Antral follicle count as a predictor of ovarian response

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Abstract
Objective: To evaluate the relationship between the number of antral follicles at baseline and the number of oocytes retrieved after ovarian stimulation treatment, and to establish the role of antral count follicles by ultrasonography as a predictor of ovarian response. As a secondary objective we assessed the correlation of antral follicle count with the age of patients and the success of treatment.

Materials and methods: We retrospectively evaluated 40 women undergoing transvaginal ultrasonography guided follicular aspiration between January and March 2015. Transvaginal ultrasonography follicle count was performed prior to antral follicles stimulation, (only follicles measuring between 3 and 8 mm were considered). All patients received hormonal stimulation and were monitored with ultrasonography and hormonal blood tests until follicle aspiration.

Results: A strong inverse correlation between patient age and antral follicle count and a very strong inverse correlation between age and oocyte retrieval was observed. A very strong positive correlation between the antral follicle count and the number of oocytes retrieved in the transvaginal aspiration was also observed. The small number of patients limited the analysis of treatment success.

Conclusion: The antral follicle count had significant associations with ovarian response and the number of oocytes retrieved. The antral follicles count by ultrasonography should be considered the first choice for ovarian reserve testing because of its low cost, good performance and accessibility.

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Keywords: Ultrasonography; Fertility; Ovarian follicle; Ovarian reserve.

Introduction

The success of in vitro fertilization (IVF) and embryo transfer depends, among other factors, on the number of oocytes retrieved after ovarian stimulation through various drugs (exogenous gonadotropins, gonadotropin-releasing hormone, clomiphene citrate, etc.)1 In turn, the number of oocytes retrieved after ovarian stimulation is associated with the number of oocytes available for ovulation in the ovary and with the chronological age of the patient2–3.

Women are born with a finite number of eggs, which are used throughout their reproductive life and decline in quality and quantity with age. The possibility of identifying the number of follicles available and measuring ovarian function would allow estimation of the patient’s reproductive potential. In this respect, the true ovarian reserve is represented by the number of primordial or resting follicles in both ovaries. Because of their small size, these follicles might only be assessed by histologic exploration4.

Primordial follicles initiate folliculogenesis in the ovary, progressing to primary, secondary or preantral follicles, which cannot be identified on ultrasound examination. A proportion of follicles develops into antral follicles, which are sensitive to follicle-stimulating hormone (FSH), can be identified by ultrasound, and progressively reaches the preovulatory status. The number of these follicles can be quantified by gonadotropins measurement (FSH, basal estradiol, luteinizing hormone), especially by the anti-Mullerian hormone (AMH), together with the determination of the number of follicles with developing antrum in the ovary by ultrasound.

AMH is produced in the adult ovary by the granulosa cells of small (primordial, primary and secondary) follicles until the preantral status. It has an inhibitory and protective effect in folliculogenesis, but most of all, it reflects in a reliable man-
ner the resting follicle pool. AMH is considered the marker of choice of the follicle count. Several authors agree that antral follicle count (AFC) by transvaginal ultrasound before ovarian stimulation is the best predictor of response to assisted reproduction treatment cycles\(^{3,9}\). Thus, AFC at the beginning of the ovarian cycle may provide information about the reserve, but also about the type of response to ovarian stimulation. The ovarian reserve is considered to be adequate when the number of antral follicles (3-8 mm) identified in each ovary is greater than or equal to 5.

The risk of hyper-response is associated with over 10 antral follicles in each ovary, while a poor response is associated with an antral follicle count of less than 10 in total (poor ovarian reserve). Another ultrasound parameter is ovarian volume measurement, although its usefulness remains controversial. This measurement is included in the initial evaluation of the patient, with an ovarian volume of less than 3 cm\(^3\) being associated with a high likelihood of failure in treatment response\(^4\).

Identifying women at a higher risk of poor ovarian response before treatment is useful for choosing an adequate thera-

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**Figure 1** Transvaginal ultrasounds for antral follicle count: (a) ovary (arrows) with no antral follicle; (b) ovary with one antral follicle (arrow); (c) round anechoic images representing antral follicles; and (d) measurement of the internal diameter of the follicle (arrow).
peutic management and adjusting the dose of medication, thus optimizing ovarian response. Once treatment has been initiated, monitoring is performed by ultrasound to assess ovarian response to gonadotropin stimulation. This follow-up is functionally supplemented with measurement of serum estradiol levels during stimulation, allowing identification of the type of response (poor or high) and of the associated risk of clinical complications. The objective of this study was to evaluate the relationship between the number of antral follicles at baseline and the number of oocytes retrieved after treatment, and to establish the role of AFC as a predictor of ovarian response. In addition, we sought to determine if there is a correlation between AFC and the age of patients and treatment success.

Materials and methods

Ethical considerations
All study procedures were standard of care and all study data were blinded and managed with the highest level of confidentiality, with access being restricted only to authorized personnel for the purpose of the study in accordance with the provisions of Personal Data Protection Act 25326/00 and Act 26529/09 currently in force in Argentina. The final manuscript was reviewed and approved by the Institutional Bioethics Committee.

Study population and equipment
In this cross-sectional retrospective study conducted in our institution, we evaluated candidates for follicular aspiration who had had a transvaginal ultrasound performed for a baseline antral follicle count before stimulation during the period between January and March 2015. The age and reason for ovarian stimulation were tabulated.

Study protocol
The transvaginal ultrasounds of patients enrolled in this study were performed by four physicians specialized in radiology with expertise in gynecologic ultrasound using a Toshiba Xario 200 ultrasound systems with 7 MHz TV transducers. Antral follicles were measured during the early follicular phase (i.e., between days 2 and 4 of the menstrual cycle) to prevent false positive cases, such as coexisting ovarian cysts or the corpus luteum. Only follicles measuring between 3 and 8 mm were considered. Size was estimated using the internal diameter of the anechoic area (fig. 1), while for counting, the systematic approach recommended by Broekmans et al was used:

1. Identify the ovary.
2. Explore the dimensions in two planes.
3. Carefully examine the entire ovary in the anterior-posterior and sagittal-parasagittal directions in order to count all visible antral follicles.

The number of follicles was quantitatively tabulated and patients were divided into four groups according to their antral follicle (AF) count, in a similar way to that reported by Huang et al.: group A, less than 5 AFs; group B, 6-10 AFs; group C, 11-15 AFs; and group D, more than 16 AFs.

Monitoring and follow-up
Following the baseline count of AFs, patients underwent stimulation with a flexible antagonist protocol. In the early follicular phase, patients received recombinant follicle-stimulating hormone (FSHr; Puregon, Organon Laboratory) in individually adjusted doses, starting on day 2 of their cycle. Follow-up ultrasound scans and estradiol measurements were performed during stimulation. With follicles larger than 14 mm (average diameter) by ultrasound and a complementary correlation with plasma estradiol levels, daily administration of gonadotropin-releasing hormone (GnRH; cetrotrelix 0.25 mg/ml, Orgalutran, Organon) antagonists was initiated, in combination with 75 IU/day of human menopausal gonadotropin (HMG; Menopur, Ferring).

With follicles larger than 17 mm in diameter on average, measured by ultrasound, ovulation trigger with human chorionic gonadotropin (hCG: 5000 to 10,000 IU) or GnRH agonists (Triptorelin Acetate, Ferring) was scheduled with the aim of achieving the ovulatory changes required. Follicle aspiration was scheduled 34 to 36 hours after trigger. The amount of oocytes retrieved in each patient was tabulated and analyzed. The presence of pregnancy after transfer was recorded.

Statistical analysis
Categorical variables were reported as proportions with confidence intervals (CI), while continuous variables were reported as mean and standard deviation or median and interquartile range, depending on their distribution. For correlation between quantitative variables, Spearman’s test was used for variables with non-normal distribution and Pearson’s test was used for variables with normal distribution. Coefficients of correlation were reported with their 95% CI. For multiple comparisons between groups, the Kruskal-Wallis test was used, given the non-normal distribution observed in small groups. The difference between continuous variables between two groups was analyzed using Mann-Whitney or
Student’s t-test for independent samples, depending on their distribution. All tests were performed with the Medcalc 12.7 software. Statistical significance was set at \( p < 0.05 \).

**Results**

During the period under review, 40 patients had an antral follicle count performed for follicle aspiration. Some of these patients were candidates for IVF (\( n = 26 \)) and others (\( n = 14 \)) for egg donation (as oocyte donors) or ovarian preservation. Median age was 38 years old (interquartile range between 31 and 41 years).

In the overall population (\( n = 40 \)), there was an important inverse correlation between the age of patients and the antral follicle count; at the same time, there was a highly marked inverse correlation between age and oocyte retrieval. A very strong positive correlation was also found between the amount of antral follicles and the amount of oocytes retrieved by transvaginal aspiration (table 1).

If we analyzed the subset of patients with fertility disorders undergoing embryo transfer (\( n = 26 \)) within the overall population, these associations would persist but to a lesser extent (table 1; fig. 2).

Based on the comparison between different groups (A, B, C and D), ages of patients in groups A and B were significantly different from those in group D (\( p < 0.05 \)). Then, group A was merged with group B, and group C with group D, with a significant difference being observed (\( p < 0.05 \)) between the ages of the two samples. The median age was 40 years for group A-B and 33 years for group C-D.

When comparison was performed only in the series of patients with fertility disorders (\( n = 26 \)), i.e., those with embryo transfer, difference in age was not significant between groups (\( p = 0.145 \)), but there was a trend in grouping. Patients in groups C and D made up a younger group than those in A and B (\( p = 0.081 \)). If the sample had been larger, this trend would probably have been confirmed.

In addition, a significant difference was observed between the number of oocytes retrieved by aspiration in group A and B vs. group C and D (\( p < 0.05 \)); such difference was also significant in the population with fertility disorders (\( p < 0.05 \)). Then data from patients in group A-B and in group C-D undergoing embryo transfer were analyzed to evaluate the success of IVF.

The pregnancy rate was 20% in patients from group A-B and 50% in patients in group C-D; however, these results were non-significant (\( p = 0.29 \)). In our sample, there was no as-

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**Table 1**: Correlation between the amount of antral follicles and oocytes retrieved.

<table>
<thead>
<tr>
<th></th>
<th>Overall population (( n = 40 ))</th>
<th>Candidates for in vitro fertilization (( n = 26 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>Age and antral follicles</td>
<td>-0.640(^a)</td>
<td>-0.793 to -0.410</td>
</tr>
<tr>
<td>Age and ovarian reserve</td>
<td>-0.755(^a)</td>
<td>-0.863 to -0.579</td>
</tr>
<tr>
<td>Antral follicles and ovarian reserve</td>
<td>0.753(^a)</td>
<td>0.577 to 0.862</td>
</tr>
</tbody>
</table>

\(^a\) \( p < 0.05 \)

**Figure 2** Relationship between the amount of antral follicles and the amount of oocytes retrieved by transvaginal aspiration in patients with fertility disorders. The line of equality shows where perfect correlation between them should lie. The chart shows how they are distributed following this trend.
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Discussion

In this study, in which antral follicles ranging from 3 to 8 mm in diameter were measured by transvaginal ultrasound in the early follicular phase, a significant correlation was found between the number of antral follicles and oocytes retrieved after treatment (with a larger number being found in groups C and D). In agreement with other authors, we can affirm that the higher the follicle count, the higher the oocyte retrieval\textsuperscript{8-11}.

We also observed a correlation with age, with patients from groups C and D being younger, with a median age of 33 years old. In these patients, a larger number of oocytes was retrieved. Thus, it can be concluded that the older the patient, the lower the antral follicle count and the lower the amount of oocytes retrieved. This finding is consistent with what was previously stated and with the literature, in that there is a relationship between quantitative ovarian reserve and the chronological age of women\textsuperscript{2,3}.

In women with fertility disorders, this correlation between age and follicle count declines (possibly due to unknown or non-evaluated factors). This is consistent with the affirmation of Scheffer et al.\textsuperscript{3}, who sustained that chronological age may be predicted according to the number of antral follicles in women with no disorders, but that this not possible in women with fertility disorders.

It is important to predict the ovarian reserve and to perform an adequate monitoring of ovulation to prevent any unnecessary use of drugs that may lead to ovarian hyperstimulation. This may occur because during a spontaneous menstrual cycle a single antral follicle is usually selected and becomes dominant, whereas during ovarian stimulation treatments, multiple antral follicles progress and become dominant (larger than 20 mm in diameter)\textsuperscript{4,5}. For this reason, if the induction protocol is not patient-tailored, multiple ovarian cysts and complications may occur, constituting a hyperstimulation syndrome.

Various studies have concluded that AFC is a good predictor of ovarian reserve. In agreement with most of these studies, we think that a low antral follicle count predicts a poor response and that a high count anticipates an adequate response to ovarian stimulation\textsuperscript{3,5,10,12}.

Even if some reports suggest that with an AFC ≥ 10 a larger number of pregnancies and births is observed than with an AFC ≤ 10, in our study this was not significantly different\textsuperscript{7,9,10,12-14}. Our outcome in patients with fertility disorders was probably not significant because of the small size of the sample, or there may be other variables, apart from antral follicle count, that may have an impact on the success of IVF, such as the uterine and hormonal status or the male factor.

There are various limitations to our study. First and foremost, the small number of patients did not allow detection of significant differences in treatment success in infertile women or obtaining narrower 95% confidence intervals. Secondly, the use of a secondary database is a potential source of bias, as some data may be lost or not recorded, as it was the case of anti-Mullerian hormone measurements, not recorded in the electronic medical record.

Conclusion

The antral follicle count allows prediction of ovarian response and of the amount of oocytes that will be retrieved, being useful for choosing the appropriate treatment for each patient. Because of its low-cost, high diagnostic performance, accessibility and ease of use, the ovarian follicle count by ultrasound should be considered the test of choice for ovarian reserve testing.

Ethical responsibilities

Protection of human subjects and animals. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Comment

At the time of this study, Dr. Lonegro was attending the residency program in the Imaging Department at Hospital Italiano de Buenos Aires.
References


