Original Article

Comparing Two- and Three-dimensional Antral Follicle Count in Patients with Endometriosis

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Abstract

Background: The purpose of the study was to compare three-dimensional (3D) ultrasound semiautomatic antral follicle count (AFC) with two-dimensional (2D) ultrasound real-time AFC to evaluate patients with deep endometriosis and/or endometrioma submitted to ovarian stimulation (OS). **Methods:** This was a retrospective cohort study assessing all women with documented diagnosis of deep endometriosis who underwent OS for assisted reproduction treatment. The primary outcome was the difference between AFC by semiautomatic 3D follicle count using 3D volume datasets and 2D ultrasound count with the number of oocytes retrieved at the end of the cycle. The 3D ultrasound AFC was obtained using sonography-based automated volume count (SonoAVC), and the 2D ultrasound AFC data was collected from the electronic medical record. **Results:** Total of 36 women had deep endometriosis documented by magnetic resonance imaging, laparoscopy, or ultrasonography and 3D ovarian volume datasets stored from their first exam. The differences between the 2D and 3D AFC and the number of oocytes retrieved at the end of the stimulation were compared, showing no significant statistical difference between both methods ($P = 0.59$). Correlations were similar using both methods when compared to the number of oocytes retrieved (2D [$r = 0.83$, confidence interval (CI) = 0.68–0.9, $P < 0.001$]); (3D [$r = 0.81$, CI = 0.46–0.83, $P < 0.001$]). **Conclusion:** 3D semiautomatic AFC can be used to access the ovarian reserve in patients with endometriosis.

Keywords: Antral follicle count, assisted reproduction, endometriosis, ovarian reserve, three-dimensional ultrasound

Introduction

Three-dimensional (3D) ultrasonography has been expanding its application in clinical practice, especially after the development of probes with automatic scanning to capture the image block and specific software for each type of exam.[1] The *SonoAVC* (*sonography-based automated volume count: GE Medical Systems, Zipf, Austria*) is a software that allows to identify and measure the average diameters and volumes of the follicles in a semi-automatic way and has been validated for the counting and monitoring of the follicles in assisted reproduction treatments.[2-4] Some authors point out that the count and volumetric measurements obtained by *SonoAVC* have a good correlation with the number of aspirated oocytes and with the volume of follicular fluid aspirated in oocyte retrieval (OR).[5-7] However, there are doubts regarding the accuracy of the new method in correctly identifying the follicles,[8] especially when the quality of the ultrasonography image is impaired by clinical conditions that promote image attenuation.[9]

Endometriosis is a very prevalent cause of infertility in human reproduction services.[10,11] In general, patients with such diagnosis have significant pelvic pain during transvaginal ultrasonography examination.[11] In addition, patients with deep endometriosis and endometriomas tend to present a challenging ultrasound evaluation, due to the frequent distortion of the pelvic anatomy and the presence of fibrotic

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processes that promote image attenuation,[12,13] as well as an underestimated count of antral follicles caused by the presence of endometriomas.[14] There is evidence that the poor image quality may have a greater impact on 3D ultrasound.[9] Because the especially short time needed to the image acquisition (on average 6 s) compared to two-dimensional (2D) ultrasound, the 3D ultrasound may reduce the patient's discomfort during the exam.[1] Despite the advantages over traditional 2D ultrasonography, *SonoAVC* antral follicle count (AFC) is not yet considered as a method of choice to count antral follicles.[8]

The AFC consists of counting all the follicles ranging from 2 to 10 mm of mean diameter by ultrasound.[15] It is the most commonly used marker to evaluate ovarian reserve because it is easy to perform and reliable. This number is important to establish the most appropriated protocol of treatment for each patient and helps to estimate the number of oocytes to be retrieved at the end of the cycle.[16-18]

The purpose of this study was to compare the use of *SonoAVC*, a more comfortable method that allows the later analysis of AFC, with the gold standard (in light of current knowledge) for the assessment of AFC in patients with deep endometriosis and endometriomas.

Materials and Methods

We conducted a retrospective study in a cohort of patients diagnosed with deep endometriosis and/or endometrioma who underwent ovarian stimulation (OS) for OR at a private reproductive medicine center in Rio de Janeiro-RJ, Brazil, between January 2016 and December 2019. The study was approved by the Local Ethics Committee (approval number: 89845818.0.0000.5243), without the need for additional consent from the participants.

Manual 2D AFC is the validated method in the literature and adopted as protocol in our service. In January 2016, we received a new ultrasound apparatus with 3D scan, allowing us to keep in our image database not only one plane of the ovary, but also the 3D ovary volume. The 3D provides the advantage to better document the ultrasound examination and it can be reviewed in a further moment. Because of that, the capture of the 3D dataset of the ovaries was recommended during the *in vitro* fertilization ultrasound scans in our routine. We observed, in the selection of patients diagnosed with endometriosis, despite of the recommendation, that some of them had only 2D images stored. These cases did not match the inclusion criteria.

We selected 63 patients who underwent OS for OR who had a diagnosis of deep endometriosis or endometrioma by Magnetic Resonance imaging (MRI), Ultrasonography or Videolaparoscopy (VDL) and whose 3D volumes of the ovaries from the first follow-up examination of the cycle were stored in the image files of the ultrasonography equipment (Voluson S6, General Electric, Healthcare, Zipf, Austria). Only one cycle for each patient was included. The stored 3D volumes were examined with the *SonoAVC* tool by a single professional with an extensive software experience, at least 2 months after the examination, having recorded the number of antral follicles (2–10 mm diameter in average) detected [Figure 1]. Subsequently, AFC obtained from 2D real-time evaluation was extracted from the medical record, as well as the number of oocytes retrieved at the end of the cycle.

The use of *SonoAVC* to count antral follicles is not yet recommended by the international guidelines and the manual 2D count continues to be recorded in the medical records as routine in our service. 3D datasets are kept in our image records without the use of *SonoAVC* software. To perform the AFC with *SonoAVC*, the first step is to collect 3D dataset displaying the three orthogonal planes of the ovary (multiplanar mode). Second, is necessary to adjust the selection boxes close to the ovary's margins in each plane. As a third step, we activate the automatic detection of the follicles and perform the postprocessing adjustments. Since 3D AFC is not displayed automatically as the 3D dataset is captured, we can be sure that the AFC recorded in medical records was not taken from 3D volumes stored. However, among the 3D datasets selected for the study, some of them that already have been rendered with *SonoAVC*. To avoid any bias and to be sure that we were comparing the two different methods, these cases were excluded. Patients with only superficial endometriosis were excluded because they usually have ultrasound examinations without any sign of the disease.

AFC is an indirect measure of the ovarian reserve used to predict the number of oocytes captured as the primary outcome of OS.[16-18] The differences between the number of OR and AFC of both methods were compared (OR-AFC). The slope coefficients of each method were calculated to determine its correlation with the number of OR and agreement between the methods and the number of OR was verified subsequently. Because the AFC methods were compared in the same cycle for the same patient, the possibility of bias was greatly reduced. The evaluation of the 3D volumes preceded the collection of data from the medical records, avoiding the influence of the number registered from the medical records in the evaluation with *SonoAVC* which is semi-automatic and requires

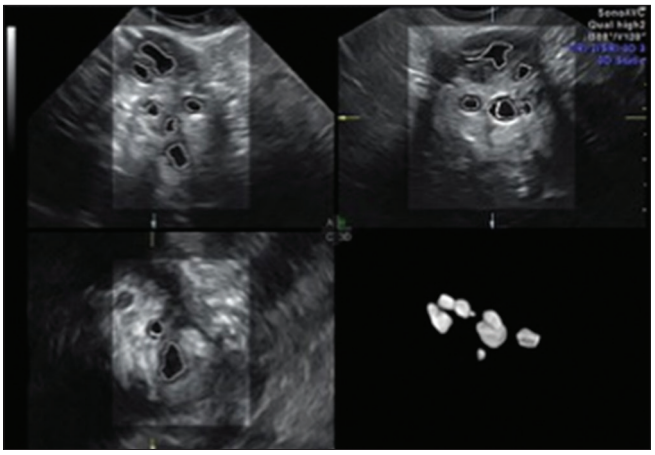

Figure 1: Sono AVC volume render of antral follicles count

postcapture adjustments. A significant time interval was taken between the examination and the volume analysis in order to minimize the chances of recall bias.

All patients who underwent OS for OR in the mentioned period who had an imaging diagnosis of deep endometriosis and/or endometrioma and who had their first image of the cycle stored in 3D volume image were considered. Statistical analysis was performed using the program R version 3.6.1, by one of the authors. The normal distribution of the numerical parameters was assessed by the Shapiro-Wilks test. The comparison between the OR-AFC with both methods was made using a *Student's t-test*. Pearson's correlation coefficients and slope coefficients of a linear regression without intercept were calculated. Bland–Altman plots were constructed to verify agreement. $P < 0.05$ was considered statistically significant.

Results

During the study period, a total of 63 patients who underwent OS to retrieve oocytes had endometriosis as probable cause of infertility, all of them with imaging diagnosis documenting the disease and its phenotype and 3D datasets from both ovaries. At the end, a total of 36 patients were included in the study analysis [Figure 2].

The mean age of the patients was 35.5 ± 3.2 years and the mean body mass index (BMI) was 23.4 ± 4.4 kg/m^2. Only 25 patients had Anti-Müllerian Hormone (AMH) registered and the levels ranged from 0.11 to 6.54, mean (± standard deviation) ± 1.88, median 1.37 mUI/L. Data of Follicle Stimulating Hormone (FSH) titles, number of mature oocytes, and embryos were not collected.

All patients were submitted to a stimulus protocol with recombinant FSH and luteinizing hormone. Four patients had only endometrioma (isolated ovarian disease) and six had endometrioma associated with deep endometriosis. Twenty-one patients were diagnosed by MRI, eleven by VDL, two by MRI and VDL and one by ultrasonography and MRI.

Comparing the two methods using a Student's *t*-test, there was no statistically significant difference between the means ($P = 0.59$). By calculating Pearson's coefficient, we demonstrate that there was a linear correlation between the variables in the dispersion diagram. The slope coefficients of a linear regression without intercept were calculated and similar results were obtained for 2D and 3D. In the 2D evaluation, $r = 0.83$ was obtained, with a confidence interval (CI) of (0.68–0.9), ($P < 0.001$); in the 3D evaluation, $r = 0.81$ was obtained, with a CI of (0.46–0.83), ($P < 0.001$). Figure 3 shows the correlations between each method and the OC. Observing the Bland Altman's plots, we concluded that both methods agree with the number of oocytes retrieved [Figure 4].

Discussion

In 2017, Peres Fagundes *et al*.[19] concluded that both 2D and 3D assessments were methods that agreed with each other.

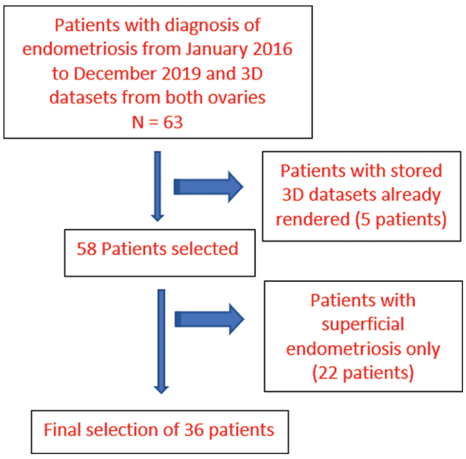

Figure 2: Flow chart of the patient's selection

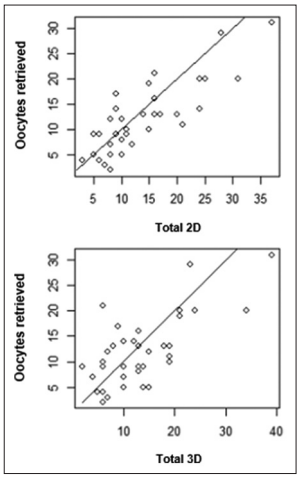

Figure 3: Correlation between two-dimensional and three-dimensional ultrasound and the number of oocytes retrieved

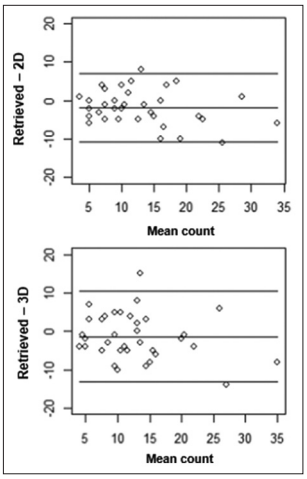

Figure 4: Bland–Altman plots of the two-dimensional and three-dimensional ultrasound methods

However, the results indicated that the 3D assessment tended to result in a higher count of antral follicles, especially in ovaries with more than 20 follicles. In our study, this difference may

not have been significant because the sample used does not contain many multifollicular ovaries. Controversially, other authors found in their results an underestimated score with the 3D ultrasound assessment.[4,20] The justification for this result would be that the new method would promote a reduction in the recount of follicles previously computed.

In a literature review on AFC of 2018, Coelho Neto *et al*.[8] recommended the technique to be manual counting with 2D ultrasound in real time, 2D ultrasound stored in the form of short videos (cine-loops) or manual 2D screening of stored 3D volumes. The argument used was that, despite the visual appeal, the evaluation of semi-automatic AFC with *SonoAVC* still needs to be improved and tested, and there are no robust studies in the literature that prove that it is actually more advantageous in any aspect. There would even be a greater risk of very different counts due to the use of the 3D tool without proper training of the examiner.

New technologies often take time to be incorporated into the clinical routine as they require financial investment and a learning curve. To support the *SonoAVC* software, it is necessary to own a General Electric ultrasound machine with automation for the acquisition and rendering of the 3D volume, demanding a significant cost. In our service, however, because of the great volume of cycles performed every month (around 100), the costs with this kind of equipment are easily covered after 3 months. Other brands already offer similar software on the market, and there are no studies to prove that they are equivalent to *SonoAVC*. The learning curve for handling the software is around 38 exams.[21] These issues are a limitation for the use of this technology in small services, with the aggravating factor that usually the reproduction specialists only have a basic training in ultrasonography. In this study, only one examiner used the software to determine the 3D AFC in all 3D data sets. This professional has been working with this machine for more than 5 years, performing *SonoAVC* render alternately with 2D-ultrasound, especially for multifollicular ovaries, having a complete learning curve for the software.

In any centre of human reproduction, however, patients with deep or ovarian endometriosis constitute a significant percentage[11,22,23] and, in general, the evaluation and monitoring of ovarian stimulus cycles in these cases are a challenge. Endometriosis is a disease that promotes an acute and chronic inflammatory process in the pelvic cavity, leading to adhesions that generate mechanical distortions, pain, and infertility.[10] Anatomical distortion and the presence of fibrosis processes, as well as cysts, hydrosalpinx and endometriomas usually make the image difficult to assess in these patients. The presence of pain during mobilization of the endocavitary probe is a frequent symptom since these patients are not submitted to a hormonal blockage, which reduces the patient's tolerance to ultrasonography evaluation. The patients that need to be submitted to human reproduction treatments are under substantial stress, being evaluated with ultrasound examination several times during the treatment. In our service, we have a special concern to promote the best experience to the patients, and this certainly includes a less painful ultrasound examination.

The count of antral follicles is one of the main indirect markers of the ovarian reserve[16] and is the parameter that most closely approximates the number of oocytes retrieved at the end of the cycle.[8] The AMH would be an alternative to the evaluation of AFC in the indirect evaluation of the ovarian reserve,[19,24] but it is also a marker of response to the ovarian stimulus and it is difficult to predict the number of oocytes at the end of its induction cycle, since there is no standardization of analysis in the various laboratories.[25,26] In comparison to ultrasonography, AMH is a more expensive test and does not replace the first, as ultrasonography assessment is essential to identify other factors that may interfere with treatment.

This study has limitations due to the fact that it is retrospective and based on data from medical records recorded by several examiners and that it has a small sample. The 3D volume evaluation was performed by only one examiner with a complete learning curve, which makes it not applicable for many services. The total analysis time of the volume was not measured, nor the time of the 2D and 3D image capture process. However, there is evidence in the literature that the use of the *SonoAVC* software reduces inter-observer variation and that the scan time for capturing the 3D image is extremely short.[4,9] The image capture technique must be performed with the probe still in the middle of the ovary, not being necessary to move the probe to observe the various planes of the ovaries.[8] When the 3D capture window is selected, a specific *preset* is activated so that the identification of the follicle is more accurate, with the possibility of postcapture adjustments. In traditional 2D ultrasonography, it is also necessary to adjust multiple parameters for correct follicle counting.[1] There are references in the literature of studies in which the evaluation with the *SonoAVC* was faster even with postcapture adjustments.[19] Other studies with similar methodology will be necessary to verify the reproducibility of our results.

Conclusion

In view of the results obtained in this study, the assessment of AFC with 3D ultrasound using the *SonoAVC* software is a method equivalent to the traditional 2D ultrasound assessment in patients with endometriosis. We believe that the 3D evaluation counting of the follicles will be, very soon, incorporated into the routine of most human reproduction services, as the trend over time is for technology to become more accessible and for software to improve. It is also very likely that other options of software with good performance will be available soon.

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Conflicts of interest
There are no conflicts of interest.

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