Article

Müllerian inhibiting substance is an accurate marker of ovarian response in women of advanced reproductive age undergoing IVF

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Abstract

While multiple investigators have demonstrated that Müllerian inhibiting substance (MIS) concentration is an accurate marker of ovarian reserve, all previous reports have focused on patients aged 36 and younger. It remains to be seen if MIS concentration is a useful marker in patients with the highest prevalence of diminished ovarian reserve: those aged 37 and over. Day 2 MIS concentrations in patients aged 37 and over undergoing IVF were examined, and the predictive value of MIS concentration was compared to that of FSH and oestradiol concentration. Three groups of patients were studied: (i) patients who experienced cycle cancellation; (ii) patients who underwent oocyte retrieval; (iii) patients who were precluded from IVF due to elevated FSH concentrations. While FSH and oestradiol concentrations were statistically similar in groups 1 and 2, MIS concentrations were significantly lower in group 1. In group 2, MIS concentration positively correlated with the number of oocytes retrieved. In group 3, 91% had low or undetectable MIS concentrations. The results indicate that MIS concentration accurately predicts cycle cancellation that is belied by normal FSH and oestradiol meaurements, and has a better correlation with the number of oocytes retrieved than FSH and oestradiol measurements.

Keywords: *advanced reproductive age, anti-Müllerian hormone, Müllerian inhibiting substance, ovarian reserve*

Introduction

Müllerian inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), has recently emerged as an accurate clinical marker of ovarian reserve. Serum concentrations significantly correlate with baseline antral follicle counts, poor response to ovarian stimulation, IVF cycle cancellation, the number of oocytes retrieved at aspiration, the risk for ovarian hyperstimulation syndrome, and oocyte and embryo quality (de Vet *et al*., 2002; Ebner *et al*., 2006; Nakhuda *et al*., 2006; Silberstein *et al*., 2006). While the value of MIS concentration has been repeatedly demonstrated as a good predictive measure of ovarian reserve, the average age of patients in these reports is relatively young, with no study reporting a mean age older than 36 years (Fallat *et al*., 1997; Seifer *et al*., 2002; van Rooij *et al*., 2002; Fanchin *et al*., 2003; Peñarrubia *et al*., 2005; Ficicioglu *et al*., 2006).

The interpretation of ovarian reserve testing is not directly comparable between younger and older patients (Barnhart and Osheroff, 1999), and therefore it remains to be seen how well MIS concentration correlates with ovarian response in an older group of patients not represented by previous studies. The ovarian response to gonadotrophins in women of advanced reproductive age is especially heterogeneous and not reliably predicted by traditional markers (Keck *et al*., 2005). Even in the presence of apparently normal ovarian reserve, older women are more likely to fail assisted reproduction (Urman *et al*.,

2005). Thus, if MIS concentration can delineate the fate of older women seeking assisted reproduction beyond that predicted by traditional markers, it could serve an important prognostic role in this group. However, before routinely incorporating MIS testing into clinical practice, it is necessary to determine if the correlations between MIS concentration and ovarian reserve, previously demonstrated in general IVF populations, can be specifically validated in older IVF patients.

To determine the predictive value of MIS concentration as a marker for ovarian reserve in women of advanced reproductive age, day-2 serum concentrations of MIS, FSH, LH and oestradiol were measured in patients 37 years and older at the initiation of their IVF cycles. Patients who demonstrated normal baseline FSH and oestradiol concentrations for cycle initiation, yet experienced cycle cancellation due to poor response (group 1), were compared to patients who successfully met criteria for oocyte retrieval (group 2). MIS concentrations were also measured in women of similar age who presented with elevated baseline concentrations of FSH, and were not allowed to begin ovarian stimulation (group 3). In the subset of patients who underwent oocyte retrieval (group 2), the relationship between baseline parameters and IVF outcome was examined. The objective of this study was to determine if the use of MIS data improved the prognostic accuracy in a cohort of IVF patients with an age-related risk for treatment failure, beyond that achieved by measurement of the traditional baseline serum markers.

Materials and methods

This study was approved by the Institutional Review Board of Columbia University Medical Centre. All patients with normal cycles (21–35 days) between the ages of 37 and 45 years who attempted an IVF cycle at the Centre for Women's Reproductive Care at Columbia University between 1 March and 1 May 2006 were considered for the study. Patients with polycystic ovarian syndrome were not included in the study, given the elevated MIS concentrations that have been observed in these patients compared with controls (Cook *et al.*, 2002). Cycle initiation required day-2 FSH, < 15 mIU/ml, oestradiol < 65 pg/ml, normal prolactin, thyroid stimulating hormone and testosterone. Serum FSH, oestradiol, and LH were measured by immunometric assays (Immulite, Los Angeles, CA, USA). Intra- and inter-assay coefficients of variation (CV) were as follows: FSH, 1.9%, 5.0%; LH, 3.6%, 5.0%; oestradiol, 9.3%, 10.5%. For consistency of sampling, MIS assays were performed with day-2 serum specimens. MIS was assayed in duplicate using a commercially available enzyme-linked immunosorbent assay (DSL, Webster, TX, USA). Precision of the MIS assay was independently confirmed in the laboratory with intra- and inter-assay CV of 5.6 and 10.5%, respectively. Assays for FSH, LH, and oestradiol were performed daily, while MIS assays were performed weekly, with serum specimens stored at –20ºC until use. Investigators were blinded to MIS results, but other parameters were available as part of routine clinical care.

All patients were undergoing their first cycle of IVF. Most patients were stimulated by a gonadotrophin-antagonist protocol, with administration of antagonist when the follicles had reached a mean diameter of 14 mm. For some 37-year-old patients, the standard 'long' protocol of GnRH agonist followed by a combination gonadotrophin administration was used, depending on physician preference. If a patient did not have at least three growing follicles, defined by an increase of mean follicular diameter of 1–2 mm per day, by day 7 of stimulation, the cycle was cancelled. When the average follicular diameter reached 18–20 mm, 10,000 IU human chorionic gonadotrophin (HCG) was administered and oocyte retrieval was performed 35 h later. Mature oocytes were fertilized by routine insemination or ICSI. Embryos were cultured for 3 days under standard embryology laboratory conditions. Embryo transfer was performed transcervically under ultrasound guidance. Serum β-HCG was assayed 12 days after transfer and repeated 2 days later if initially positive. β-HCG was then followed weekly until viable pregnancy was confirmed by transvaginal sonogram.

SPSS 13.0 for Windows was used for data analysis. Descriptive statistics are presented as median ± SEM (range). The Mann–Whitney *U*-test or analysis of variance (ANOVA) with Bonferroni correction was used to compare means where appropriate. Spearman correlation coefficients were calculated where appropriate. Using cycle cancellation status as the state variable, receiver operating characteristic (ROC) curves were calculated for the predictor variables. Univariate and multivariate logistic regression were used to determine the best model for prediction of cycle cancellation. Within the group of patients that completed the stimulation cycle, linear regression was performed to calculate a model for predicting the number of oocytes retrieved, and logistic regression was used to determine the best model for predicting clinical pregnancy. Statistical significance was considered to be reached with a *P*-value < 0.05.

Results

Patient characteristics are summarized in **Table 1**. Cancelled patients (group 1, $n = 22$) and those who had successful oocyte retrievals (group 2, $n = 44$) were statistically identical in age, concentration of FSH, oestradiol and LH, and initial gonadotrophin dose. As expected, days of stimulation, total gonadotrophin dose and peak oestradiol concentration were significantly different between these groups ($P < 0.001$). MIS concentrations were significantly lower in patients who experienced cycle cancellation compared with those who met criteria for retrieval (0.20 ± 0.06 ng/ml, 95% CI 0.14–0.26; 1.1 ± 0.17 ng/ml, 95% CI, 0.69–1.49, respectively). There was no difference between groups in regard to the stimulation protocol used (agonist: group 1, $n = 2$; group 2, $n = 4$; antagonist: group 1, $n = 20$; group 2, $n = 40$; Fisher's test).

Using binary logistic regression, MIS concentration was the only significant covariate to correlate with cycle cancellation ($P = 0.003$). To determine predictive values for cycle cancellation, ROC curves were constructed (**Figure 1**). The area under the curve was the greatest for MIS (0.936), followed by age (0.592), FSH concentration (0.598), oestradiol concentration (0.488), and LH concentration (0.448). According to the ROC curve, an MIS cut-off concentration of 0.35 ng/ml rendered the most optimal combination of sensitivity and specificity at 90.1% and 81.8%, respectively. When the MIS concentration was below the threshold value of 0.35 ng/ml, the odds ratio for cycle cancellation was 45 (95% CI 8.7–232). Using multivariate logistic regression, it was determined that no