and AFC in this study, which was different from the observation of Scheffer et al. (2003). The reason for this discrepancy is unknown. Similar FSH and inhibin B levels across different age groups in this study support the concept that age-dependent hormonal changes are a relatively late phenomenon and only occur when the number of follicles is much reduced (te Velde and Pearson, 2002). Hormonal levels following some sort of stimulation may be a better reflection of AFC, as the stimulated inhibin B
level after a GnRH agonist was highly correlated with AFC (Scheffer et al., 2003). Serum anti-Müllerian hormone, which is involved in control of primordial follicle recruitment in the mouse (Durlinger et al., 1999), appears to be an early marker for ovarian reserve (Seifer et al., 2002; Van Rooij et al., 2002; Fanchin et al., 2003).

In conclusion, AFC is the only ovarian reserve marker that was significantly different among the four age groups. AFC had the best correlation with age, followed by FSH level and ovarian volume. The decline of AFC over age was linear, at 3.8% per year (95% CI = 2.7–4.9%) or 0.35 follicles per year (95% CI = 0.26–0.45%).

Acknowledgements
This study was funded by the Hong Kong Research Grant Council (HKU 7280/01M).

References


Submitted on May 12, 2003; accepted on June 19, 2003