Measurement of antral follicle count in patients undergoing in vitro fertilization treatment: results of a worldwide web-based survey

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Abstract

Purpose The purpose of the present study was to identify trends in the therapeutic approaches used to measure antral follicle count (AFC) in patients undergoing in vitro fertilization (IVF) treatment worldwide.

Methods A retrospective evaluation utilizing the results of a web-based survey, IVF-Worldwide (www.IVF-Worldwide.com), was performed.

Results Responses from 796 centers representing 593,200 cycles were evaluated. The majority of respondents (71.2 %) considered antral follicle count as a mandatory part of their practice with most (69.0 %) measuring AFC in the follicular phase. Most respondents (89.7 %) reported that they would modify the IVF stimulation protocol based on the AFC. There was considerable variation regarding a limit for the number of antral follicles required to

initiate an IVF cycle with 46.1 % designating three antral follicles as their limit, 39.9 % selecting either four or five follicles as their limit, and 14.0 % reporting a higher cutoff criteria. With respect to antral follicle size, 61.5 % included follicles ranging between 2 and 10 mm in the AFC. When asked to identify the best predictor of ovarian hyper-response during IVF cycles, AFC was selected most frequently (49.4 %), followed by anti-Mullerian hormone level (42.7 %). Age was selected as the best predictor of ongoing pregnancy rate in 81.7 % of respondents.

Conclusions While a large proportion of respondents utilized AFC as part of their daily practice and modified IVF protocol based on the measurement, the majority did not consider AFC as the best predictor of ongoing pregnancy rate.

Keywords Antral follicle count · Ovarian reserve · In vitro fertilization

Capsule A worldwide survey of IVF centers demonstrates most utilize antral follicle count as part of their practice but most do not consider it the best predictor of ongoing pregnancy rate.

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Introduction

Over the past two decades, ovarian reserve has been identified as a key factor in the success of assisted reproductive technologies (ARTs). As a result, markers of ovarian reserve have evolved to become part of routine diagnostic testing performed prior to in vitro fertilization (IVF). The goal of such diagnostic procedures and assays is to identify women at high risk of having a poor response to controlled ovarian stimulation during IVF cycles, with subsequent risk of cancellation. Likewise, such testing can identify patients at risk for ovarian hyper-stimulation syndrome [1]. Therefore, ovarian reserve testing is useful for individually calculating gonadotropin doses prior to IVF cycles, correctly identifying poor responders, and counseling patients for whom donor egg may be a more suitable option [2–5]. It is well known that a woman's ability to conceive naturally decreases after the age

of 40. As women continue to postpone childbearing, thereby increasing the prevalence of age-related fertility, ovarian reserve testing remains an important diagnostic tool in daily practice for IVF providers [6]. Several methods have been established to predict IVF response, including basal follicle-stimulating hormone (FSH) levels, inhibin B, anti-Müllerian hormone (AMH), and antral follicle count (AFC).

Antral follicle count is typically defined as the number of follicles measuring 2 to 10 mm in greatest diameter. It has been shown that the number of antral follicles in the ovaries is proportionally related to the size of the primordial follicle pool from which the follicles are recruited [7, 8]. Additionally, antral follicles are responsive to FSH and may be considered a predictor of ovarian response to gonadotropins [7, 8]. A “low” AFC ranges from four to ten follicles and is used as a predictor of both low ovarian reserve and a poor response in IVF cycles. Thresholds for AFC prior to starting IVF and for dosing gonadotropins vary among centers [9, 10, 2, 11]. A potential advantage of AFC is that it can be measured readily in the clinic, generating results immediately. A downfall, on the other hand, is lack of standardization in ultrasound equipment, technique, and antral follicle size, which results in challenges with cross-comparing results from studies and different centers [10]. To our knowledge, no studies to date have evaluated the contemporary clinical use of antral follicle count by IVF centers worldwide.

IVF-Worldwide (www.IVF-Worldwide.com) is a comprehensive IVF-focused website for physicians, embryologists, nurses, and social workers. It allows members to locate IVF centers anywhere in the world to communicate directly, facilitating the sharing of ideas and discussion of treatment and medication regimens. This non-commercial, non-profit website has an advisory panel of 52 leaders in the fertility field and routinely performs surveys focusing on various aspects of ART.

The purposes of our web-based survey were to identify trends in the therapeutic approaches used to measure the antral follicle count in patients undergoing IVF treatment, and to correlate these results with current evidence-based literature. This information may prove useful to providers seeking guidance in utilizing antral follicle count for planning fertility treatment protocols and allow them to compare their clinical use of antral follicle count with that of a large cohort of IVF providers.

Materials and methods

A 16-item survey entitled “Anti-Müllerian Hormone and Antral Follicular Count IVF-Worldwide Survey” was compiled and posted on the IVF-Worldwide website from July 15, 2014 to August 31, 2014. Please refer to the questions

stems specific to AFC and analyzed in this study in [Appendix](#). Survey questions focused on various aspects of antral follicle count technique, including timing of AFC measurement, the AFC cutoff threshold use for IVF, size of follicles in AFC measurement, and the methods used for calculating IVF dosing using AFC. Attitudes of IVF units were also assessed regarding the best predictor of hyper-response in IVF and the best predictor of ongoing pregnancy rate. This study was determined to be exempt from institutional review board approval by Johns Hopkins University School of Medicine.

Quality assurance methods

Minimization of duplicate reports from a clinical unit as well as possible false data was achieved via a computerized software program that assessed the consistency of four parameters from self-reported data of the unit surveyed with existing data of units registered on the IVF-Worldwide website. These parameters included the name of the unit, name of the unit director, country, and e-mail address. At least three of the parameters from the survey had to match archived data on the website in order for the data reported by the clinical unit to be included in the study.

Statistical analysis

The analysis was based on the number of IVF cycles reported by the unit and not on the number of units in the study. Thus, the relative proportion of answers reflects the total proportion of IVF cycles represented rather than the proportion of individual respondents to the survey questions. The survey was structured as a sequence of multiple choice questions, in which respondents could select a single answer. For example, for a question with four possible answers (a, b, c, d), results were calculated by using the following formulas as described in previously reported research from the IVF-Worldwide network [12]:

$$\%a' = \frac{\sum \text{Number of cycles of units who answered 'a'}}{\sum \text{Number of cycles of all the units}} * 100$$

$$\%b' = \frac{\sum \text{Number of cycles of units who answered 'b'}}{\sum \text{Number of cycles of all the units}} * 100$$

$$\%c' = \frac{\sum \text{Number of cycles of units who answered 'c'}}{\sum \text{Number of cycles of all the units}} * 100$$

$$\%d' = \frac{\sum \text{Number of cycles of units who answered 'd'}}{\sum \text{Number of cycles of all the units}} * 100$$

Results

A total of 796 IVF centers from 102 countries responded to the survey and met the computerized system's quality assurance standards. Overall, respondents reported on 593,200 cycles. International representation of the cycles was diverse: 47 % European ($n=275,900$), 24 % Asian ($n=140,300$), 11 % North American ($n=64,800$), 6.6 % South American ($n=39,100$), 6 % African ($n=35,300$), and 6.4 % from Australia and New Zealand ($n=37,800$).

Respondents representing the majority of cycles (71.2 %, $n=422,100$) consider the measurement of AFC as a mandatory part of their practice. Among remaining respondents, most (22.1 %, $n=131,100$) reported that they consider AFC “welcome but not mandatory,” and only 6.3 % ($n=37,100$) did not consider measuring AFC as mandatory. Likewise, the majority of respondents, 82.1 % ($n=487,000$), report documenting AFC levels in the medical chart. In terms of measuring antral follicle count, 69.0 % ($n=409,400$) do so in the follicular phase. The remaining 24.0 % ($n=142,100$) measure AFC throughout the menstrual cycle and 7.0 % ($n=41,700$) report never measuring AFC. Nearly 90 % of respondents (89.7 %, $n=532,100$) report that they would modify the IVF stimulation protocol based on the AFC. Of the remaining, 7.2 % ($n=43,000$) do not change protocols based on AFC and 3.1 % ($n=18,100$) did not know if they would.

There was considerable variation among centers regarding a cutoff level for the number of antral follicles required to initiate an IVF cycle. While 46.1 % ($n=258,500$) of respondents designated three antral follicles as their cutoff level, 39.9 % ($n=223,700$) selected either four or five follicles as their cutoff level. Only 14.0 % ($n=78,500$) indicated that their antral follicle count cutoff criteria was higher (six or seven antral follicles). With respect to defining antral follicle size, the majority of respondents, 61.5 % ($n=346,600$), included follicles ranging between 2 and 10 mm in the AFC. Other size ranges for the AFC included 5–10 mm (19.7 %, $n=110,800$), 2–5 mm (17.1 %, $n=96,300$), or other ranges (1.7 %, $n=9600$).

Several clinical-outcome-based parameters were also surveyed. When asked to identify the best predictor of ovarian hyper-response during IVF cycles, AFC was selected most frequently (49.4 %, $n=292,800$), followed closely by AMH (42.7 %, $n=253,300$). Categories selected less frequently as predictors of ovarian hyper-response included age (4.3 %, $n=25,800$) and other (3.6 %, $n=21,300$). Finally, age was selected as the best predictor of ongoing pregnancy rate in the majority of cases, chosen by 81.7 % of respondents. Other factors

chosen less often as predictors of ongoing pregnancy rate included AMH (3.7 %, $n=22,200$), AFC (2.6 %, $n=15,300$), FSH level (3.0 %, $n=18,000$), and other factors (8.9 %, $n=53,000$).

Discussion

To our knowledge, this is the only study to date addressing the practices among an international cohort of fertility centers regarding the use of antral follicle count in treating IVF patients. Ovarian biomarkers such as AFC are valuable in predicting ovarian response and in optimizing the live birth rate. Our survey identified many common practices, as well as variations, in the approach to using AFC in patients undergoing IVF. Although a large majority of respondents utilized AFC as part of their daily practice and modified IVF protocols based on the number of antral follicles, the majority did not consider AFC as the best predictor of ongoing pregnancy rate.

A question that has persisted since the advent of IVF is which measurement of ovarian reserve is most predictive of ovarian response, particularly for poor responders. AMH is a dimeric glycoprotein produced by the granulosa cells of preantral and small antral follicles and has been noted to have strong correlation with the primordial follicle pool [13]. Since AFC is tied to AMH level and vice versa, a number of studies have compared AFC with AMH level as predictors of ovarian response in IVF. Three recent multicenter trials of good prognosis IVF patients showed that the AMH level was a better predictor of ovarian response than AFC in both gonadotropin-releasing hormone (GnRH) agonist and antagonist cycles [14–16]. In contrast, Mutlu et al. (2013) conducted a prospective study which demonstrated that AFC determines poor ovarian response better than AMH [17]. Additionally, Broer et al. (2009) performed a systematic review to assess the effectiveness of AFC versus AMH as predictors of poor ovarian response and pregnancy occurrence after IVF. The authors identified 17 studies reporting outcomes on AFC and 13 on AMH and found no significant differences between AFC and AMH in predicting poor ovarian response [18]. Findings which contrasted the systematic review of Broer et al. (2009) were demonstrated by Anderson et al. (2011) through a multicenter randomized controlled trial (RCT) which compared the predictive value of AMH to AFC of ovarian response in a GnRH antagonist cycle. They failed to show an independent association between AFC and oocyte yield in either treatment arm, whereas AMH was the strongest predictor of ovarian response [16]. More recently, Nelson et al. (2015) compared AFC with AMH as predictors of

ovarian response in a secondary analysis of two multicenter trials involving 1205 patients undergoing GnRH antagonist and long-luteal agonist cycles. They found that AMH was more strongly correlated with oocyte yield than AFC [19]. It should be noted that the value of AFC will likely be clinic-dependent. As our survey demonstrates variability in AFC ranges and cutoffs that respondents used in managing IVF patients, the predictive value of AFC is likely to be more subjective and variable than that of AMH, which will have values consistent among centers.

Using AFC as a predictor of excessive response in controlled ovarian hyper-stimulation is also critical. In our survey, AFC was selected most frequently as the top factor predictive of ovarian hyper-response followed closely by AMH level. Broer et al. (2010) performed a meta-analysis to assess the accuracy of AMH and AFC as predictors of excessive response in IVF/ICSI treatment. They identified nine studies reporting on AMH, and five on AFC, reporting no significant difference between the two parameters in predicting excessive response [20]. These findings are consistent with our survey, since both AMH and AFC appear to have clinical value in predicting ovarian hyper-response.

Additionally, while ovarian response is critical, the most important outcome in any IVF cycle is the live birth rate. In our survey, age was selected as the best predictor of ongoing pregnancy rate by over 80 % of respondents, with AMH and AFC selected by only a few (3.7 and 2.6 %, respectively). This clinical trend demonstrated in our study supports existing research that predicting pregnancy by AFC alone is poor. For instance, Mutlu et al. showed in their prospective study that age was the only independent predictor of live birth in IVF as compared to AMH, AFC, and other ovarian reserve markers [17]. Hsu et al. (2011), in a retrospective cohort study of 1049 IVF cycles, showed that while AFC did predict ovarian response, it did not predict pregnancy or live birth rate [21]. Li et al. (2013) demonstrated in a retrospective study of 1156 women undergoing their first IVF cycle that while both AMH and AFC revealed significant correlation with age and ovarian response, regression analysis showed that both AMH and AFC were not significant predictors of cumulative live birth after adjusting for age [22].

One recognized advantage of AFC is that it can be readily measured by an individual provider in the IVF clinic with immediate results. However, variability among clinicians can also lead to intra-provider inconsistencies in its measurement and use. Indeed, these inconsistencies were demonstrated in our study. One key issue in using antral follicle count as a

marker for ovarian reserve is defining the number of antral follicles as well as the accepted size range. Practical recommendations for use of AFC define the size range for antral follicles between 2 and 10 mm [2]. In our study, nearly 40 % of respondents defined an AFC range which differs outside this accepted range. Additionally, 46.1 % of respondents used a cutoff of three antral follicles for moving forward with IVF treatment, while the remaining used alternative cutoff values. These variations will likely bring about different reported results and variability among centers that use AFC as a predictive factor for ovarian response. There is currently a lack of standardization of ultrasound equipment, training, and technique that leads to heterogeneity in the interpretation and clinical utility of antral follicle count, as demonstrated in our study as well in [10, 23].

The strengths of this study are the comprehensive nature of the survey, focusing on several aspects of antral follicle use in IVF cycles, ranging from clinical indications to treatment protocols to perceived impact on patient outcomes. An additional strength is the large number of IVF centers and cycles represented, as well as diversity in the geographic locations represented. Our study exhibits weakness as well, in that there is the potential for selection bias. Units that chose to participate in a worldwide survey may have different practice techniques than other centers worldwide that chose not to participate. However selection bias is inherent in all data collected from surveys and should always be considered when interpreting results. Additionally, we controlled for many inherent errors that can result from anonymously completed surveys by verifying the representative identity of the respondent clinic and eliminating potential redundant responses.

In conclusion, AFC is a key modality for estimating ovarian reserve. Our survey of IVF centers worldwide demonstrates that the AFC is used widely as a predictive factor for ovarian response prior to IVF and for customizing gonadotropin dosing. There appears to be variability among IVF providers in terms of measuring AFC and in the use of predetermined limits for AFC required in order to commence an IVF cycle. The variability we demonstrate in this survey may explain in part why AFC may fail in its predictability for ovarian response in comparison with AMH, a lab value which produces a metric much less subjective, as it is not as easily influenced by provider ability, technique, or ultrasound equipment. While AFC will likely continue to be a valuable tool for predicting ovarian response in IVF cycles, its role in predicting live birth rate is limited.

Appendix: Question stems pertaining to antral follicle count used for analysis in this study with responses as percentages of units and cycles

1) Have the majority of patients visiting your IVF clinic already undergone endocrine function testing?					
	Yes	No	I do not know		
% of Units	71.4	27.9	0.8		
% of Cycles	75.0	23.4	1.6		
2) Do you consider AFC testing mandatory in your practice?					
	Yes	No	Welcome but not mandatory	I do not know	
% of Units	70.2	5.5	23.7	0.5	
% of Cycles	71.2	6.3	22.1	0.5	
3) Do you routinely document the AFC in your patients' charts?					
	Yes	No	I do not know		
% of Units	81.8	15.7	2.5		
% of Cycles	82.1	15.7	2.2		
4) When do you measure the AFC?					
	During the early follicular phase	Any time in the cycle	I do not measure AFC on a routine basis		
% of Units	74.2	19.6	6.2		
% of Cycles	69.0	24.0	7.0		
5) Would you change your stimulation protocol based on AFC results?					
	Yes	No	I do not know		
% of Units	87.8	8.9	3.3		
% of Cycles	89.7	7.2	3.1		
6) What is your AFC cut-off level for poor responders?					
	Three follicles or less	Four to five follicles	Six to seven follicles	I do not measure AFC	
% of Units	45.6	38.1	11.4	4.9	
% of Cycles	43.6	37.7	13.2	5.5	
7) What size of follicles (in mm) would you include in the AFC?					
	Two to five mm in diameter	Five to ten mm in diameter	Two to ten mm in diameter	Other	
% of Units	21.1	19.0	53.6	1.8	
% of Cycles	16.2	18.7	58.4	1.6	
8) In your opinion, which is the best predictor of hyper-response to ovarian induction?					
	Age	AFC	AMH	Other	
% of Units	4.6	50.6	39.7	5.0	
% of Cycles	4.3	49.4	42.7	3.6	
9) What do you think is the best predictor of on-going pregnancy rate?					
	Age	FSH	AFC	AMH	Other
% of Units	80.3	2.9	2.8	6.2	7.8
% of Cycles	81.7	3.0	2.6	3.7	8.9

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