Assessment of ovarian function by 3D ultrasound

Throughout the entire female reproductive lifespan, the ovary relies on the reserve of resting follicles or ‘ovarian reserve’ which is a measure of ovarian ageing. It is defined by the quantity and quality of the remaining primordial follicular pool within the paired ovaries at any given time (Broekmans et al, 2006). Ovarian reserve declines gradually with women’s age (Gougeon 1998; Baird et al, 2005) and this decline in the quantitative and qualitative ovarian reserve dictates the onset of different age-related reproductive events occurring in women, such as shortening of the length of the menstrual cycle, loss of menstrual regularity (Treloar et al, 1967; Treloar 1981) and the onset of the menopause (Faddy et al, 1992). The well established observation of age-related decreases in fecundity and conception rates per menstrual cycle (Menken et al, 1986; Van Noord-Zaadstra et al, 1991) are also primarily related to declining ovarian reserve rather than uterine ageing, as evident by the equivalent pregnancy rates seen in women of all ages undergoing assisted reproduction treatment (ART) with oocytes donated from younger women (Navot et al, 1994; Van Voorhis 2007).

Why do we assess ovarian function?
Ovarian reserve clearly reflects the fertility potential of a woman and strongly influences the possibility of conception, either spontaneously or in conjunction with fertility treatment. However, neither ovarian ageing nor reduced ovarian reserves are currently listed as a cause of sub-fertility. A subset of couples with signs of ovarian ageing could potentially benefit from undergoing assisted reproduction treatment such as IVF/ICSI, as a first line treatment or at an earlier stage as it offers the maximum chance of conception and avoids wasting time and money on other options which may also be assessed directly using ultrasound to quantify the total number of antral follicles (Tomas et al, 1997), mean ovarian volume (Lass et al, 1997) and ovarian vascularity (Zaidi et al, 1996). All these tests have different sensitivities, specificities and predictive values, but there is a lack of consensus as to the best single test or combination of tests (Broekmans et al, 2006). Basal FSH is the most widely used marker of ovarian reserve worldwide (Hendriks et al, 2005). However, recent evidence has suggested that the best predictors are anti-Müllerian hormone (AMH) (La Marca et al, 2010) and antral follicle count (AFC) and ovarian vascularity. Most investigators have used conventional 2D ultrasound to assess ovarian morphology and quantify these variables. The recent use of 3D ultrasonography and quantitative 3D power Doppler angiography (3D-PDA) as a diagnostic modality has an important role in improving the predictive accuracy of ultrasound assessment of ovarian reserve.

Three-dimensional ultrasound assessment of ovarian reserve
Over the last two decades, various ultrasound markers have been investigated to evaluate their role in the prediction of ovarian function and, hence, reserve. The three most common markers that have been specifically addressed are ovarian volume, antral follicle count (AFC) and ovarian vascularity. Most investigators have used conventional 2D ultrasound to assess ovarian morphology and quantify these variables. The recent use of 3D ultrasonography and quantitative 3D power Doppler angiography (3D-PDA) as a diagnostic modality has an important role in improving the predictive accuracy of ultrasound assessment of ovarian reserve.

3D ultrasound or ‘volume sonography’ involves the acquisition of a series of 2D images from a pre-selected region of interest (Benacerraf et al, 2005). These data have relative positional information within the acquired volume dataset defined in a Cartesian format. The acquired 3D data may be displayed in a variety of ways and analysed in a virtual real-time manner as the user can obtain any image plane they desire. The multiplanar view provides the user with a simultaneous display of three mutually related, perpendicular planes: The sagittal (A), transverse (B), and coronal (C) planes (figure 1). These planes maximise the information available and improve spatial awareness of the area of interest (Merz 1999). The coronal plane, perpendicular to the ultrasound beam and parallel to the transducer face, is unique to 3D ultrasound and facilitates the identification of surface irregularities, which can then be accounted for during volume measurement (Maymon et al, 2000).

Antral follicle count (AFC)
Assessment of the antral follicle count has been commonly made using two-dimensional ultrasound, which is simply used to identify and count the number of follicles within each ovary. Assessment of follicle size requires measurement of each follicle in two dimensions and calculation of the mean diameter (Haadsma et al, 2007). This can be very

al, 1992). The direct assessment of ovarian reserve to evaluate the growing follicle population can be performed by taking an ovarian biopsy but this is not practical for obvious reasons. As a result, several endocrine and ultrasound markers of ovarian reserve, all based on the biological mechanisms involved in reproductive ageing, have been adopted into clinical practice. Primarily, all these tests aim to estimate the number of gonadotropin-responsive or ‘selectable follicles’, which are assumed to be reflective of the primordial follicle population. Ovarian reserve is typically assessed indirectly through measurement of serum follicle-stimulating hormone (FSH) levels (Sharif et al, 1998) during the early follicular phase of the cycle or by quantification of the endocrine factors produced by the developing follicles including oestradiol (Smotrich et al, 1995), inhibin B (Seifer et al, 1999) and anti-Müllerian hormone (AMH) (van Rooij et al, 2002). Developing follicles and, consequently, ovarian reserve may also be assessed directly using ultrasound to quantify the total number of antral follicles (Tomas et al, 1997), mean ovarian volume (Lass et al, 1997) and ovarian vascularity (Zaidi et al, 1996). All these tests have different sensitivities, specificities and predictive values, but there is a lack of consensus as to the best single test or combination of tests (Broekmans et al, 2006). Basal FSH is the most widely used marker of ovarian reserve worldwide (Hendriks et al, 2005). However, recent evidence has suggested that the best predictors are anti-Müllerian hormone (AMH) (La Marca et al, 2010) and antral follicle count (AFC) (Broer et al, 2009). AMH has the added advantage of demonstrating a lower variability in its serum levels within and between menstrual cycles, allowing it to be measured at any stage of the menstrual cycle as opposed to antral follicles which are generally counted in the early follicular phase.

In the last few years, the use of 3D ultrasound has become increasingly more widespread. Although the introduction of 2D ultrasound improved the ability to assess ovarian morphology and thus the ovarian reserve, the quantification of these parameters is still limited. The problems of ultrasound assessment of ovarian morphology and follicular number were firstly addressed using 3D ultrasound or ‘volume sonography’ involves the acquisition of a series of 2D images from a pre-selected region of interest (Benacerraf et al, 2005). These data have relative positional information within the acquired volume dataset defined in a Cartesian format. The acquired 3D data may be displayed in a variety of ways and analysed in a virtual real-time manner as the user can obtain any image plane they desire. The multiplanar view provides the user with a simultaneous display of three mutually related, perpendicular planes: The sagittal (A), transverse (B), and coronal (C) planes (figure 1). These planes maximise the information available and improve spatial awareness of the area of interest (Merz 1999). The coronal plane, perpendicular to the ultrasound beam and parallel to the transducer face, is unique to 3D ultrasound and facilitates the identification of surface irregularities, which can then be accounted for during volume measurement (Maymon et al, 2000).

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labour intensive and the reliability and validity of such measures are likely to be reduced when there are numerous follicles. Ultrasonographic assessment of the total number of antral follicles measuring 2-10mm is a reliable determinant of ovarian reserve (Jayaprakasan et al, 2008, Scheffer et al, 2003). The total AFC is performed by counting the number of antral follicles measuring 2-10mm in both ovaries and can be estimated using 2D (Scheffer et al, 2002, Jayaprakasan et al, 2008) or 3D ultrasound (Jayaprakasan et al, 2007).

3D ultrasound has two main advantage. It allows the display of an image in three perpendicular planes, simultaneously giving more spatial orientation, and offline assessment of these data along with the facility to render the image. 3D ultrasound has recently been demonstrated to offer a significant advantage over 2D imaging in terms of measurement reliability (Jayaprakasan et al, 2008).

More recently, Jayaprakasan et al demonstrated that the number of antral follicles measuring 2 to 6mm is most reflective of the quantitative status of ovarian reserve (Jayaprakasan et al, 2010). The number of small antral follicles is strongly correlated with other ovarian reserve tests, such as AMH (Anti-Müllerian Hormone), supporting the concept that these smaller follicles represent the functional ovarian reserve (Haadsma et al, 2007).

There is a linear decline in the number of antral follicles with age (Gougeon 1994) and this is more apparent in the smaller antral follicles (<6.0mm) than the larger ones (>6.0mm) (Scheffer et al, 2003; Haudsma et al, 2007) and their total number is, therefore, more reflective of the primordial follicle pool. These findings indicate that the method by which the antral follicles are counted may influence its performance as a predictive test of ovarian function, reserve and response.

A recent development is the introduction of a 3D automated technique; sonography-based Automated Volume Count (SonoAVC; GE Healthcare Ultrasound, Zipf, Austria) (Raine-Fenning et al, 2008), where mathematical algorithms allow the definition and differentiation of hypoechoic, fluid-filled areas within the acquired volume (Raine-Fenning et al, 2007). Sono-automatic volume calculation (SonoAVC; GE Medical Systems, Zipf, Austria) also provides automatic estimation of the absolute dimensions of each three-dimensional fluid-filled area (Raine-Fenning et al, 2008b).

Each individual volume is given a specific colour and the automated measurements of its mean diameter (relaxed sphere diameter), maximum dimensions (x, y, z diameters) and volume are displayed in descending order from the largest to the smallest (Raine-Fenning et al, 2008a).

Theoretically, an unlimited number of volumes can be quantified and the software lends itself, therefore, to the examination of follicles within the ovary. Studies in patients undergoing ovarian stimulation have shown that SonoAVC provides automatic measurements of follicular diameter and volume that are more reliable and more accurate than comparable estimations made from two dimensional data (Raine-Fenning et al, 2007, 2008b).

Recent studies evaluating various ovarian reserve markers and response have demonstrated that the AFC may be considered as the test of choice in the assessment of diminished ovarian reserve (Verhagen et al, 2008).

### Ovarian volume

The first ultrasound marker of ovarian reserve to be evaluated was ovarian volume. It is easily determined and can be calculated from 2D images, using the principle of the volume of an ellipsoid and the formula \( \frac{4}{3} \pi \times \text{length} \times \text{width} \times \text{depth} \). or with 3D ultrasound (figure 3) (Raine-Fenning et al, 2003), which seems to provide a more reliable and valid method of volume calculation. The three-dimensional ultrasound allows an objective assessment of ovarian volume and enhances measurement accuracy and both intra- and interobserver reliability (Raine-Fenning et al, 2003).

There are two basic methods employed to calculate volume from a 3D dataset – the conventional ‘full planar’ or ‘contour’ method and the recently-introduced ‘rotational’ method possible through Virtual Organ Computer-aided AnaLysis (VOCAL™), which also generates a 3D model of the object of interest (Bordes et al, 2002, Raine-Fenning et al, 2002). Both techniques involve manual delineation of the object of interest in the multiplanar display that shows the three perpendicular planes characteristic of 3D ultrasound.

While volume measurements using both the methods have been shown to more reproducible than 2D measurements (Raine-Fenning et al, 2003a), the VOCAL technique is less time consuming (Bordes et al, 2002). VOCAL allows rotation of the 3D dataset about a central axis through a
number of pre-defined rotation steps (Bordes et al, 2002; Raine-Fenning et al, 2002).

Both the reproducibility and validity of volume calculation using rotational method were better than that made using conventional trapezoid formula. The most appropriate rotation step for its use in a clinical or research setting recommended is 9° as it provides the best compromise between reliability, validity and time taken for measurement (Raine-Fenning et al, 2003c).

**Ovarian blood flow**

There are only a few studies reporting on the ability of measures of ovarian vascularity or blood flow to predict ovarian response or the occurrence of pregnancy (Zaidi et al, 1996, Ng et al, 2005, Jayaprakasan et al, 2008). Kupesic et al used 3D power Doppler angiography to evaluate ultrasound-derived ovarian predictors of ovarian response and outcome in 56 women with normal basal serum follicle-stimulating hormone concentrations (<10mIU/ml) undergoing their first cycle of IVF (Kupesic et al, 2003). Multiple regression analysis of the six most predictive variables, including the peak estradiol on the day of human chorionic gonadotropin administration, total ovarian volume, total ovarian stromal area, age and total AFC, revealed AFC to be the best predictor of the number of mature oocytes collected and pregnancy, followed by the ovarian stromal flow index, which is a measure of the intensity of blood flow.

Similar findings were reported in larger subsequent studies by Ng et al and Jayaprakasan et al in which the AFC achieved the best predictive value in relation to the number of oocytes retrieved, although the 3D ovarian vascular indices were not predictive of ovarian response or pregnancy (Ng et al, 2005, Jayaprakasan et al, 2008).

**Conclusion**

Over the last two decades, many studies have suggested that 3D ultrasound offers more reliable and valid information than conventional 2D ultrasonography. 3D ultrasound may provide a more objective assessment of the ultrasound markers of ovarian reserve and may therefore improve the performance of these measures in determining the response to controlled ovarian stimulation. These measures do not appear to be able to predict the chance of pregnancy, however, and we are still a long way from being able to accurately subjectively or objectively quantify true ovarian function with ultrasound.

**References**


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