Original Articles

Reproductive Biology Section

Anti-müllerian hormone is the best predictor of poor response in ICSI cycles of patients with endometriosis

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Summary

Purpose: To correlate ovarian reserve (OR) markers with response in assisted reproduction techniques (ART) and determine their ability to predict poor response among patients with endometriosis (EDT). *Methods:* We evaluated ART cycles of 27 women with EDT and 50 with exclusive male factor. Basal follicle stimulating hormone (FSH) and anti-müllerian hormone (AMH) levels were determined. Ovarian response to gonadotropin stimulation was assessed and correlation coefficients calculated between the variables and reserve markers. Areas under the curve (AUC) determined ability of tests to predict poor response. *Results:* AMH was significantly correlated with response in both groups and it was the only marker with significant discriminative capacity to predict poor response among EDT (AUC = 0.842; 95% CI: 0.651-0.952) and control group (AUC = 0.869; 95% CI: 0.743-0.947). *Conclusion:* Infertile patients with endometriosis can benefit from the pre-therapeutic assessment of OR markers. However, regardless of disease presence, only AMH predicts poor response to stimulus.

Key words: Ovarian Reserve; Endometriosis; Assisted reproduction; Follicle-stimulating hormone; Anti-müllerian hormone; Infertility.

Introduction

Identification of potentially poor responders in assisted reproduction techniques (ART) remains a great challenge, since there is no sufficient evidence supporting an ideal marker of ovarian reserve (OR) or predictor of ovarian response to be routinely used as a counseling tool [1]. Also, the literature reveals a predominance of studies correlating OR markers to ovarian response in groups with heterogeneous causes of infertility [2].

The proposal of individualized complementary assessment opens the possibility of choosing markers of greater or lesser effectiveness for a given population of infertile women, such as those with endometriosis. Sixty percent of women with the disease are estimated to be infertile [3] and even though *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have provided some hope to this population [4-6], worse results are still expected due to negative interference of endometriosis with follicular quality, number of aspirated oocytes, fertilization and embryo implantation [7].

On that basis, we hypothesized that OR markers in infertile patients with endometriosis should present with specific patterns of behavior. This study, then, aimed to correlate basal serum levels of follicle stimulating hormone (FSH) and anti-müllerian hormone (AMH) with ovarian response in intracytoplasmic sperm injection (ICSI) cycles, and to determine their ability to predict poor response among women with the disease.

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Materials and Methods

With approval of the Institutional Research Ethics Committee, we prospectively assessed 227 ICSI cycles in the Sector of Human Reproduction, Faculty of Medicine of Ribeirão Preto, University of São Paulo, from June 1, 2006 to February 28, 2008. Inclusion criteria were: infertility associated with endometriosis and/or male factor; age ≤ 40 years; regular menses; absence of endocrine diseases; and presence of both ovaries. All patients underwent laparoscopy with the same search strategy no more than a year preceding the study; those with endometriosis were classified in stages I/II (minimal/mild) and III/IV (moderate/severe) [8]. Twenty-nine patients with endometriosis (study group) and 50 patients with exclusive male factor (control group) were primarily included; two patients from the study group were later excluded due to previous endometriotic cystectomy; diathermic treatment alone was not considered for exclusion.

Blood samples were collected in a menstrual cycle preceding treatment. FSH was determined by chemoluminescence (Immulite 2000, DPC, Los Angeles, CA) with interassay coefficients of variation from 2.9% to 4.2% and intra-assay coefficients of variation of 4.2%. AMH was determined by an ultrasensitive ELISA (Immunotech Inc, Marseille, France) with inter-asay and intra-assay coefficients of variation of 14.2% and 12.3%, respectively.

All patients were submitted to ovulation induction by a long protocol with leuprolide acetate plus recombinant α-follitropin; endovaginal ultrasound monitoring was started on the seventh day of treatment and doses were readjusted by a step-down protocol, if necessary. A single dose of recombinant chorionic gonadotropin (hCG) was administered when at least three dominant follicles had reached a mean diameter ≥ 18 mm, and oocyte retrieval was performed after 34 to 36 hours.

Table 1. — *Correlations between the basal endocrine markers of ovarian reserve and the response to the exogenous gonadotropic stimulus in assisted reproduction cycles of women with endometriosis and controls (exclusive male factor).*

	Controls (exclusive male factor)						Endometriosis					
	Follicles ≥ 18 mm		Oocytes		Mature Oocytes		Follicles ≥ 18 mm		Oocytes		Mature Oocytes	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Age	–0.3616	0.0125	–0.462	0.0007	–0.3866	0.0055	–0.2284	0.2518	–0.3422	0.0806	–0.3792	0.0511
FSH (mUI/ml)	0.0397	0.7912	–0.0786	0.5875	–0.0743	0.6082	–0.4912	0.0093	–0.5014	0.0077	–0.5318	0.0043
AMH (pmol/l)	0.3793	0.0085	0.5853	< 0.0001	0.5044	0.0002	0.3806	0.0502	0.4386	0.0221	0.4530	0.0177

R = correlation coefficient; FSH = follicle-stimulating hormone; AMH = anti-Müllerian hormone.

Response in ART was analyzed by number of oocytes aspirated [9-13] and counting of dominant follicles on the day of hCG administration [14, 15]. A poor response was considered to be the development of fewer than four dominant follicles on the day of hCG administration or the retrieval of less than four oocytes. Mature oocyte retrieval was also analyzed.

Using GraphPad Prism Software version 5.00 (GraphPad Software, La Jolla, CA), samples with normal distribution were analyzed by the unpaired t-test and Pearson correlation coefficient; the Mann-Whitney test and the Spearman coefficient were used for non-parametric data. Areas under receiver operating characteristic (ROC) curves (AUC) were obtained using the MedCalc Statistical Software version 9.3.7.0 (MedCalc Software, Mariakerke, Belgium); $p < 0.05$ was set as the level of significance.

Results

Patients with endometriosis in Stages I/II and III/IV presented statistically similar results and, therefore were pooled into the study group. There was no significant difference between the study group and controls with regard to age (33.85 ± 3.37 vs 32.98 ± 4.3; $p = 0.4834$), BMI (23.69 ± 3.8 kg/m^2 vs 23.88 ± 3.5 kg/m^2; $p = 0.8397$), or dose of recombinant FSH used (2167 ± 1149 IU vs 2055 ± 709.7 IU; $p = 0.6535$).

A significant negative correlation between FSH and variables of ovarian response was obtained for the study group, but not for controls. AMH, by instance, was positively and significantly correlated with response variables in both groups. A significant correlation was observed between age and response, but only for the control group (Table 1).

Since correlations between OR markers and different criteria of response showed similar behaviors, we adopted the retrieval of fewer than four oocytes as a criterion of poor ovarian response. AMH was significantly lower in poor responders, regardless of the presence of endometriosis; age and FSH were statistically similar for both groups (Table 2).

AMH was the only individual marker with a significant ability for determination of poor response in the study group (AUC = 0.842 [95% CI: 0.651-0.952]), with 87.50% sensitivity and 73.68% specificity, for levels ≤ 10.831 pmol/l, and among controls (AUC = 0.869 [95% CI: 0.743-0.947]), with 88.89% sensitivity and 80.49% specificity, for levels ≤ 9.147 pmol/l. AUC for age and FSH were not significant for either group (Figure 1).

Table 2. — *Age and endocrine markers of ovarian reserve in infertile patients with endometriosis and controls (exclusive male factor), according to total number of retrieved oocytes.*

	< 4 oocytes	≥ 4 ooctes	*p*
Endometriosis			
Age	35.38 ± 3.54	33.21 ± 3.172	0.13
Basal FSH (mIU/ml)	12.27 ± 5.74	8.05 ± 4.1	0.2762
Basal AMH (pmol/l)	4.7 ± 2.11	16.31 ± 2.27	0.0052
Controls (male factor)			
Age	35.22 ± 4.055	32.49 ± 4.243	0.0743
Basal FSH (mIU/ml)	6.26 ± 1.41	6.3 ± 2.7	0.7332
Basal AMH (pmol/l)	4.32 ± 4.91	17.68 ± 12.58	0.0006

The number of oocytes is reported as mean ± standard deviation; FSH = follicle-stimulating hormone; AMH = anti-Müllerian hormone.

Discussion

In the present study we have demonstrated that AMH and FSH levels were significantly correlated with ovarian response in ART cycles for patients with endometriosis, but only AMH was significant for women with male factor for infertility. Studies evaluating normal patients [16], infertile patients due to male factor [10] or pooling heterogeneous groups of infertile patients [17] demonstrated strong correlations between AMH and number of oocytes yielded. In fact, a previous study demonstrated lower basal AMH levels among women with endometriosis, but did not correlate them with response to ART [18]. A weaker but significant correlation between FSH levels and response to stimulus was also noted, in partial agreement with our findings.

We did not detect any study in the literature correlating reserve markers with the response to stimulus or determining the ability to predict a poor response specifically in women with endometriosis. Considering the putative interference of the disease with fertility and the need for better patient counseling, determining not only markers that significantly correlate with the response but also those with the best ability to predict a poor response remains an attractive goal. Our results demonstrated a sharp and significant reduction of basal AMH in poor responders, and AMH was the only marker with the ability to discriminate a poor response among those tested, and reduction occurred regardless of the presence of endometriosis.

An interesting aspect of AMH use as an OR marker should be discussed: even though it has been considered to be the best independent predictor of poor ovarian response to the exogenous stimulus [17, 19], a consensual cut-off value has not been proposed [2, 20]. Our data for

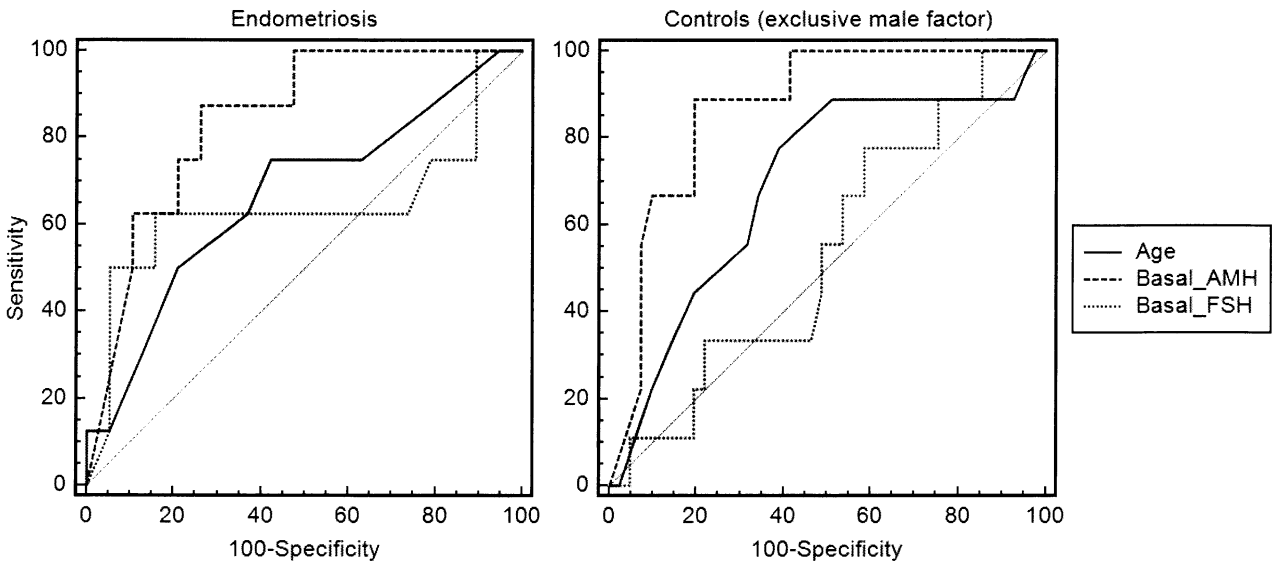

Figure 1. — ROC curves for basal FSH, E2, AMH and age as predictors of a poor response in ART cycles (< 4 oocytes retrieved after exogenous gonadotropic stimulus) for women with endometriosis and exclusive male factor (controls).

the controls are similar to those reported in other studies [12, 21, 22], but due to discrepancies in our data and the small sample we could not determine if there is a specific cut-off value for the population with endometriosis.

We conclude that pre-therapeutic determination of basal AMH and FSH levels can benefit infertile patients with endometriosis. However, only basal AMH presents a significant capacity to pre-therapeutically identify potential poor responders to stimulation in ART cycles, regardless of the presence of endometriosis.

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