Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography

W. Hamish Wallace¹,³ and Thomas W.Kelsey²

¹Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh and
²School of Computer Science, University of St Andrews, UK
³To whom correspondence should be addressed at: Department of Haematology/Oncology, Royal Hospital for Sick Children, 17 Millerfield Place, Edinburgh EH9 1LW, UK. E-mail: Hamish.Wallace@luht.scot.nhs.uk

BACKGROUND: The human ovary contains a fixed number of primordial follicles that decreases bi-exponentially with age, culminating in the menopause at an average age of 50–51 years. There currently is no reliable test of ovarian reserve for individual women that will accurately predict their remaining reproductive lifespan.

METHODS AND RESULTS: We use the Faddy–Gosden model of human primordial follicle population decline to describe the natural decay of the ovarian follicle pool. Assuming that the wide distribution for age at menopause is due to the wide variation in number of primordial follicles at birth, we describe follicle population decline for early and late menopausal women. Using published data on age-related ovarian volume as measured by transvaginal sonography, we have obtained a highly significant correlation between primordial follicle population and ovarian volume. We show that ovarian volume in women aged 25–51 years accurately reflects the number of primordial follicles remaining, and describe how measurement of ovarian volume by transvaginal sonography may determine ovarian reserve and reproductive age. CONCLUSIONS: The accurate assessment of ovarian reserve will revolutionize the management of women requesting assisted conception, those who have had treatment for childhood cancer and those who are considering delaying a family for personal or professional reasons.

Key words: fertility counselling/human primordial follicle/ovarian reserve/ovarian volume/reproductive age

Introduction

The human ovary contains a fixed pool of primordial follicles, maximal at 5 months of gestational age, which declines with increasing age in a bi-exponential fashion, culminating in the menopause at an average age of 50–51 years. For any given age, the size of the follicle pool can be estimated based upon a mathematical model of decline (Faddy and Gosden, 1996; Wallace et al., 2003). The rate of follicle decline represents an instantaneous rate of temporal change based upon the remaining population pool which increases around age 37 years when ~25 000 follicles remain and precedes the menopause by 12–14 years (Richardson et al., 1987). Reproductive ageing in women is due to ovarian follicle depletion, with ~1000 follicles remaining at the menopause (Faddy et al., 1992).

Several studies have reported the number of primordial ovarian follicles at different ages in humans. These data have been used to construct complex mathematical models of follicle decline (Block, 1952, 1953; Baker, 1963; Gougeon, 1984; Richardson et al., 1987). During embryo development, several million germ cells are formed in the ovarian rudiment, several hundred thousand are present at birth and some 300 000 are present at menarche (Block, 1952; Baker, 1963). The precise number of primordial follicles remaining at menopause is unclear, but is of the order of 1000. Ovarian follicles were counted in the ovaries of 43 females aged 6–44 years, following accidental death, and, using linear extrapolation, the number of follicles present at menopause was predicted to be 2200 (Block, 1952). This is now considered an overestimate, as further studies of follicle numbers present in the ovaries of pre- peri- and post-menopausal women have demonstrated that <1000 ovarian follicles remain in peri-menopausal women, indicating that follicle decline accelerates in the decade preceding menopause (Richardson et al., 1987). With only an estimated 400 ovulations occurring during the reproductive period, this progressive reduction is attributed to follicle death by apoptosis. Follicle depletion as a result of atresia and recruitment towards ovulation leads to premature exhaustion of the follicle pool and menopause long before death (te Velde and Pearson, 2002).

There currently are no reliable markers or clinical methods to assess ovarian reserve accurately in the normally menstruating pre-menopausal woman. Follicular density measured in ovarian biopsies from infertile women shows a significant negative correlation with increasing age. Women >35 years of
age have a mean ovarian volume significantly smaller than in women <35 years, and have been shown to have only a third of the follicles of younger women (Lass et al., 1997a). An ovarian volume of <3 ml was predictive of a poor response to ovulation induction by HMG for IVF, very suggestive of reduced ovarian reserve (Lass et al., 1997b). Furthermore, women with a low number of retrieved oocytes at ovulation induction for IVF were more likely to become post-menopausal at an earlier age than women with a higher number of retrieved oocytes (De Boer et al., 2002).

Following the rapid increase in the use of transvaginal sonography, the measurement of ovarian volume has become quick, accurate and cost-effective. Ovarian volume measurement has become a potentially useful tool in the screening, diagnosis and monitoring of the treatment of conditions such as polycystic ovarian syndrome and ovarian cancer, and in the prediction of superovulation during IVF (Lass and Brinsden, 1999). The aim of this work is to describe a methodology for determining a woman’s reproductive age and ovarian reserve by measurement of ovarian volume by transvaginal sonography.

Methods and Results

Natural decay of the ovarian follicle pool and solution of the Faddy–Gosden equation

Graphical representation of ovarian follicle number, expressed logarithmically against age, suggests that ovarian follicle decline is bi-exponential (Faddy et al., 1992). An increase in the rate of exponential decline appeared to occur at a follicle pool of ~25 000, corresponding to an average age of 37 years. However, biologically, an abrupt change when the primordial follicle population falls to 25 000 is unlikely, and more plausibly the change represents an instantaneous rate of temporal change based on the remaining population pool, which is expressed mathematically as a differential equation. Faddy and Gosden (1996) provide a revised model in terms of the differential equation: $\frac{dy}{dx} = -y \left[ 0.0595 + 3716/ (11780 + y) \right]$, where $x$ denotes age and $y$ denotes primordial follicle population, with initial value $y(0) = 701 200$.

This equation expresses the rate of change in the population from birth, and we consider it to be the best model currently available. To find the population at a given age, $y(x)$, we must solve the equation. This can be done either numerically or analytically. A numeric solution is inexact, but computation is designed to bound local errors, and, hopefully, minimize global errors. An analytical solution need not exist, and may be hard to find. It will, however, be exact (subject to careful evaluation of the terms involved). We solved the Faddy–Gosden equation using a 7th–8th continuous Runge–Kutta numerical method (Butcher, 1987), and used the solution to estimate the radiosensitivity of the human oocyte (Wallace et al., 2003). We have since solved the differential equation analytically using the Maple (Char, 1991) computer algebra system; the two solutions agree to within 10 primordial follicles. Application of the Faddy–Gosden model for healthy women from birth to 51 years old and 2.1 (±0.01) ml in women aged 60–69 years. Mean ovarian volume was 4.9 ml in post-menopausal women and 2.2 ml in post-menopausal women (Pavlík et al., 2000).

Using age of menopause distribution to describe primordial follicle population decline in early, average and late menopausal women

There is a wide variation in age of menopause; a prospective study of 529 Western women (Treloar, 1981) gives a mean age of 50.4 years with an SD of 3.9 years. This gives a 95% confidence interval (CI) for the cessation of menses of 42.8–58.0 years (Figure 2, upper panel). A more recent study of 4686 women (Van Noord et al., 1997) is in close agreement, with a mean age of 50.16 years and an SD of 4.15 years.

If we assume that the wide variation in age at menopause is due to a wide variation in primordial follicle population at birth, such that women with an early menopause have fewer primordial follicles at birth than women with an average or late menopause, then the expected primordial follicle population decline for early, average and late age of menopause in women is illustrated in Figure 2 (lower panel). This assumption, supported by the histological studies of Baker (1963), is discussed in depth in the Discussion.

Ovarian volume decreases with increasing age

Adult ovaries are ovoid, measure ~3–5 cm (D1) × 1.5–3 cm (D2) × 0.6–1.5 cm (D3) and weigh 5–8 g (Clement, 1991). During transvaginal sonography, the ovaries are measured in three planes and ovarian volume is calculated from the prolate ellipsoid formula $V = D1 \times D2 \times D3 \times 0.523$. There is good evidence that adult ovarian volume decreases with increasing age as the remaining pool of primordial follicles becomes exhausted. As part of the University of Kentucky Ovarian Cancer Screening programme, 13 963 women between 25 and 91 years of age underwent annual transvaginal sonography. From 58 673 observations of ovarian volume, a statistically significant decrease in ovarian volume was shown with each decade of life from age 30 to 70 years (Figure 3). Mean (±SEM) ovarian volume was 6.6 (±0.19) ml in women <30 years old, 6.1 (±0.06) ml in women 30–39 years, 4.8 (±0.03) ml in women aged 40–49 years, 2.6 (±0.01) ml in women 50–59 years old and 2.1 (±0.01) ml in women aged 60–69 years. Mean ovarian volume was 4.9 ml in pre-menopausal women and 2.2 ml in post-menopausal women (Pavlík et al., 2000).
Correlation between primordial follicle population and ovarian volume

For each chronological age from 25 to 51 years, we can determine the mean primordial follicle population from our solution of the Faddy–Gosden equation (Figure 1). If we relate 表情 mean primordial follicle population at each chronological age to mean ovarian volume at each chronological age from Pavlik et al. (2000) (Figure 3), we derive a highly significant correlation as shown in Figure 4 (r = 0.97). If, in addition, we plot the 表情 primordial follicle population for late menopause women (Figure 2) with 95% upper confidence limit for ovarian volume (Pavlik et al., 2000) for ages 25–58 years, we derive a similar highly significant correlation (r = 0.96) (Figure 4). We have therefore demonstrated a significant correlation between primordial follicle population (for both average and late menopausal women) and ovarian volume. This provides good evidence that ovarian volume in women aged 25–51 years is strongly associated with the remaining primordial follicle population.

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Using ovarian volume to predict reproductive age

We now define a method using ovarian volume as a surrogate measure for the remaining primordial follicle pool to predict reproductive age. For each chronological age from 25 to 50, we define the minimum expected reproductive age as chronological age minus 1.96 SDs of the distribution of ages at

Figure 2. Upper panel: distribution of ages at menopause in 529 women followed prospectively. Mean age at menopause 50.4 years, SD 3.9 years (Treloar, 1980). Y is the mean age at menopause (50.4) years. X is 42.8 years; the 95% lower confidence limit for age at menopause (50.4 – 1.96 × 3.9) years. Z is 58.0 years; the 95% upper confidence limit for age at menopause (50.4 ± 1.96 × 3.9) years. Here 1.96 is the Student’s t critical point for a 95% CI for a normal distribution. Lower panel: combining primordial follicle population decline and age at menopause for early (X), average (Y) and late menopause (Z). Points X, Y and Z are derived as in the upper panel, and all occur at a primordial follicle population of 1000 (best estimate of number of remaining follicles at menopause) represented by the horizontal dotted line. The lines terminating at X and Z are the solutions for the Faddy–Gosden equation where 1000 follicles remain at 42.8 and 58.0 years, respectively. For example, a 35-year-old woman is expected to have a primordial follicle population of between 10 000 (LP) and 90 000 (UP). This corresponds to a reproductive age of between 27.4 (LA) and 42.6 (UA) years. LA = lower reproductive age; UA = upper reproductive age; LP = lower primordial follicle population; UP = upper primordial follicle population.

Figure 3. Mean ovarian volume and 95% upper confidence limit related to age for ages 25–51. Redrawn from Pavlik et al. (2000). The number of observations are as follows: 25–29 years, 444; 30–39 years, 3259; 40–49 years, 9963; and 50–51 years, 4300.

Figure 4. Upper panel: correlation between log mean primordial follicle population from our solution of the Faddy–Gosden equation (Figure 1) and mean ovarian volume for each chronological age from 25 to 51 years (from Pavlik et al., 2000) (Figure 3). Using a least squares fit: log(10)y = 0.53x + 1.3, with r = 0.97. Example data points are as follows: age 48 years, ovarian volume 4.0 ml, primordial follicle population 2300; age 28 years, ovarian volume 6.7 ml, primordial follicle population 85 100. Lower panel: correlation between (log) primordial follicle population for late menopause women (Figure 2) with 95% upper confidence limit for ovarian volume (Pavlik et al., 2000) (Figure 3) for ages 25–58 years. Using a least squares fit: log(10)y = 0.19x + 2.5, with r = 0.96. Example data points are as follows: age 51 years, ovarian volume 6.0 ml, primordial follicle population 7300; age 41 years, ovarian volume 12.0 ml, primordial follicle population 43 900.
menopause. This is 1.96 × 3.9 years, or 7.6 years. Here, 1.96 is the Student’s t critical point for a 95% CI for a normal distribution, and 3.9 years is the SD given by Treloar (1981).

To relate reproductive age to ovarian volume for a given chronological age, we have three points, A, B and C. Point A has the coordinates minimum expected reproductive age and upper limit on 95% CI on ovarian volume. Point B has coordinates chronological age and mean ovarian volume. Point C has coordinates average age of menopause and average ovarian volume for post-menopausal women. For a given ovarian volume and chronological age, we estimate reproductive age from a plot through the three points (Figure 5).

Two examples are given, both for women with chronological age 40 years, in Figure 5. The first has an ovarian volume of 9.0 ml, and hence a reproductive age of 36 years. The second has an ovarian volume of 5.0 ml, giving a reproductive age of 42 years. This is equivalent to saying that the first individual can expect ovarian failure to occur in 14–15 years (50–51 minus 36 years), and the second in 8–9 years (50–51 minus 42 years).

In Figure 6, we describe the relationship between ovarian volume (as a surrogate measure for the remaining primordial follicle pool) and reproductive age for women of chronological ages 25–45 years at 5 yearly intervals. The age at menopause for each age group is fixed at 50.4 years (Treloar, 1981), corresponding to an ovarian volume of 2.2 ml being the mean ovarian volume for post-menopausal women (Pavlik et al., 2000). More specifically, if a woman of chronological age 25–51 years has her mean ovarian volume measured by transvaginal sonography, then, taking ovarian volume data for her age from Pavlik et al. (2000), we can estimate her reproductive age using ovarian volume as a surrogate for her ovarian reserve. We can then predict her age of menopause.

This prediction will only apply to women who have no evidence of ovarian disease and who are not on hormonal contraception.

Discussion

We have shown that ovarian reserve and reproductive age in healthy pre-menopausal women, who are not using hormonal contraception, can be determined from the measurement of ovarian volume by transvaginal sonography. There are two inherent assumptions in our methodology that require justification: (i) that the observed variation in age at menopause is due to a wide difference in the primordial follicle population at birth; and (ii) that ovarian volume between the ages of 25 and 50 years is directly associated with the remaining primordial follicle population.

Evidence to support our first assumption that the observed variation in age at menopause is due to wide variation in the number of primordial follicles present at birth comes from the histological studies of the number of follicles in human ovaries at different ages. Reproductive ageing in women is due to exhaustion of the remaining pool of primordial follicles, with <1000 ovarian follicles remaining in peri-menopausal women (Richardson et al., 1987). It has been accepted for some time that it is a critical number of primordial follicles rather than a critical age that determines the timing of the menopause (Gosden, 1987). The studies (Block, 1952, 1953; Baker, 1963; Gougeon, 1984; Richardson et al., 1987; Gougeon et al., 1994) which counted primordial follicles in human ovaries provided the basis on which the Faddy–Gosden mathematical model was derived, but also clearly showed a wide variation in the number of primordial follicles present at birth and at all ages until the menopause. Furthermore, the age related-decline in the number of follicles is bi-exponential and more than doubles when numbers fall below a critical number of ~25 000 (Faddy et al., 1992). The wide variation in age at menopause of otherwise healthy Western women (Treloar, 1981; Van Noord et al., 1992).
1997) must be due to either variation in the rate of primordial follicle loss, for which there is no evidence, or a wide variation in the number of primordial follicles present at birth, for which there is good histological evidence (Baker, 1963). The Faddy–Gosden solution for those women with an average age at menopause (Figure 1) can therefore be applied to those who are pre-programmed through a reduced (increased) number of primordial follicles at birth to have an early (late) menopause (Figure 2, lower panel).

Our second assumption is that ovarian volume between the ages of 25 and 50 years is directly related to the remaining primordial follicle population. The human ovary changes in size, shape and activity throughout life. At birth, the ovary is ~1 cm in length and weighs <0.3 g (Clement, 1991); there is continuous slow growth of the ovaries throughout childhood, and by puberty they have reached the size and shape of the adult ovary (Ivarsson et al., 1983). The largest published study of ovarian volume related to age (Pavlik et al., 2000) showed a statistically significant decrease in ovarian volume with each decade of life from 30 to 70 years. As we have shown, this steady decrease in ovarian volume throughout reproductive life significantly correlates with the number of primordial follicles present in the ovary as calculated from our solution of the Faddy–Gosden equation. Further evidence to support this relationship comes from studies of the ability of the ovary to respond to exogenous gonadotrophins for successful IVF. Women who have a mean ovarian volume of <3 ml have a very high chance of failure to respond to ovulation induction, implying significantly reduced ovarian reserve (Lass et al., 1997b). In a further study of 60 infertile women aged 19–45 years, which included an ovarian biopsy (Lass et al., 1997a), a significant negative correlation between increasing age, ovarian volume and the density of primordial follicles in the ovarian cortex was found. A recent study (Erdem et al., 2003) of both fertile (n = 53) and infertile (n = 62) women (aged 35–45 years) showed a significant negative correlation between mean ovarian volume and age in both groups. Interestingly, basal FSH and antral follicle counts did not differ between infertile and fertile women, whereas mean ovarian volume was significantly smaller in the infertile women. These studies support a strong direct association between mean ovarian volume and remaining ovarian reserve. For ovarian volume to be used as a surrogate measure of the remaining primordial follicle pool, it is important for women to be assessed when they are not taking hormonal contraception because it appears that oral contraception reduces the volumes of both ovaries in all phases of the menstrual cycle (Christensen et al., 1997).

The assessment of ovarian reserve in the otherwise healthy pre-menopausal woman remains a challenge. FSH in the early follicular phase of the menstrual cycle reflects the sum of both hypothalamic drive and ovarian feedback, but is not elevated until the peri-menopausal period (Wallach, 1995). Direct products of the ovary including inhibin B and anti-Mullerian hormone (AMH) have been investigated (De Vet et al., 2002; Van Rooij et al., 2002) as markers of a diminished ovarian reserve. The value of inhibin B is significantly increased following the administration of a single dose of FSH to stimulate granulosa cell function in small healthy follicles (Yong et al., 2003). AMH, a member of the transforming growth factor-β family, is produced by granulosa cells of early developing follicles and is postulated to have a role in the regulation of human folliculogenesis (Durlinger et al., 1999). Concentrations decrease over time in young normo-ovulatory women before other markers of ovarian ageing (De Vet et al., 2002), and reduced baseline levels of AMH are associated with a poor response to ovarian stimulation for IVF (Van Rooij et al., 2002). Interestingly, levels show little fluctuation over the menstrual cycle (Cook et al., 2000). The measurement of AMH in the circulation is certainly a promising marker of ovarian ageing, but as yet it has not been shown to be helpful in assessing ovarian reserve for the individual.

The number of small antral follicles measuring between 2 and 10 mm in the early follicular phase of the menstrual cycle on transvaginal sonography was found to have the best correlation with chronological age in a study of 162 healthy female volunteers with proven normal fertility and regular menstrual cycles (Scheffer et al., 2003). The women were divided into three age groups; young (n = 49; 25–34 years), middle (n = 53; 35–40 years) and old (n = 60; 41–46 years), and the number of small antral follicles fell significantly from median (range) 15 (3–30), 9 (1–25), 4 (1–17) in each group, respectively. Antral follicle counts have been used successfully to predict the ovarian response and pregnancy results of patients undergoing assisted reproductive technologies, with no pregnancies in the women with an antral follicle count of ≤3 (Chang et al., 1998). Undoubtedly the number of small antral follicles reflects the remaining primordial follicle pool, but the wide range within each age group, and the large variation over concurrent cycles, makes interpretation difficult for the individual and it therefore remains at best an indirect test.

Fertility is a major concern for women who have survived cancer during childhood, and is of increasing importance because 70% of children treated for malignant disease will become long- term survivors (Mertens et al., 2001; Wallace et al., 2001). Some women may develop an early menopause, but others may progress through puberty normally and have regular menstrual cycles with normal endocrine profiles (Wallace et al., 1989a,b, 1993). As the agents used to treat childhood malignancy will destroy a greater or lesser number of ovarian primordial follicles, it would be of value to be able to assess accurately the effect of treatment on the ovarian reserve, particularly in those women with apparently normal ovarian function. Assessment of ovarian reserve in childhood cancer survivors with regular menstrual cycles and basal FSH <10 IU/l by repeated transvaginal sonography has shown that female survivors had significantly smaller ovarian volumes and a lower number of small antral follicles per ovary than controls (Larsen et al., 2003). In a recent study, we have confirmed significantly reduced ovarian volumes and shown reduced serum AMH concentrations (Bath et al., 2003), providing clear evidence for diminished ovarian reserve in regularly menstruating pre-menopausal childhood cancer survivors.

The ability to make a direct and accurate assessment of ovarian reserve would be of enormous benefit to women who are being considered for assisted reproductive technologies, for
young women who are long-term survivors of childhood cancer and for women who are considering delaying starting a family for personal or professional reasons (Nikolaou and Templeton, 2003). Demographic (Wood, 1989) and clinical (Noord-Zaadstra et al., 1991) studies have shown that women experience their optimal fertility before the age of 30–31 years, and it gradually declines towards the age of 40 years. The possibility of providing a direct and easily reproducible assessment of ovarian reserve and reproductive age through the transvaginal measurement of ovarian volume for all interested women is a real advance, and opens the door to the possibility of screening women for ‘early ovarian ageing’ which currently affects 10% of the general population.

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