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Counting ovarian antral follicles by ultrasound: a practical guide

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KEYWORDS: AFC; antral follicle count; FNPO; follicle number per ovary; ovarian reserve

ABSTRACT

This Consensus Opinion summarizes the main aspects of several techniques for performing ovarian antral follicle count (AFC), proposes a standardized report and provides recommendations for future research. AFC should be performed using a transvaginal ultrasound (US) probe with frequency ≥ 7 MHz. For training, we suggest a minimum of 20–40 supervised examinations. The operator should be able to adjust the machine settings in order to achieve the best contrast between follicular fluid and ovarian stroma. AFC may be evaluated using real-time two-dimensional (2D) US, stored 2D-US cine-loops and stored three-dimensional (3D) US datasets. Real-time 2D-US has the advantage of permitting additional maneuvers to determine whether an anechoic structure is a follicle, but may require a longer scanning time, particularly when there is a large number of follicles, resulting in more discomfort to the patient. 2D-US cine-loops have the advantages of reduced scanning time and the possibility for other observers to perform the count. The 3D-US technique requires US machines with 3D capability and the operators to receive additional training for acquisition/analysis, but has the same advantages as cine-loop and also allows application of different imaging techniques, such as volume contrast imaging, inversion mode and semi-automated techniques such as sonography-based automated volume calculation. In this Consensus Opinion, we make certain recommendations based on the available evidence. However, there is no strong evidence that any one method is better than another; the operator should choose the best method for counting ovarian follicles based on availability of resources and on their own preference and skill. More studies evaluating how to improve the reliability of AFC should be encouraged. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

Ovarian antral follicles can be identified and counted using transvaginal ultrasound (US)[1,2]. Since there is no test available to evaluate the true ovarian reserve[3], ovarian antral follicle count (AFC) is accepted as a good surrogate marker[3,4]. AFC is frequently assessed in women of reproductive age, for various reasons: it is helpful in infertility and assisted reproduction technique work-up[5], in predicting the risk of menopause[6,7], to raise suspicion of ovulatory dysfunction secondary to hyperandrogenism anovulation[8,9] and in certain other specific clinical situations[1,10]. It is a non-invasive, easily performed technique[11] that can be used as a surrogate marker for the reserve of each ovary separately[8,12]. However, the use of transvaginal US to count ovarian follicles is not totally standardized and consequently there can be variability between observers and as a result of the equipment used[13–15].

Anti-Müllerian hormone (AMH), a glycoprotein secreted by the granulosa cells of primordial follicles, is also used as a surrogate marker of ovarian reserve, as

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this hormone is produced mainly by the ovarian antral follicles. AMH and AFC apparently have similar accuracy in providing information regarding ovarian reserve and in predicting response to ovarian stimulation[8,12,14,16]. Limitations of AMH concentration assessment are the absence of international standardization[12,17] and higher costs; standardization combined with a stable automated assay is likely to enhance its performance in the prediction of ovarian response[15].

The greatest advantage of AFC over AMH is that, when assessing the functional ovarian reserve by AFC, the observer is able to evaluate many other important aspects of the ovaries, such as their position and the presence of endometrioma or other ovarian lesions, as well as gaining important information regarding the Fallopian tubes (e.g. presence of hydrosalpinx) and the uterus (e.g. presence of endometrial polyps or submucous leiomyomas). Adding AMH to AFC improves only the prediction of excessive response to ovarian stimulation, and not the prediction of poor ovarian response[16]. In cases in which there are technical difficulties in performing AFC (e.g. poor view of the ovaries, restricted mobility of the ovaries or presence of ovarian lesions) or problems arising from limited operator experience or US equipment, evaluation of AMH can help to predict ovarian response.

The primary objective of this Consensus Opinion was to summarize the most important aspects of several techniques to count ovarian follicles, aiming to help less experienced observers to choose the best technique, and to specify minimum standards with the aim of maximizing the reliability of AFC while minimizing scan time and patient discomfort. We also aimed to propose a standardized report to describe antral follicle evaluation and to obtain perspective to direct future research on sonographic evaluation of ovarian follicles.

METHODOLOGY

Elements of Glaser's method[18] and of the nominal group technique[19] were used in the development of this Consensus. The group consisted of clinicians and sonologists focused on the application of US in gynecology, reproductive medicine, gynecological endocrinology and gynecological surgery as well as the reliability and accuracy of US tools. The initial manuscript was written by three authors (M.A.C.N., A.L., W.P.M.) following a review of the literature and provided to the other authors with a request for comments and corrections. The most important comments were incorporated and a second version was sent to all authors, along with questions regarding the more controversial points. A third version of the manuscript, incorporating their responses, was sent to all authors, asking for more suggestions; any changes at this point were performed only if they were approved by the majority of the authors. A fourth and final version of the manuscript was circulated to all authors' for their approval prior to completion.

CONSENSUS

Terms and definitions

Ovarian follicles contain oocytes and are covered by granulosa cells. Ovaries include four different types of follicle at distinct stages of development: primordial, primary, secondary and tertiary (or antral)[1,20]. The number of primordial follicles, which is the true ovarian reserve, is determined in the fetus and declines throughout life[21]. However, primordial follicles cannot be seen by US since they are too small (< 0.05 mm in diameter)[22]. Primordial follicles consist of a dormant single layer of granulosa cells covering an oocyte. They are quiescent, but initiate growth depending on a sensitive balance between the factors that promote proliferation and apoptosis[20,23,24]. When changing to primary follicles, the granulosa cells start to duplicate and become cuboidal. A glycoprotein polymer capsule, the zona pellucida, forms around the oocyte, separating it from the granulosa cells. When becoming secondary follicles, stroma-like theca-cells that surround the outer layer of the follicle undergo cytodifferentiation to become theca externa and theca interna, which are separated by a network of capillary vessels[24]. The formation of a fluid-filled cavity adjacent to the oocyte, the antrum, defines the tertiary, or antral, follicle. However, initially, antral follicles are not identifiable by US as they measure < 1.0 mm in diameter. The antral follicles become more easily identifiable by US when they reach 2 mm in diameter, coinciding with higher sensitivity to follicle-stimulating hormone (FSH)[1,20,23]. Antral follicles measuring between 2 and 10 mm are 'recruitable', while antral follicles > 10 mm are usually referred to as 'dominant' follicles.

The AFC generally includes follicles with a mean diameter ranging from 2 to 10 mm. Although some antral follicles < 2 mm in diameter might be identified during transvaginal US, it is debatable as to whether these should be included in the count, as doing so might increase the risk of counting as follicles certain other small anechoic structures, such as vessels or artifacts[25]. Moreover, such follicles are less responsive to FSH[1,26]. It is believed by some that the number of follicles measuring only up to 5–6 mm represents more accurately the best cohort of recruitable follicles and correlates better with the true ovarian reserve[3]; however, distinguishing follicles that measure 5–6 mm from those measuring 7–9 mm may increase the time needed to count them, without evidence of clinical benefit. Hence, in clinical practice, counting all identifiable follicles 2–10 mm in diameter provides the most practical method for AFC, without having to measure several follicles[1].

Influence of timing, hormones and ovarian cysts on AFC

Although it has been recommended to count the follicles preferentially in the early follicular phase of the menstrual cycle, in order to prevent the effect of intracycle fluctuation[1,27,28], evaluation of ovarian reserve by follicle

count may be performed at any point in the menstrual cycle[29]. While variation in AFC during a menstrual cycle may be as much as 30%, this difference does not affect the choice of stimulation protocol for *in-vitro* fertilization (IVF)[28,30]. Furthermore, allowing follicles to be counted at any point in the menstrual cycle not only reduces patient inconvenience due to the discomfort of an examination during the menstrual period, but also removes a logistical burden for IVF centers[30,31]. The recommendation of this Consensus is that AFC may be performed at any point in the menstrual cycle. However, it should be borne in mind that counting follicles in the early follicular phase is frequently easier, as this reduces the likelihood of the presence of an ovarian cyst or a corpus luteum, which might mask some antral follicles[1,32].

With respect to the effect on follicle count of hormone use, there are several points to consider. First, follicle count assessed by US might be reduced in women using hormones that interfere with the menstrual cycle (e.g. hormonal contraceptives or gonadotropin-releasing hormone agonists)[33], but the degree of reduction will differ considerably depending on the type of hormone and on the individual's susceptibility. Second, the follicle count in women using hormones, particularly when the count is normal or high, might still be useful in clinical practice. Third, asking a woman to stop using contraceptives purely in order to increase the accuracy of an AFC will make the test less likely to be accepted and might expose her to the risk of an undesired pregnancy. We recommend that follicle count is performed preferentially during the natural menstrual cycle; however, follicles can still be counted in women using hormonal contraceptives. In this case, women should be advised before the scan that hormone use might reduce the number of follicles identified by US and that a small count under such circumstances should be confirmed by another scan performed after 2–3 months without hormone use before any clinical decision can be made.

Additionally, observers and clinicians should be careful when counting and when interpreting the ovarian follicle count in the presence of cysts, particularly endometriomas, and when the woman has had pelvic surgery[1]. Endometriomas, local inflammation, fibrosis and adhesions are likely to increase the distance between the ovarian tissue and the US probe, resulting in attenuation and impaired visualization of the follicles, particularly small ones[12,32,34].

The total number of follicles seen in both ovaries, the 'total AFC', is frequently used in assisted reproduction centers[1,35]; however, the number of follicles in the ovary with more follicles, the 'follicle number per ovary' (FNPO), is often more useful in gynecological clinical practice[8].

Why count ovarian follicles?

AFC is accepted as being one of the best markers for assessing the functional ovarian reserve[3,4], for predicting response to gonadotropin stimulation[36–39] and for assessing the chance of pregnancy following IVF[29]. AFC can predict both poor ovarian response and exaggerated response[16,38], and is therefore useful to individualize optimal gonadotropin dosage[5,29,38,40,41]. AFC $< 5–7$ is associated with small number of oocytes retrieved[42,37] and reduced pregnancy rate[43]. A total AFC ≥ 20 is related to exaggerated ovarian response and a higher risk of ovarian hyperstimulation syndrome[12,39,44–46].

Indications for counting ovarian follicles include age > 35 years in a woman attempting pregnancy for more than 6 months[10] and risk for diminished ovarian reserve i.e. history of cancer treated with gonadotoxic drugs and/or irradiation[10,47,48], or ovarian surgery for endometriomas[10,49,50]. AFC might be useful to improve the prediction of risk of fetal aneuploidy[51,52]. Furthermore, the number of antral follicles might help to predict age at menopause, AFC ≤ 4 being related to an increased risk of menopause within 7 years (35%) when compared to women with AFC > 4 (13%)[6,7].

AFC helps to predict whether there is an increased risk of retrieving a small number of oocytes following ovarian stimulation, which results in reduced rate of pregnancy following IVF[10,13,29,53–55] and lower cumulative pregnancy rate[56]. It is important to note that having a poor ovarian reserve should not be interpreted as being the same as having a reduced chance to conceive spontaneously[10,13,29,53].

FNPO has been used for more than a decade as a criterion to diagnose ovulatory dysfunction secondary to hyperandrogenism, or 'polycystic' pattern[5,9]. However, the suggested FNPO cut-off of ≥ 12 follicles, published in 2004[9], is no longer considered valid. Improvements in imaging technologies allow visualization of a much higher number of follicles: the median FNPO in women of reproductive age reported by recent studies is between 13 and 16[8]. Therefore, using FNPO ≥ 12 as a cut-off will result in suspicion of hyperandrogenism in more than half of young women. Based on more recent results, a reasonable cut-off for suspecting hyperandrogenism would be FNPO ≥ 25[8,45].

Minimum ultrasound training and machine requirements

Antral follicles are seen as round or oval sonolucent structures[1,57]. However, they might not be identified equally easily in all women. Practical difficulties include determination of whether a sonolucent structure is a follicle and, if so, whether it is a single follicle or two adjacent ones[58].

Although follicle count is often evaluated by two-dimensional (2D)-US, three-dimensional (3D)-US might also be used[59]. 3D-US imaging has the advantage of a shorter examination time, as it enables storage of acquired data for offline analysis[57,60], and better interobserver reliability[59,60].

In order to maximize reliability, the follicle count should be performed only by a competent observer; however, there is no specific training or certification[1].

Furthermore, there is no consensus regarding how many scans a person should perform before being considered technically competent. A study on learning curves in follicle monitoring during controlled ovarian stimulation[61] suggests that 20–40 exams are required to perform this analysis well; we believe this should also be valid for counting antral follicles.

Although the impact on AFC reproducibility of the quality of particular US equipment is poorly defined[1], it is clear that appropriate US equipment and technique are necessary to achieve an accurate AFC. Scans should be transvaginal, and with a minimum frequency of 7 MHz[58]. The transabdominal route should be employed when it is the only way to assess the ovaries by US[8], for example when the ovary is located cranially and anteriorly in the pelvis/abdomen, or when a transvaginal approach is not recommended (e.g. virgin women) or causes pain (vaginism). For the latter two situations, transrectal scanning might be considered, as it offers an image quality comparable to that of transvaginal US and superior to that of transabdominal US for imaging female pelvic structures[62–64].

Ultrasound techniques for counting ovarian antral follicles

Both 2D- and 3D-US may be employed to perform AFC (Figures 1 and 2). Ovarian follicles are frequently counted on 2D-US using either real-time or stored cine-loops. When using 3D-US, the most common technique is to count the follicles manually in the multiplanar mode; however, the count might also be performed using rendered modes, particularly inversion mode or semi-automatically, using sonography-based automated volume calculation (SonoAVC™). The main characteristics of the different US techniques for counting ovarian follicles are summarized in Table 1.

Real-time 2D ultrasound

When performing AFC with real-time 2D-US, the woman should be positioned in the lithotomy position, with an empty bladder. The operator should scan the ovary in both longitudinal and coronal planes, to identify which offers the better image. After this decision is made, the ovary is centered on the screen and the US machine is adjusted to optimize image quality, trying to maximize the contrast between the follicular fluid and the ovarian stroma (including adjustments of gain, depth, magnification and, most importantly, the use of harmonics; see below). The ovary should occupy at least 50% of the screen along its largest axis. All follicular structures 2–10 mm in diameter identified when scanning from one ovarian margin to the other should be included in the count (Figures 1a and 2a). When it is doubtful whether a follicle lies within the 2–10-mm range, follicular size is measured using the internal diameter of the sonolucent area. For round follicles, only one measurement is required; for oval follicles, the mean of two diameters is calculated (greatest diameter and greatest diameter perpendicular to it)[1]. The number of follicles measuring < 2 mm or > 10 mm is subtracted from the total number of identifiable follicles. If the observer is not sure about the count, the process should be repeated in the other scanning plane. The count is then performed on the other ovary and reported separately.

Harmonic imaging may improve image quality by reducing artifacts[25] (Figures 1b and 2b). The minimal noise and clutter when harmonics are used reduces artifacts in liquid cavities, which appear as clean and dark (anechoic) regions[65], and with better contrast resolution compared with standard US imaging[66]. However, harmonic imaging suffers more from attenuation and might not be useful when evaluating ovaries that are not close to the US probe.

Real-time 2D-US imaging is generally adequate for counting ovarian follicles in clinical practice[1]. However, although usually quick, it requires the presence of the woman during the entire examination since the follicular dimensions are obtained one at a time. Confusion may arise during the examination regarding whether a particular sonolucent structure is a single follicle or two follicles side by side, or the same follicle may be counted twice[58]. Hence, 2D-US is highly dependent upon the observer[59]. On the other hand, real time is better than offline evaluation for determining whether an adjacent anechoic structure is a follicle, a paraovarian cyst or a hydrosalpinx, as pressure can be applied to check whether the structure slides in relation to the ovary[25]. Additionally, it allows the use of color/power Doppler, which might help in differentiating follicles from vascular structures.

Cine-loop 2D ultrasound

Cine-loop or clip is a tool used in 2D-US evaluation. Images can be analyzed in the presence of the patient, allowing reacquisition when the images are not satisfactory, or they can be stored for later evaluation. The same procedure is followed as for 2D real-time AFC, except that a single continuous sweep of each ovary is performed and the loops are saved, thus shortening the duration of the US exam[25]. It also has the advantage that, in case of doubt, clips can be replayed. However, the quality of the acquired clips is operator-dependent, with some of the same limitations as 2D conventional mode, and analysis of the clip without the patient present precludes performing any additional maneuvers to resolve any doubts.

3D manual mode

Using 3D manual mode or multiplanar view, AFC can be performed while visualizing three perpendicular planes simultaneously[35,57,59] (Figures 1c and 2c). The images of the ovaries are acquired automatically, and the resulting volumes are stored and analyzed subsequently by using the three orthogonal planes simultaneously[35,57]. The three planes are linked, so that the movement of one plane shifts the other two planes accordingly, with a central point being the intercept for all three planes[35,67]. Multiplanar

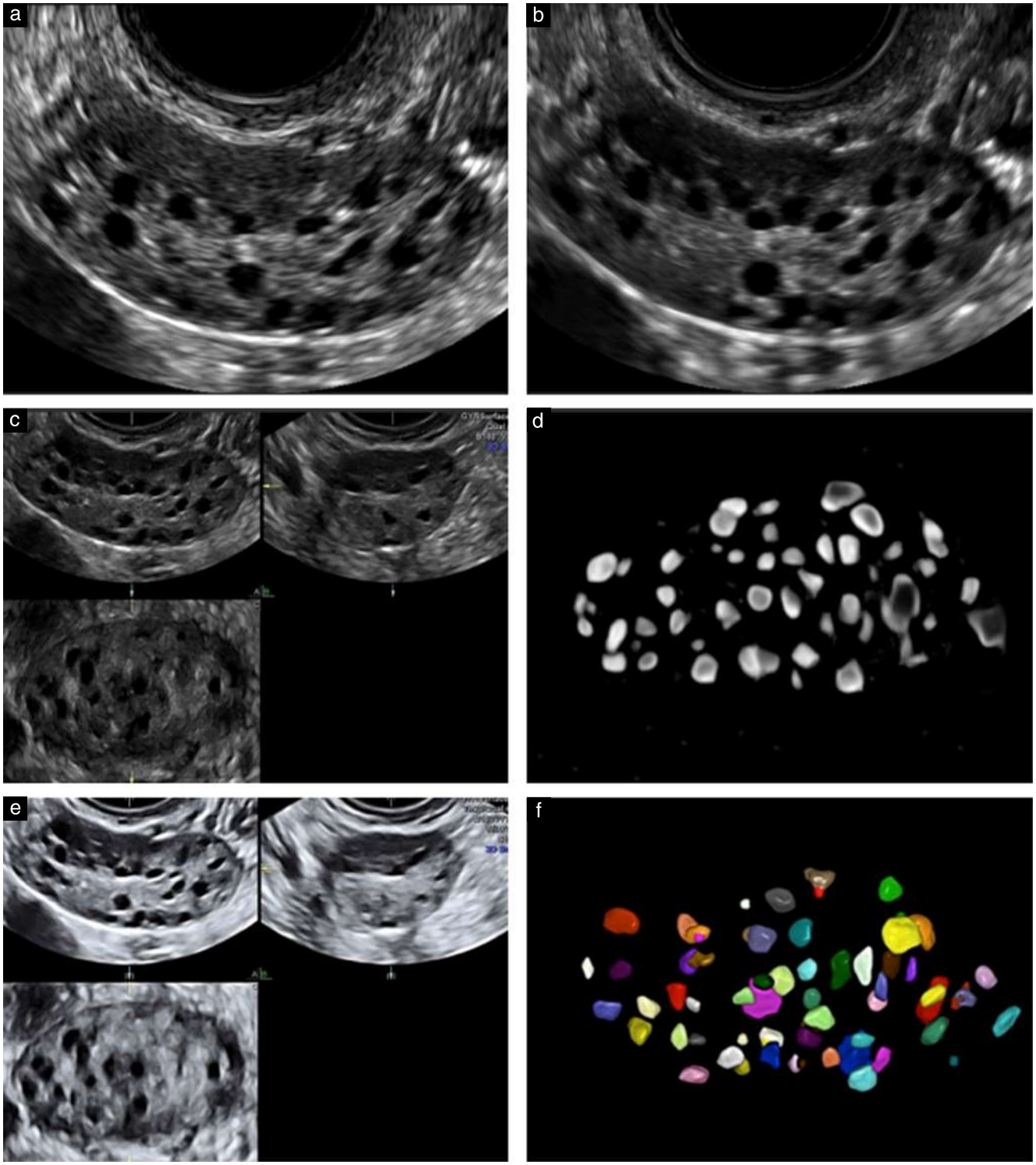

Figure 1 Ultrasound imaging of ovary with several follicles: (a) two-dimensional (2D) ultrasound (US) without harmonics; (b) 2D-US with harmonics; (c) multiplanar view without volume contrast imaging (VCI); (d) three-dimensional inversion mode; (e) multiplanar view with VCI; (f) sonography-based automated volume calculation (SonoAVC).

mode allows follicles to be cross-checked in different planes, so this method might provide better reliability compared with 2D methods [35,57].

Each ovary should be scanned in two planes to determine which provides the better image quality for the entire tissue (i.e. which suffers less from interposition of bowels); if both planes provide equal image quality, the one in which the ovary appears more elongated should be chosen, as this will facilitate acquisition of the entire ovarian tissue during the sweep: the maximum sweep angle is 120° while the original view angle is almost 180°. A longitudinal section of the ovary is centered within the 3D sonographic sector on the screen. Using the chosen plane, the transducer is held at the center of the ovary. The

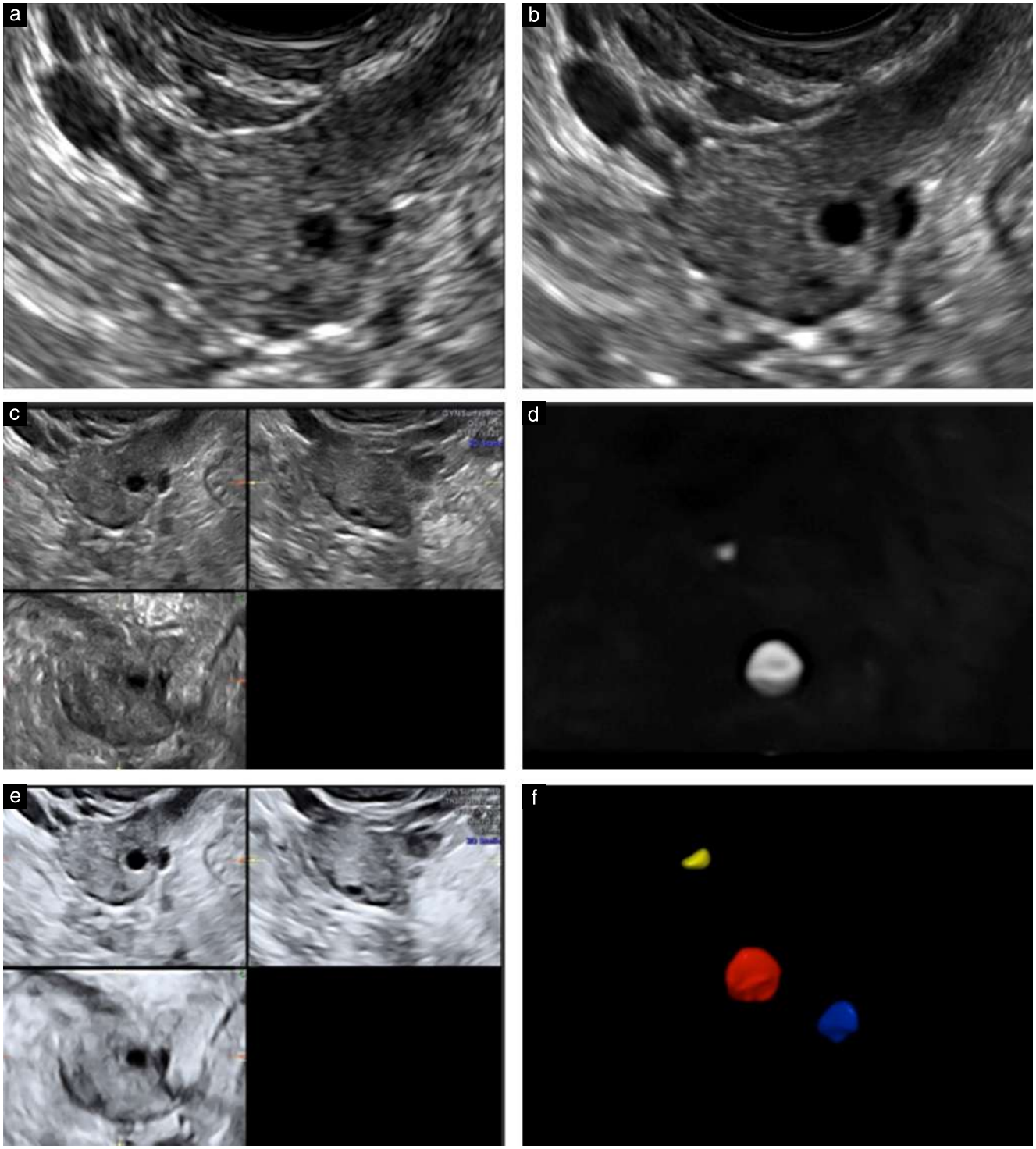

Figure 2 Ultrasound imaging of ovary with few follicles: (a) two-dimensional (2D) ultrasound (US) without harmonics; (b) 2D-US with harmonics; (c) multiplanar view without volume contrast imaging (VCI); (d) three-dimensional inversion mode; (e) multiplanar view with VCI; (f) sonography-based automated volume calculation (SonoAVC).

maximum image quality and maximum acquisition angle (normally 120°) are selected, the latter to ensure that the whole ovarian tissue is included during acquisition. Since the original maximum view angle on B-mode is larger than the acquisition angle (176° *vs* 120°), if the entire ovary is not included in the first acquisition, the observer should try to elongate it as far as possible in the B-mode, leaving the shorter diameter to be included in the 120° sweep. After the 3D dataset is acquired and stored, the follicles can be counted offline, either on the US machine or on a personal computer[25]. Although some training is needed, acquisition of 3D datasets of the ovaries is quick, reducing scanning time. If the observer believes that vessels might be confused with follicles, power Doppler may be used; however, this will make the 3D volume acquisition somewhat slower and its potential benefit has not been addressed.

Table 1 Ultrasound techniques for counting ovarian follicles

Technique	*Characteristics*
Real-time 2D-US	- Standard use in routine clinical practice - Relatively quick - Allows assessment of whether structures 'slide' on application of pressure, and therefore discernment of intra- to paraovarian structures - Allows application of Doppler ultrasound; useful in case of doubt as to whether structure is follicle or vessel - Relatively long scan time - Does not allow offline analysis, which would be useful for training, to resolve uncertainties and for audit purposes
Cine-loop 2D-US	- Same characteristics as real-time 2D-US - Reduces scan time - Allows offline analysis
3D manual mode	- Better reproducibility compared with 2D methods - Requires specifically trained sonologists and particular ultrasound systems (less widely available and more expensive than standard machines) - Allows use of rendering mode with thin slice, which enhances contrast, making follicles easier to identify - Inversion mode allows complete view of all follicles within volume, but requires specifically trained sonologists and particular ultrasound systems (less widely available and more expensive than standard machines)
SonoAVC	- Semi-automatic method - Counts and measures all follicles - Frequently requires correction of problems by manual post-processing: e.g. to include follicles that have not been identified, to exclude extraovarian structures or tissue incorrectly identified as follicles, or to separate follicles incorrectly identified as being only one - Requires specifically trained sonologists and particular ultrasound systems (less widely available and more expensive than standard machines)

2D, two dimensional; 3D, three dimensional; SonoAVC, sonography-based automated volume calculation; US, ultrasound.

Table 2 Suggestion of how to interpret follicle count in general gynecological practice and before ovarian stimulation in fertility centers

Nomenclature	*FNPO*	*Interpretation in clinical practice*
Oligofollicular or low follicle count	1–3	Low ovarian reserve and increased risk of menopause in next 7 years*
Normofollicular or normal follicular count	4–24	Normal follicle count for women of reproductive age
Multifollicular or high follicle count	≥ 25	High risk of hyperandrogenism
Nomenclature	*Total AFC*	*Interpretation for ovarian stimulation*
Very low functional ovarian reserve or very small number of recruitable follicles	0–4	Very high risk of poor response to ovarian stimulation and reduced chance of pregnancy
Low functional ovarian reserve or small number of recruitable follicles	5–8	High risk of poor response to ovarian stimulation
Normal functional ovarian reserve or normal number of recruitable follicles	9–19	Expected normal response to ovarian stimulation
High functional ovarian reserve or large number of recruitable follicles	≥ 20	High risk of excessive ovarian response and OHSS

*35% *vs* 13%[7]. AFC, antral follicle count (number of follicles in both ovaries); FNPO, follicle number per ovary (number of follicles in ovary with more follicles); OHSS, ovarian hyperstimulation syndrome. Adapted from Martins *et al.*[5].

Volume contrast imaging (VCI) is a 3D-US tool that permits use of a rendered mode that simulates the multiplanar view, in order to improve contrast enhancement and speckle suppression, providing better definition between tissues and/or organs[68] and improving the contrast between fluid and surrounding structures[69] (Figures 1e and 2e). While the basis of the tools is completely different, VCI seems to improve image quality on 3D-US in a way similar to that achieved by harmonics on 2D-US (Figure 1b and 2b)[25].

Inversion mode, a rendering mode, inverts the echogenicity, turning the darkest points into the brightest ones and vice versa. Combining this with any surface-rendered mode (which eliminates the darker voxels from the region of interest) it is possible to visualize the 3D arrangement of follicles[70]. This method offers a different way to count follicles, since all of them can be seen at the same time[60] (Figures 1d and 2d). Any surrounding hypoechogenic tissue in the original image will make it difficult to identify the follicles, as this will not be eliminated in rendered mode; in such cases, Virtual Organ Computer-Aided anaLysis (VOCAL™) can be used, in order to exclude from the region of interest all tissue surrounding the ovary, facilitating the identification of the ovarian follicles. One study[60] found that AFC performed using inversion mode was better correlated with the

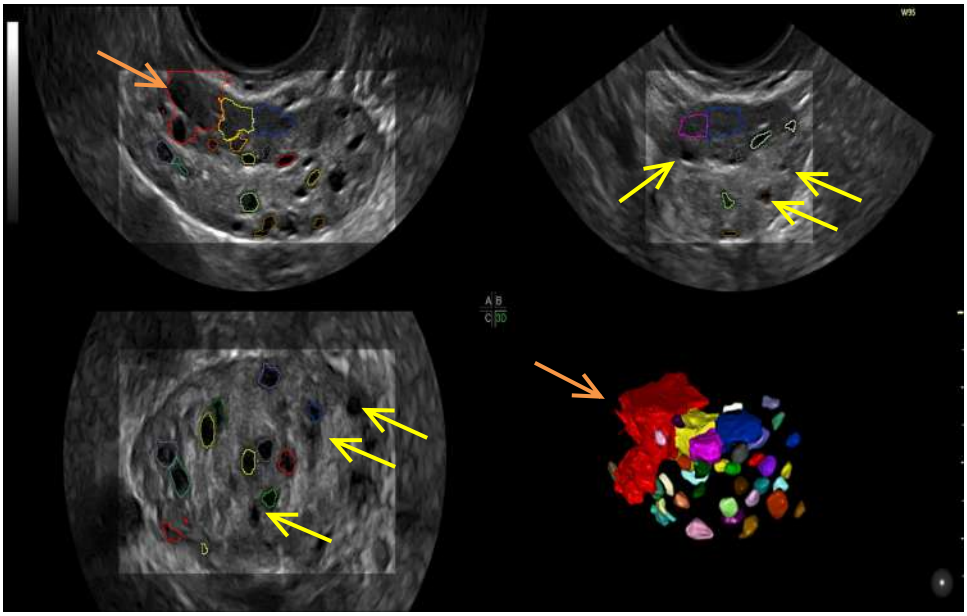

Figure 3 Illustration of problems encountered when measuring follicles using sonography-based automated volume calculation (SonoAVC) technique: orange arrows show coalescence of follicles and extraovarian tissue that were identified incorrectly as being only one follicle; yellow arrows show follicles not included in count.

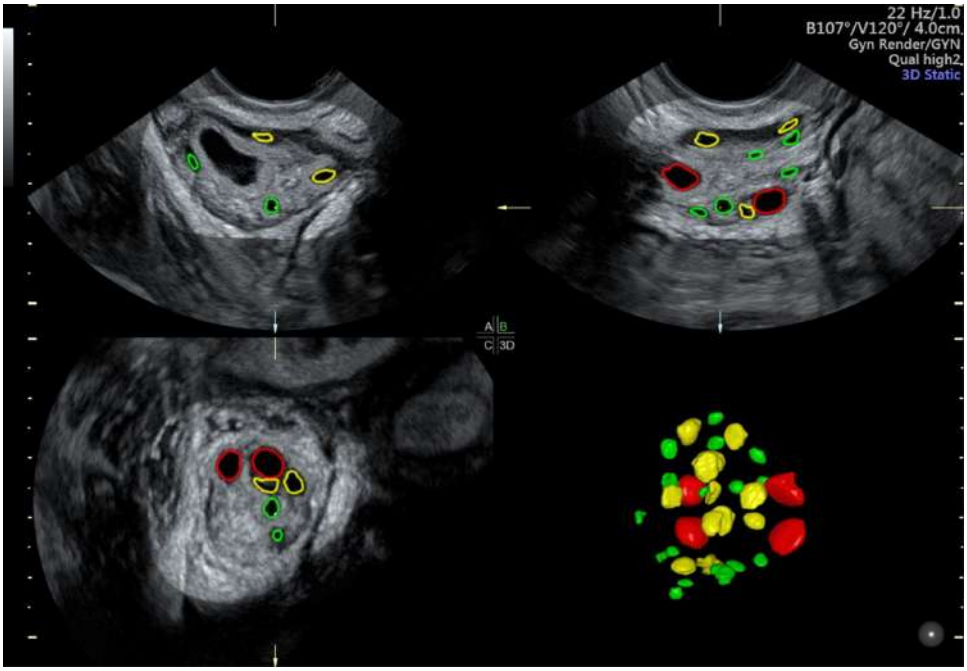

Figure 4 Using 'sonography-based automated volume calculation (SonoAVC) antral', follicles of similar size are shown with the same color and are counted automatically.

number of oocytes retrieved following ovarian stimulation than was AFC using 2D-US or multiplanar view. However, the use of inversion mode is much more time consuming and requires specifically trained observers[60,70].

SonoAVC

SonoAVC allows counting and measuring of diameters and volumes of anechoic structures within a region of interest of an acquired 3D dataset[35]. The algorithm used to calculate volume is based on the sum of all of the volume elements within a given hypoechoic region[60], and it is thought to reduce the interobserver variability of follicle count[35,59].

To perform AFC using SonoAVC, the region of interest should be adjusted in order to exclude as much extraovarian tissue as possible, while ensuring that the whole ovary is included[35]. The automated analysis takes around 6 s, and the individual follicles are then displayed in a 3D colored rendered mode, along with the

number of follicles and their dimensions (Figures 1f and 2f)[60]. However, the automatic evaluation is frequently imperfect (Figure 3), although there are various options to correct such problems manually[60,71]. These include clicking on a follicle that has not been identified as well as clicking on non-follicular structures that have been identified erroneously. It is also sometimes necessary to separate different follicles that have been identified incorrectly as a single follicle, or to merge areas of the same follicle that were identified incorrectly as being different follicles (Figure 3). Additionally, there are two functions that can assist with automatic identification of follicles: 'separation' and 'growth'. Increasing the separation will reduce the problem of merging of different follicles, while increasing the growth function will reduce that of follicular fluid not being included fully.

SonoAVC can reduce the time needed to count the follicles compared with manual counting, particularly when there are several follicles[72]. Other advantages are that it allows use of the volume-based diameter rather than an average of two or three diameters, thereby reducing the variability of measurements in non-spherical follicles, and it avoids double-counting of the same follicle[12]. The main limitation of SonoAVC is that the initial evaluation is frequently not perfect and additional training is necessary to be able to deal with potential problems[25,60,73], and manual correction is time-consuming[35]. Additionally, if the observer is unaware of this common problem, the reported number of follicles might be very different from the actual one. Recently, GE Medical Systems released 'SonoAVC antral', which counts the number of follicles which lie within prespecified size ranges (e.g. 2–5 mm, 6–7 mm and 8–10 mm), coloring all follicles within a particular size range the same color (Figure 4). Compared with the traditional SonoAVC technology ('SonoAVC follicle'), 'SonoAVC antral' facilitates the visualization and interpretation of an unstimulated ovary and also the counting of follicles of specific size.

Suggestion for a standardized report

- Identify day of cycle and whether woman is using hormones (particularly oral contraceptive pills).
- Report number of follicles with diameter between 2 and 10 mm in each ovary and also sum of follicles considering both ovaries.
- Record which technique was used for evaluation (real-time 2D-US, assessment of 2D-US cine-loops, 3D-US, SonoAVC follicle) and specify maximum frequency of probe.
- Note presence of dominant follicle, cyst or tumor.
- Comment on accessibility of each ovary for transvaginal egg collection (e.g. some ovaries are fixed to posterior serosa of uterus or situated more cranially than usual, resulting in more difficult/risky transvaginal puncture).

Our proposal for interpretation of ovarian follicle count in gynecological practice and fertility centers is presented in Table 2[5]. In the examination report, a comment on how to interpret the AFC can be added if necessary, explaining that ovarian reserve tests do not predict failure to conceive naturally[10,55].

Recommendations for future research and final considerations

There is no consensus on the best US technique with which to perform follicle count. Future research should include reproducibility studies that consider the effect of the acquisition of 3D datasets stored for later evaluation and interpretation. In many of the available studies[35,57,59,71,74–78], acquisition of the dataset was performed only once, with the data then being analyzed by more than one observer to evaluate the interobserver reliability of reading the same acquired dataset. Since there are sources of variability intrinsic to the acquisition, including pressure, interposition of bowel and machine settings used to maximize the contrast, new studies should assess different datasets, acquired ideally a few minutes apart by different observers.

This Consensus has several limitations that should be considered. Due to the scarcity of studies, most of the recommendations are based on expert opinion rather than being evidence-based. There are several ways to count ovarian follicles, and, although reduction of observer-dependence is desirable, we believe that the process will always depend on the operator's skill. Proper adjustment of the machine settings and pattern recognition are also crucial for an accurate count. Additionally, one might argue that Delphi methods or Delphi-like methods are considered more reliable, but the results depend also on the participants and would suffer from the absence of robust evidence to guide choices[79].

All methods currently available have pros and cons and are affected by the operator's preference and skill. Despite the appeal of semi-automatic follicle counting, this process still needs improvement and should be tested properly to evaluate whether it saves time, reduces interobserver variability and provides a more accurate count. Additionally, there is a need for studies assessing how many examinations are necessary before competence is achieved in each technique. Because of the limitations of the semi-automated technique, particularly the risk of the unwary observer reporting a totally wrong number of follicles, we suggest that follicles be counted manually in clinical practice, using any of the following techniques: real-time 2D-US, pre-acquired 2D-US cine-loops or 3D-US datasets.

REFERENCES

1. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. *Fertil Steril* 2010; **94**: 1044–1051.
2. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. *Hum Reprod Update* 2014; **20**: 124–140.
3. Haadsma ML, Bukman A, Groen H, Roeloffzen EM, Groenewoud ER, Heineman MJ, Hoek A. The number of small antral follicles (2–6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. *Hum Reprod* 2007; **22**: 1925–1931.

4. Amanvermez R, Tosun M. An Update on ovarian aging and ovarian reserve tests. *Int J Fertil Steril* 2016; **9**: 411–415.
5. Martins WP, Kollmann M, Raine-Fenning N. Counting ovarian follicles: updated threshold for diagnosis of hyperandrogenic anovulation. *Ultrasound Obstet Gynecol* 2014; **44**: 131–134.
6. Depmann M, Broer SL, van der Schouw YT, Tehrani FR, Eijkemans MJ, Mol BW, Broekmans FJ. Can we predict age at natural menopause using ovarian reserve tests or mother's age at menopause? A systematic literature review. *Menopause* 2016; **23**: 224–232.
7. Wellons MF, Bates GW, Schreiner PJ, Siscovick DS, Sternfeld B, Lewis CE. Antral follicle count predicts natural menopause in a population-based sample: the Coronary Artery Risk Development in Young Adults Women's Study. *Menopause* 2013; **20**: 825–830.
8. Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, Escobar Morreale HF. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update* 2014; **20**: 334–352.
9. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; **19**: 41–47.
10. ACOG. Committee opinion no. 618: ovarian reserve testing. *Obstet Gynecol* 2015; **125**: 268–273.
11. Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril* 1999; **72**: 845–851.
12. Lima ML, Martins WP, Coelho Neto MA, Nastri CO, Ferriani RA, Navarro PA. The assessment of ovarian reserve by antral follicle count in ovaries with endometrioma. *Ultrasound Obstet Gynecol* 2015; **46**: 239–242.
13. ASRM. Testing and interpreting measures of ovarian reserve: a committee opinion. *Fertil Steril* 2015; **103**: e9–e17.
14. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: antral follicle count versus anti-Mullerian hormone. *Reprod Biomed Online* 2015; **31**: 486–496.
15. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Mullerian hormone and antral follicle count as biomarkers of ovarian response. *Hum Reprod Update* 2015; **21**: 698–710.
16. Farquhar C, Marjoribanks J, Brown J, Fauser B, Lethaby A, Mourad S, Rebar R, Showell M, van der Poel S. Management of ovarian stimulation for IVF: narrative review of evidence provided for World Health Organization guidance. *Reprod Biomed Online* 2017; **35**: 3–16.
17. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update* 2014; **20**: 370–385.
18. Glaser EM. Using behavioral science strategies for defining the state-of-the-art. *J Appl Behav Sci* 1980; **16**: 79–92.
19. Fink A, Kosecoff J, Chassin M, Brook RH. Consensus methods: characteristics and guidelines for use. *Am J Public Health* 1984; **74**: 979–983.
20. Craig J, Orisaka M, Wang H, Orisaka S, Thompson W, Zhu C, Kotsuji F, Tsang BK. Gonadotropin and intra-ovarian signals regulating follicle development and atresia: the delicate balance between life and death. *Front Biosci* 2007; **12**: 3628–3639.
21. Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. *Biol Reprod* 1994; **50**: 653–663.
22. Charleston JS, Hansen KR, Thyer AC, Charleston LB, Gougeon A, Siebert JR, Soules MR, Klein NA. Estimating human ovarian non-growing follicle number: the application of modern stereology techniques to an old problem. *Hum Reprod* 2007; **22**: 2103–2110.
23. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Hum Reprod Update* 2012; **18**: 73–91.
24. Field SL, Dasgupta T, Cummings M, Orsi NM. Cytokines in ovarian folliculogenesis, oocyte maturation and luteinisation. *Mol Reprod Dev* 2014; **81**: 284–314.
25. Martins WP, Jokubkiene L. Assessment of the functional ovarian reserve. In *Managing Ultrasonography in Human Reproduction* (Guerriero S, Alcazar JL, Martins WP, eds). Springer, 2016; 3–12.
26. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 1996; **17**: 121–155.
27. Deb S, Campbell BK, Clewes JS, Pincott-Allen C, Raine-Fenning NJ. Intracycle variation in number of antral follicles stratified by size and in endocrine markers of ovarian reserve in women with normal ovulatory menstrual cycles. *Ultrasound Obstet Gynecol* 2013; **41**: 216–222.
28. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010; **25**: 221–227.
29. Jayaprakasan K, Chan Y, Islam R, Haoula Z, Hopkisson J, Coomarasamy A, Raine-Fenning N. Prediction of in vitro fertilization outcome at different antral follicle count thresholds in a prospective cohort of 1,012 women. *Fertil Steril* 2012; **98**: 657–663.
30. Mavrelos D, Al Chami A, Talaulikar V, Burt E, Webber L, Ploubidis G, Yasmin E. Variation in antral follicle counts at different times in the menstrual cycle: does it matter? *Reprod Biomed Online* 2016; **33**: 174–179.
31. Rombauts L, Onwude JL, Chew HW, Vollenhoven BJ. The predictive value of antral follicle count remains unchanged across the menstrual cycle. *Fertil Steril* 2011; **96**: 1514–1518.
32. Lima ML, Nastri CO, Coelho Neto MA, Ferriani RA, Navarro PA, Martins WP. Antral follicle count might be underestimated in the presence of an ovarian endometrioma. *Hum Reprod* 2015; **30**: 250.
33. Bentzen JG, Forman JL, Pinborg A, Lidegaard O, Larsen EC, Friis-Hansen L, Johannsen TH, Nyboe Andersen A. Ovarian reserve parameters: a comparison between users and non-users of hormonal contraception. *Reprod Biomed Online* 2012; **25**: 612–619.
34. Kitajima M, Dolmans MM, Donnez O, Masuzaki H, Soares M, Donnez J. Enhanced follicular recruitment and atresia in cortex derived from ovaries with endometriomas. *Fertil Steril* 2014; **101**: 1031–1037.
35. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. *Ultrasound Obstet Gynecol* 2009; **33**: 477–483.
36. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil Steril* 2002; **77**: 328–336.
37. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril* 2005; **83**: 291–301.
38. Ng EH, Tang OS, Ho PC. The significance of the number of antral follicles prior to stimulation in predicting ovarian responses in an IVF programme. *Hum Reprod* 2000; **15**: 1937–1942.
39. Polyzos NP, Tournaye H, Guzman L, Camus M, Nelson SM. Predictors of ovarian response in women treated with corifollitropin alfa for in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2013; **100**: 430–437.
40. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. *Hum Reprod Update* 2011; **17**: 46–54.
41. Nastri CO, Teixeira DM, Moroni RM, Leitao VM, Martins WP. Ovarian hyperstimulation syndrome: pathophysiology, staging, prediction and prevention. *Ultrasound Obstet Gynecol* 2015; **45**: 377–393.
42. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 2011; **26**: 1616–1624.
43. Coelho Neto MA, Martins WP, Lima ML, Barbosa MA, Nastri CO, Ferriani RA, Navarro PA. Ovarian response is a better predictor of clinical pregnancy rate following embryo transfer than is thin endometrium or presence of an endometrioma. *Ultrasound Obstet Gynecol* 2015; **46**: 501–505.
44. Jayaprakasan K, Jayaprakasan R, Al-Hasie HA, Clewes JS, Campbell BK, Johnson IR, Raine-Fenning NJ. Can quantitative three-dimensional power Doppler angiography be used to predict ovarian hyperstimulation syndrome? *Ultrasound Obstet Gynecol* 2009; **33**: 583–591.
45. Kollmann M, Martins WP, Raine-Fenning N. Examining the ovaries by ultrasound for diagnosing hyperandrogenic anovulation: updating the threshold for newer machines. *Fertil Steril* 2014; **101**: e25.
46. Leitao VM, Moroni RM, Seko LM, Nastri CO, Martins WP. Cabergoline for the prevention of ovarian hyperstimulation syndrome: systematic review and meta-analysis of randomized controlled trials. *Fertil Steril* 2014; **101**: 664–675.
47. Gracia CR, Sammel MD, Freeman E, Prewitt M, Carlson C, Ray A, Vance A, Ginsberg JP. Impact of cancer therapies on ovarian reserve. *Fertil Steril* 2012; **97**: 134–140.e1.
48. Hill MJ, Cooper JC, Levy G, Alford C, Richter KS, Decherney AH, Katz CL, Levens ED, Wolff EF. Ovarian reserve and subsequent assisted reproduction outcomes after methotrexate therapy for ectopic pregnancy or pregnancy of unknown location. *Fertil Steril* 2014; **101**: 413–419.
49. Coccia ME, Rizzello F, Mariani G, Bulletti C, Palagiano A, Scarselli G. Ovarian surgery for bilateral endometriomas influences age at menopause. *Hum Reprod* 2011; **26**: 3000–3007.
50. Urman B, Alper E, Yakin K, Oktem O, Aksoy S, Alatas C, Mercan R, Ata B. Removal of unilateral endometriomas is associated with immediate and sustained reduction in ovarian reserve. *Reprod Biomed Online* 2013; **27**: 212–216.
51. Grande M, Borobio V, Bennasar M, Stergiotou I, Mercade I, Masoller N, Penarrubia J, Borrell A. Role of ovarian reserve markers, antimullerian hormone and antral follicle count, as aneuploidy markers in ongoing pregnancies and miscarriages. *Fertil Steril* 2015; **103**: 1221–1227.e2.
52. Grande M, Borobio V, Jimenez JM, Bennasar M, Stergiotou I, Penarrubia J, Borrell A. Antral follicle count as a marker of ovarian biological age to reflect the background risk of fetal aneuploidy. *Hum Reprod* 2014; **29**: 1337–1343.
53. Hvidman HW, Bentzen JG, Thuesen LL, Lauritsen MP, Forman JL, Loft A, Pinborg A, Nyboe Andersen A. Infertile women below the age of 40 have similar anti-Mullerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. *Hum Reprod* 2016; **31**: 1034–1045.
54. Nelson SM. Biomarkers of ovarian response: current and future applications. *Fertil Steril* 2013; **99**: 963–969.
55. Tremellen K, Savulescu J. Ovarian reserve screening: a scientific and ethical analysis. *Hum Reprod* 2014; **29**: 2606–2614.
56. Drakopoulos P, Blockeel C, Stoop D, Camus M, de Vos M, Tournaye H, Polyzos NP. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? *Hum Reprod* 2016; **31**: 370–376.
57. Scheffer GJ, Broekmans FJ, Bancsi LF, Habbema JD, Looman CW, Te Velde ER. Quantitative transvaginal two- and three-dimensional sonography of the ovaries: reproducibility of antral follicle counts. *Ultrasound Obstet Gynecol* 2002; **20**: 270–275.
58. Jayaprakasan K, Campbell B, Hopkisson J, Johnson I, Raine-Fenning N. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. *Fertil Steril* 2010; **93**: 855–864.
59. Jayaprakasan K, Walker KF, Clewes JS, Johnson IR, Raine-Fenning NJ. The interobserver reliability of off-line antral follicle counts made from stored three-dimensional ultrasound data: a comparative study of different measurement techniques. *Ultrasound Obstet Gynecol* 2007; **29**: 335–341.

60. Jayaprakasan K, Hilwah N, Kendall NR, Hopkisson JF, Campbell BK, Johnson IR, Raine-Fenning NJ. Does 3D ultrasound offer any advantage in the pretreatment assessment of ovarian reserve and prediction of outcome after assisted reproduction treatment? *Hum Reprod* 2007; **22**: 1932–1941.
61. Rodriguez A, Guillen JJ, Lopez MJ, Vassena R, Coll O, Vernaeve V. Learning curves in 3-dimensional sonographic follicle monitoring during controlled ovarian stimulation. *J Ultrasound Med* 2014; **33**: 649–655.
62. Lee DE, Park SY, Lee SR, Jeong K, Chung HW. Diagnostic usefulness of transrectal ultrasound compared with transvaginal ultrasound assessment in young Korean women with polycystic ovary syndrome. *J Menopausal Med* 2015; **21**: 149–154.
63. Sun L, Fu Q. Three-dimensional transrectal ultrasonography in adolescent patients with polycystic ovarian syndrome. *Int J Gynaecol Obstet* 2007; **98**: 34–38.
64. Timor-Tritsch IE, Monteagudo A, Rebarber A, Goldstein SR, Tsymbal T. Transrectal scanning: an alternative when transvaginal scanning is not feasible. *Ultrasound Obstet Gynecol* 2003; **21**: 473–479.
65. Tranquart F, Grenier N, Eder V, Pourcelot L. Clinical use of ultrasound tissue harmonic imaging. *Ultrasound Med Biol* 1999; **25**: 889–894.
66. Desser TS, Jeffrey RB. Tissue harmonic imaging techniques: physical principles and clinical applications. *Semin Ultrasound CT MR* 2001; **22**: 1–10.
67. Raine-Fenning N, Jayaprakasan K, Clewes J, Joergner I, Bonaki SD, Chamberlain S, Devlin L, Priddle H, Johnson I. SonoAVC: a novel method of automatic volume calculation. *Ultrasound Obstet Gynecol* 2008; **31**: 691–696.
68. Jouannic JM, Rosenblatt J, Demaria F, Jacobs R, Aubry MC, Benifla JL. Contribution of three-dimensional volume contrast imaging to the sonographic assessment of the fetal uterus. *Ultrasound Obstet Gynecol* 2005; **26**: 567–570.
69. Ruano R, Benachi A, Aubry MC, Dumez Y, Dommergues M. Volume contrast imaging: A new approach to identify fetal thoracic structures. *J Ultrasound Med* 2004; **23**: 403–408.
70. Lee W, Goncalves LF, Espinoza J, Romero R. Inversion mode: a new volume analysis tool for 3-dimensional ultrasonography. *J Ultrasound Med* 2005; **24**: 201–207.
71. Jokubkiene L, Sladkevicius P, Valentin L. Number of antral follicles, ovarian volume, and vascular indices in asymptomatic women 20 to 39 years old as assessed by 3-dimensional sonography: a prospective cross-sectional study. *J Ultrasound Med* 2012; **31**: 1635–1649.
72. Ata B, Seyhan A, Reinblatt SL, Shalom-Paz E, Krishnamurthy S, Tan SL. Comparison of automated and manual follicle monitoring in an unrestricted population of 100 women undergoing controlled ovarian stimulation for IVF. *Hum Reprod* 2011; **26**: 127–133.
73. Rodriguez-Fuentes A, Hernandez J, Garcia-Guzman R, Chinea E, Iaconianni L, Palumbo A. Prospective evaluation of automated follicle monitoring in 58 in vitro fertilization cycles: follicular volume as a new indicator of oocyte maturity. *Fertil Steril* 2010; **93**: 616–620.
74. Lamazou F, Arbo E, Salama S, Grynberg M, Frydman R, Fanchin R. Reliability of automated volumetric measurement of multiple growing follicles in controlled ovarian hyperstimulation. *Fertil Steril* 2010; **94**: 2172–2176.
75. Leonhardt H, Gull B, Stener-Victorin E, Hellstrom M. Ovarian volume and antral follicle count assessed by MRI and transvaginal ultrasonography: a methodological study. *Acta Radiol* 2014; **55**: 248–256.
76. Merce LT, Gomez B, Engels V, Bau S, Bajo JM. Intraobserver and interobserver reproducibility of ovarian volume, antral follicle count, and vascularity indices obtained with transvaginal 3-dimensional ultrasonography, power Doppler angiography, and the virtual organ computer-aided analysis imaging program. *J Ultrasound Med* 2005; **24**: 1279–1287.
77. Salama S, Arbo E, Lamazou F, Levailllant JM, Frydman R, Fanchin R. Reproducibility and reliability of automated volumetric measurement of single preovulatory follicles using SonoAVC. *Fertil Steril* 2010; **93**: 2069–2073.
78. Servaes K, Van Schoubroeck D, Welkenhuysen M, D'Hooghe T, De Neubourg D. How reproducible are 2-dimensional ultrasonographic follicular diameter measurements from stored 3-dimensional files of ovarian scanning? *Gynecol Obstet Invest* 2014; **77**: 163–168.
79. Hutchings A, Raine R, Sanderson C, Black N. A comparison of formal consensus methods used for developing clinical guidelines. *J Health Serv Res Policy* 2006; **11**: 218–224.

