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ARTICLE

Variation in antral follicle counts at different times in the menstrual cycle: does it matter?

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Abstract Antral follicle count (AFC) variation was examined across the menstrual cycle and its effect on clinical management assessed. In 79 women, AFC was documented in early (iAFC) and late follicular phase (sAFC). Absolute agreement between iAFC and sAFC and agreement for classification into categories of risk of extremes of ovarian response were examined. Ovarian stimulation protocols designed with iAFC and sAFC, and the predictive value of iAFC and sAFC for extremes of ovarian response, were compared in women undergoing ovarian stimulation. Significant differences were found between iAFC and sAFC (16 [IQR 9–24] versus 13 [IQR 7– 21]; $P = 0.001$), with moderate agreement for the classification into at risk of extremes of response ($k = 0.525$). Agreement for protocol selection based on either AFC ($k = 0.750$) and starting gonadotrophin dose was good (concordance correlation coefficient 0.970 [95% CI 0.951 to 0.982]). Predictive value for iAFC and sAFC was maintained for poor ovarian response and risk of ovarian hyperstimulation syndrome (OR 0.634 [0.427 to 0.920], 0.467 [0.233 to 0.935]) and (OR 1.049 [0.974 to 1.131], 1.140 [1.011 to 1.285]). Across the cycle, AFC varies but does not significantly affect ovarian stimulation protocol design and prediction of extreme ovarian response.

KEYWORDS: AFC, AMH, ovarian reserve testing, ultrasound

Introduction

Assessment of the biomarkers of ovarian response is integral to the work-up of women presenting with subfertility. Biomarkers used in routine clinical practice include anti-Müllerian hormone (AMH), early follicular phase FSH and early follicular phase antral follicle count (AFC). The marker of ovarian reserve with least intracycle variability is AMH (Deb

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et al., 2013), making it the most convenient as it can be carried out at any point in the cycle (Iliodromiti et al., 2014); FSH is a late marker of poor ovarian response (Broekmans et al., 2006; Iliodromiti et al., 2014) and alone is not considered adequate to design a protocol of ovarian stimulation (Broer et al., 2013). Transvaginal ultrasound is used to measure AFC in the early follicular phase of the cycle, and continues to be part of initial investigations for women presenting with subfertility, as the test can be easily carried out directly by the treating clinician with immediately available results (Scheffer et al., 2002).

An inherent concern with use of AFC as a biomarker is its operator dependence (Iliodromiti and Nelson, 2015). Another concern has been timing of AFC determination in the menstrual cycle. Current recommendations limit AFC determination to the early follicular phase in an attempt to standardize measurements (Broekmans et al., 2010). This restriction, however, creates anxiety for patients as it creates a narrow time window within which to complete their assessments and results in considerable administrative burden for clinics to schedule appointments. It also increases the number of visits for the patient to the clinic.

The aim of ovarian response biomarker determination is to identify patients at risk of extremes of ovarian response and to individualize ovarian stimulation protocols to obtain an optimal result (Bosch and Ezcurra, 2011). A degree of intracycle variability that does not jeopardize these objectives may be acceptable in clinical practice. Indeed some data have been published on this subject: a retrospective study of over 3000 patients, for example, argued that the clinical usefulness of AFC remains unchanged across the cycle (Rombauts et al., 2011).

The aim of our study was to examine whether AFC determination in the late follicular phase of the cycle would affect selection of ovarian stimulation protocol and the accuracy of AFC to predict extremes of ovarian response.

Materials and methods

The study took place in the Reproductive Medicine Unit of University College London Hospital between April 2014 and June 2015. Opinion was sought from the Joint Research Office of the hospital and formal ethics approval was not required as the project involved no change in routine clinical practice.

Women with a regular 28–34 day cycle referred for fertility investigation were included. All women referred to the clinic undergo a transvaginal ultrasound scan between days 2–5 of their menstrual cycle for examination of the pelvis, uterus and assessment of the AFC (Voluson E8 Expert, GE Medical Systems, Zipf, Austria). Women undergo a second ultrasound examination on day 8–12 of their cycle for hysterosalpingo-contrast-sonography (HyCoSy) or three-dimensional saline infusion sonohysterography. At second examination, the ovaries are routinely examined and a repeat AFC is carried out. Determination of the antral follicle count is in accordance with internationally agreed guidance (Broekmans et al., 2010). Briefly, each ovary is identified and examined in two planes to determine its limits. The complete ovary is then swept in the transverse plane to identify and count all follicles 2–10 mm in diameter. Abdominal pressure is applied in cases of difficult visualization. All examinations were carried out by three operators (DM, AA, and VT).

Further routine investigations in our unit for women with subfertility are serum AMH (Beckman Coulter AMH Gen II ELISA) and day 2–5 FSH determinations. The AMH and FSH results were archived on a database, which is not accessed during ultrasound examinations thereby blinding operators to these results.

A spreadsheet was created (Excel 11 for Mac, Microsoft Corp.) to record the women's demographic details, day of the cycle of the first examination and the early follicular phase AFC (iAFC) in each ovary. A second spreadsheet was created to document the day of the cycle of the second examination and the late follicular phase AFC (sAFC) in each ovary as well as the presence and mean diameter of a dominant follicle in either ovary. Operators examining AFC only accessed the relevant database (i.e. iAFC or sAFC) and were therefore blinded to other AFC determinations. Once all examinations were complete, the databases were amalgamated and the AMH and FSH results were added.

The 2013 National Institute for Health and Care Exellence fertility guideline was used to define categories of "at risk of low response", "normal response" and "at risk of high response" after ovarian stimulation (NICE, 2013) (i.e. total AFC ≤4 and ≥16; AMH ≤5.4 pmol/l; and ≥25.0, respectively). Each woman was classified into the various categories based on AMH, iAFC and sAFC.

During the study, women with amenorrhoea or irregular cycles were excluded as AFC determination would not be able to be timed. Women with known pathologies, such as endometriosis or large fibroids that displace the ovaries and affect the accuracy of AFC, were also excluded, as were women aged over 40 years with a body mass index greater than 30 and those who did not tolerate HyCoSy or three-dimensional saline infusion sonohysterography examination.

To examine the interobserver variability in AFC determination in our unit, a set of ovarian three-dimensional volumes were obtained by DM and AA from a different cohort of patients and stored. The volumes were anonymized and each operator was asked to examine the volumes and record the total AFC for each woman in individualized Excel spreadsheets. Each operator was blinded to the other operators' findings.

Ovarian stimulation protocols in our unit are individualized according to age, body mass index, AMH, AFC, FSH and clinician preference (Bosch and Ezcurra, 2011). To further explore the potential effect of AFC in the late follicular phase criteria, two Excel spreadsheets were created with clinical and ovarian reserve testing (ORT) data for each case. Both spreadsheets contained age, AMH, FSH and a total AFC value. One spreadsheet contained the iAFC and the other sAFC. The cases were arranged in random order with no identifiers. EY was blinded to whether the spreadsheet contained iAFC or sAFC and was asked to select an ovarian stimulation protocol. The protocols routinely used are long agonist; long agonist with withdrawal of GnRHa on day 3 of stimulation; antagonist; and no ovarian stimulation. EY also selected starting dose of gonadotrophin dose (HMG) for each case.

For women who underwent an ovarian stimulation cycle, the following data were collected: IVF protocol, total

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