American Journal of Obstetrics and Gynecology
Volume 196 • Number 1 • January 2007

Research

Reproductive endocrinology and infertility

Müllerian inhibiting substance and disrupted folliculogenesis in polycystic ovary syndrome

Jeff G. Wang, MD
Gary S. Nakhuda, MD
Michael M. Guarnaccia, MD
Mark V. Sauer, MD
Rogerio A. Lobo, MD

Department of Obstetrics & Gynecology, Division of Reproductive Endocrinology, College of Physicians & Surgeons, Columbia University, New York, NY.

* Reprints: Jeff Wang, MD, Department of Obstetrics & Gynecology, Columbia University, 622 W 168th St PH-16, New York, NY 10032

E-mail address: jw781@columbia.edu

Manuscript received April 16, 2006 , revised manuscript received June 23, 2006 , accepted July 28, 2006

Cite this article as: Wang JG, Nakhuda GS, Guarnaccia MM, et al. Müllerian inhibiting substance and disrupted folliculogenesis in polycystic ovary syndrome. Am J Obstet Gynecol 2007;196:77.e1-77.e5

PII S0002-9378(06)01159-8

Objective

This study determines whether pretreatment levels of müllerian inhibiting substance/antimüllerian hormone (MIS/AMH) would reflect ovarian response to exogenous gonadotropin in women with polycystic ovary syndrome (PCOS) and ovulatory controls matched by age and weight.

Study design

Case-control study of 20 women with PCOS and 10 normoovulatory women undergoing controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) at an academic medical center.

Results

Baseline serum MIS/AMH levels in PCOS were higher than those of normoovulatory women ($P < .001$). MIS/AMH levels increased after gonadotropin-releasing hormone (GnRH) agonist pituitary suppression; 0.5 ng/mL in PCOS ($P = .12$) and 0.7 ng/mL in controls ($P < .02$). In normoovulatory women, MIS/AMH at baseline, after pituitary suppression, and the interval change after pituitary suppression all correlated closely to the number of mature oocytes retrieved ($P < .005$). In PCOS, however, levels of MIS/AMH at baseline and after pituitary suppression did not show this correlation, whereas only the interval change correlated with the number of mature oocytes retrieved.

Conclusion

Baseline MIS/AMH is a good predictor of the ovarian response to COH in normoovulatory women but not in PCOS.

Key words
in vitro fertilization
müllerian inhibiting substance/antimüllerian hormone
polycystic ovary syndrome

In women undergoing treatment for infertility, the number of follicles available for gonadotropin selection at the time of controlled ovarian hyperstimulation (COH) determines the response to ovulation induction. With increasing age, there is a decline in ovarian reserve, and various endocrinologic markers have been used to assess ovarian reserve in an attempt to predict the outcome with assisted reproductive technology. However, the predictive value of these serum hormone parameters such as follicle stimulating hormone (FSH), estradiol (E_2), and inhibin B levels have been imprecise. Serum levels of antimüllerian hormone (AMH), also referred to as müllerian inhibiting substance (MIS), have been introduced as a novel measure of ovarian reserve.[1], [2], [3] Our previous data have shown that higher levels of baseline MIS/AMH, on the second day of the menstrual cycle, is predictive of the response to COH, with higher levels seen in women having hyperstimulation.[4]

MIS/AMH is secreted by granulosa cells of follicles undergoing gonadotropin independent development.[4], [5] Its production begins when follicles are recruited from the primordial pool to become primary oocytes and ends when they reach the final size and differentiation state available for selection by pituitary FSH.[6] On ultrasonography, the number of small antral follicles correlate closely with serum MIS/AMH levels.[7] The highest values of MIS/AMH in women are attained after puberty and subsequently decrease with age, likely reflecting the age-related decline in ovarian reserve.[8] As a marker for ovarian reserve, MIS/AMH correlates positively with ovarian response to COH in normoovulatory women,[1], [2], [3], [4] but this has not been assessed in women with polycystic ovary syndrome (PCOS). In general, however, women with PCOS have exaggerated responses to gonadotropin and have starting doses of gonadotropin modified accordingly.

Women with PCOS are known to have elevated baseline MIS/AMH levels when compared with age-matched normoovulatory women.[9], [10] The elevation begins during puberty and persists throughout the reproductive lifespan.[10], [11] The magnitude of the increase has been found to correlate positively with insulin resistance, serum androstenedione and testosterone levels, and the number of small antral follicles (2- to 5-mm) at ultrasonography.[7] Disturbed dominant follicle selection leading to an excess accumulation of preantral and small antral follicles in women with PCOS presumably causes the elevated MIS/AMH levels. To gain further insight into the relationship between MIS/AMH and ovarian responsivity in PCOS, we investigated the association of MIS/AMH levels at baseline, after pituitary suppression, and the interval change between the 2 time points with the number of mature oocytes retrieved after COH in women with PCOS and in normoovulatory controls.

Materials and Methods

We retrospectively studied 20 women with PCOS undergoing in vitro fertilization (IVF) for ovulatory dysfunction. The Institutional Review Board of Columbia University approved the study protocol. Diagnosis of PCOS was made according to the Rotterdam consensus.[12] They all had oligo- or amenorrhea with normal thyroid-stimulating hormone (TSH), prolactin, day 2 FSH, and E_2 levels, and classic polycystic ovaries on ultrasound. The control group consisted of normoovulatory women with normal ovarian morphology on ultrasound undergoing IVF for male and tubal factors concurrent to the PCOS subjects. They were selected to match the PCOS women group by age and weight. As noted previously, women with PCOS received less gonadotropins.

All women were treated with the long protocol of down-regulation initiated in the early follicular phase that consisted of norethindrone acetate (Duramed Pharmaceuticals, Inc, Pomona, NY) for 7 days and leuprolide acetate (1 mg/d subcutaneously, TAP Pharmaceuticals Inc, Lake Forest, IL) for 2 weeks starting after the first 2 days of norethindrone acetate to minimize potential flare response. Complete pituitary desensitization was confirmed after 14 days by the detection of serum E_2 less than 30 pg/mL with ultrasound examination to exclude ovarian cysts. A standard protocol of recombinant FSH Gonal-F (Serono Inc, Rockland, MA) and Repronex (Ferring Inc, Suffern, NY) was then initiated at a total dosage of 225 IU/d, whereas daily GnRH agonist was continued until the day of human chorionic gonadotropin (hCG) administration. Modification to the

standard protocol was made when there was a risk of hyperstimulation. Ultrasound and blood sampling for E_2 levels were used for monitoring follicular development. The daily FSH doses and timing of hCG administration were adjusted according to the usual criteria of follicular maturation. hCG (10,000 units) was administered when at least 3 follicles with a diameter of 18-20 mm were detected. Oocytes were retrieved 36 hours after hCG administration by transvaginal ultrasound-guided aspiration. All embryo transfers occurred 72 hours later.

Basal hormone levels were determined on day 2-3 of spontaneous or induced menses. Blood samples were obtained for E_2 measurement on the day pituitary desensitization was confirmed. Serum samples from each patient were stored at –20°C for later measurement of MIS/AMH. Serum levels of FSH, luteinizing hormone (LH), E_2, and testosterone were measured by using Immulite 2000 (Diagnostic Products Corp, Los Angeles, CA). MIS/AMH levels from all patients were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratory, Houston, TX) in a single batch. Inter- and intra-assay coefficients of variation were 8% and 6%, respectively.

Statistical analysis was performed with Student t test, Mann-Whitney U test, Fischer exact test, and Wilcoxon signed ranks test as appropriate. Results are expressed as mean ± SEM. Significant relationships between MIS/AMH hormone and the number of oocyte retrieved were evaluated by the Spearman correlation coefficient. A *P* value less than .05 was considered statistically significant. Data were analyzed by Statistical Package for Social Sciences (SPSS version 11.5, SPSS Inc, Chicago, IL).

Results

Patient characteristics, the endocrine profile at baseline and after pituitary suppression, and ovarian response to COH are depicted in Table 1. Women with PCOS were comparable to controls in age, body mass index (BMI), basal FSH, LH, and E_2 levels. Testosterone was significantly higher in PCOS compared with controls both at baseline and after pituitary suppression ($P < .05$ and $P < .05$, respectively). Similarly testosterone/E_2 ratios in women with PCOS were elevated before and after pituitary suppression compared with controls ($P < .05$ and $P < .05$, respectively). Baseline serum MIS/AMH levels in PCOS and controls were 7.0 ± 1.3 ng/mL and 2.1 ± 0.42 ng/mL, respectively ($P < .001$). After pituitary suppression, MIS/AMH remained significantly higher among women with PCOS compared with controls (7.48 ± 1.2 ng/mL vs 2.8 ± 0.6 ng/mL, respectively, $P < .001$). Compared with baseline, MIS/AMH levels during pituitary suppression increased on average by 0.5 ng/mL in PCOS ($P = .12$) and by 0.7 ng/mL in controls ($P < .02$).

TABLE 1 -- Clinical, endocrine parameters, and outcomes of COH (mean and SEM in normoovulatory control subjects compared with women with PCOS

	Controls n = 10	PCOS n = 20	*P*
Clinical parameters			
Age (y)	30 ± 1	30 ± 1	.72
BMI	25.8 ± 2.1	25.2 ± 0.8	.85
Endocrine parameters on day 2			
FSH (IU/L)	3.8 ± 0.5	4.1 ± 0.2	.47
LH (IU/L)	3.9 ± 0.8	6.4 ± 0.9	.08
Estradiol (pg/mL)	36.3 ± 04.5	30.4 ± 2.9	.23
Testosterone (ng/dL)	34.9 ± 3.3	55.5 ± 6.9	.04
Testosterone/E_2	1.1 ± 0.2	1.9 ± 0.3	.04
MIS (ng/mL)	2.l ± 0.4	6.9 ± 1.3	.0001
Endocrine parameters after pituitary suppression			
Testosterone (ng/dL)	29.1 ± 3.2	39.6 ± 3.8	.05
E_2 (pg/mL)	31.0 ± 4.0	28.6 ± 2.2	.63
Testosterone/E_2	1.1 ± 0.2	1.7 ± 0.2	.04
MIS (ng/mL)	2.8 ± 0.6	7.4 ± 1.2	.001
ΔMIS (ng/mL)	0.7 ± 0.2[□]	0.5 ± 0.3	—

Outcomes of COH			
Total gonadotropin used (IU)	2258 ± 83	1793 ± 120	.03
Maximum E_2 (pg/mL)	3109.4 ± 422.5	3814.0 ± 394.1	.31
Number of mature oocytes	18.3 ± 2.6	20.4 ± 2.4	.85
Fertilization rate	0.72 ± 0.04	0.76 ± 0.04	.70
No. of embryos transferred	2.7 ± 0.1	2.4 ± 0.1	.13
Implantation rate[†]	0.32	0.41	.60
Clinical pregnancy rate[†]	0.50	0.55	>.99

P value by Mann-Whitney *U* test unless otherwise specified.

H0 : ΔMIS = 0, *P* < .02 by Wilcoxon signed ranks test.

† *P* value by Fisher exact test.

Women with PCOS received significantly less gonadotropin stimulation than controls (1790 ± 123 IU vs 2258 ± 83 IU, *P* <.03). However, the dose of gonadotropin did not correlate with the number of mature oocytes retrieved in either group (Figure 1). Furthermore, there were no differences in the maximum E_2 (3814.0 ± 394.1 pg/mL vs 3109.4 ± 422.5 pg/mL, *P* = .31), the number of mature oocytes (20.4 ± 2.4 vs 18.3 ± 2.6, *P* = .85) retrieved during COH, the fertilization rates (0.76 ± 0.04 vs 0.72 ± 0.04, *P* = .70), the number of embryos transferred (2.7 ± 0.1 vs 2.4 ± 0.1, *P* = .13), the implantation rates (0.32 vs 0.41, *P* = .60), or the clinical pregnancy rates (0.50 vs 0.55, *P* > .99) between the PCOS or normoovulatory groups, respectively.

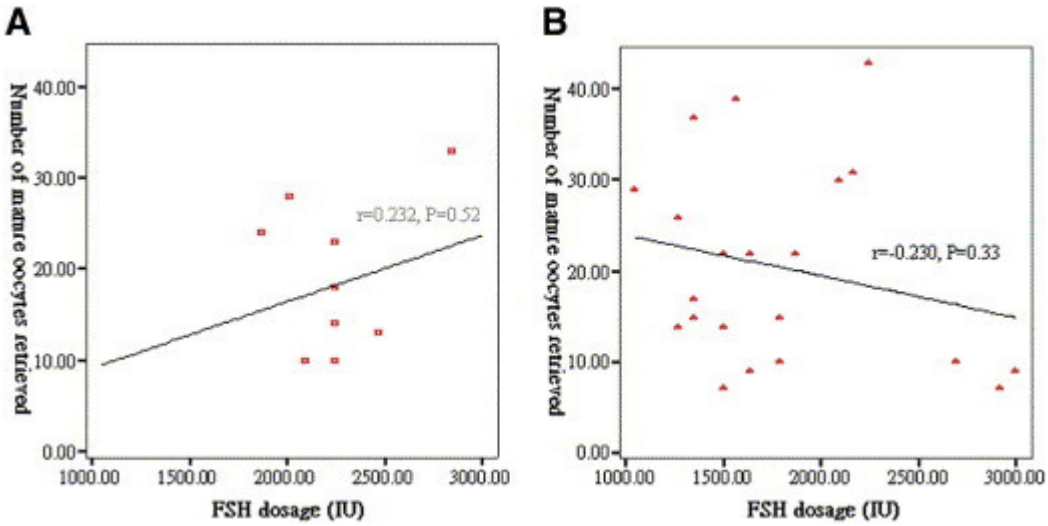

FIGURE 1 Relationship between gonadotropin dosage and total number of oocytes retrieved during COHThe dosage of gonadotropin does not correlate with the number of mature oocytes retrieved during COH in (**A**) normoovulatory women and in (**B**) PCOS. Neither correlation is statistically significant.

No relationship was found between MIS/AMH and BMI, day 2 FSH, E_2, testosterone, or the testosterone/E_2 ratio in either group (data not shown). However, day 2 LH correlated with day 2 MIS/AMH levels in normoovulatory women as well as in PCOS (*r* = 0.74, *P* = .01 and *r* = 0.53, *P* = .02, respectively).

In normoovulatory women, basal MIS/AMH, MIS/AMH after pituitary suppression, and ΔMIS/AMH during pituitary suppression all correlated strongly with the number of mature oocytes retrieved (*r* = 0.80; *P* < .005, *r* = 0.86; *P* < .001, and *r* = 0.89; *P* < .001, respectively). In the PCOS group, COH in patients with MIS/AMH levels in the upper tertile (MIS/AMH > 6.83 ng/mL) did not result in greater numbers of mature oocytes retrieved compared with that of patients in the mid- and lower tertiles (MIS/AMH < 3.88 ng/mL) despite similar dosage of gonadotropins (Table 2). Only ΔMIS/AMH during pituitary suppression was related to the number of mature oocytes retrieved in PCOS (*r* = 0.59, *P* < .006) (Figure 2).

TABLE 2 -- Relationship between MIS levels (in tertiles), gonadotropin dosage, and number of mature oocytes retrieved from COH in PCOS women

MIS levels intertiles (cutoff values)	Average day 2 MIS in each tertile (ng/mL)	Total gonadotropin dose (IU)	Number of mature oocytes retrieved
Lowest tertile (≤ 3.87 ng/mL)	3.31 ± 0.29	1961 ± 283	19 ± 5
Middle tertile (≥3.88 ng/mL, <6.42 ng/mL)	5.32 ± 0.30	1682 ± 122	22 ± 5
Highest tertile (≥ 6.43 mg/mL)	9.06 ± 0.64	1725 ± 241	20 ± 3

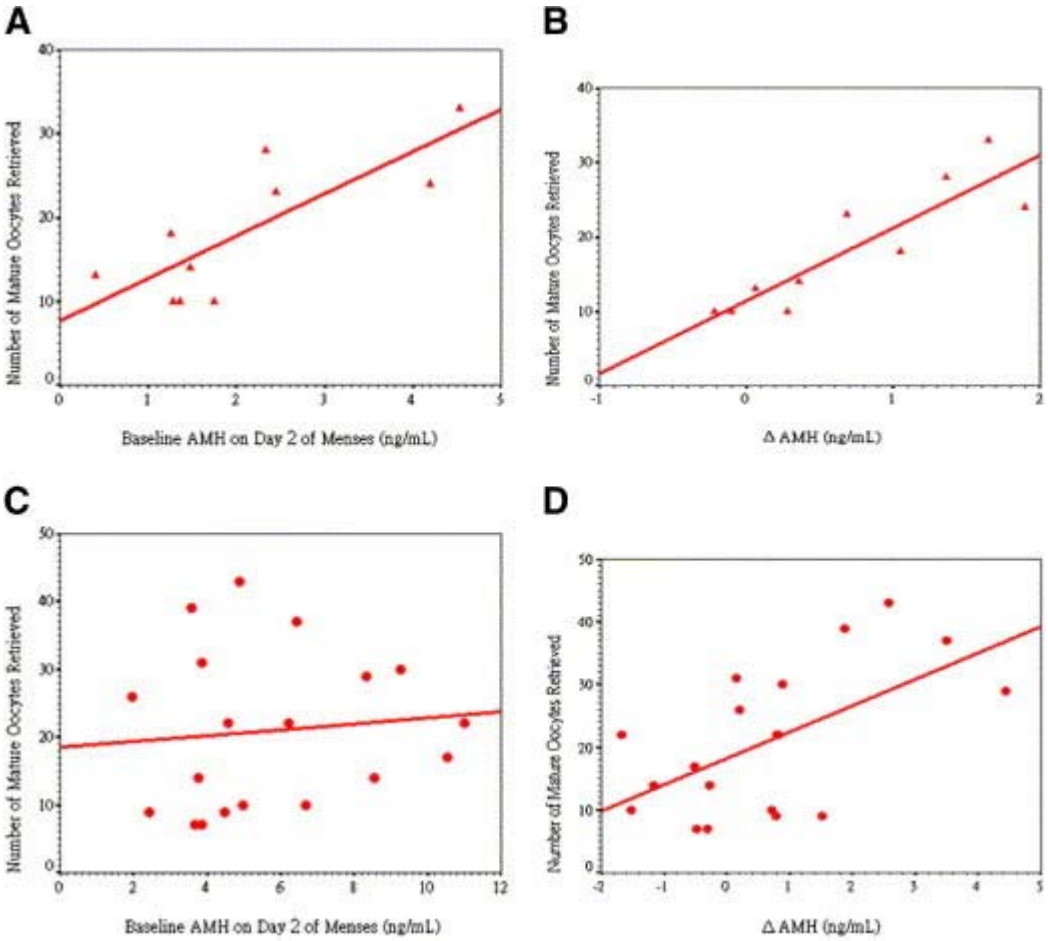

FIGURE 2 Relationship between 2 MIS/AMH, ΔMIS/AMH, and number of mature oocytes retrievedIn normoovulatory controls, the number of mature oocytes retrieved is plotted against (**A**) day 2 MIS/AMH (r = 0.80, P < .005) and (**B**) ΔMIS/AMH (r = 0.89, P < .001). Similarly, in PCOS, the number of mature oocytes is plotted against (**C**) day 2 MIS/AMH (r = –0.06, P < .790) and (**D**) ΔMIS/AMH (r = 0.594, P < .006).

Comments

This study confirms our own and previous data that MIS/AMH levels are increased in PCOS compared with normoovulatory women.[7], [8], [13], [14], [15], [16], [17] The elevated levels in PCOS persisted after pituitary suppression with leuprolide acetate. In accordance with prior studies, MIS/AMH correlated positively with baseline LH and not with BMI.[7], [9], [14] However, the negative relationship between MIS/AMH and basal FSH[7], [14], [17] was not observed in our study. Although some studies have observed a high correlation between MIS/AMH and androgens,[7], [14], [17] others have not.[11], [13], [16] This study did not find a significant relationship between baseline MIS/AMH levels and testosterone. These discrepancies with previous findings may be due to the limited sample size in our study.

As a marker of ovarian responsiveness to COH, our study confirmed the findings of previous studies that pretreatment MIS/AMH is highly associated with the number of mature oocytes retrieved during COH in normoovulatory women.[1], [2], [3], [4], [18] The correlation in this study was strong and achieved a high level of statistical significance despite a small sample size. In addition, we found that MIS/AMH after leuprolide

suppression is also highly correlated with the number of mature oocytes in normoovulatory women.

In contrast to controls, basal MIS/AMH in PCOS showed no relationship with the number of mature oocytes retrieved from COH. The disparity in the dosage of gonadotropins between the 2 groups is a potential confounder that may have contributed to this observation. Theoretically, lowering of the gonadotropin dosage to minimize the well-recognized risk of ovarian hyperstimulation syndrome in the PCOS group may compromise the development of follicles both quantitatively and qualitatively during COH. According to this premise, a true relationship between baseline MIS/AMH levels and ovarian response to COH may not be realized if the maximum potential is not evoked. However, this theory assumes that the lower dosage of gonadotropins used in the PCOS group is below the threshold needed to select all available antral follicles at the inception of the menstrual cycle.

The validity of this assumption is questionable on the basis of the observation that higher doses of gonadotropins during COH did not result in greater number of mature oocytes retrieved in either the normoovulatory or the PCOS group, suggesting that the gonadotropin stimulation dosage in PCOS women, albeit lower, was sufficient for optimal follicular development. In PCOS women, stratification of baseline MIS/AMH levels showed no relationship with the number of oocytes retrieved from COH despite similar gonadotropin dosage across all groups.

Hence, we hypothesize that the lack of correlation between basal MIS/AMH and the number of mature oocytes retrieved after COH in the PCOS group is, at least in part, due to the disruption in folliculogenesis leading to an excess accumulation of preantral and small antral follicles,[19] which may not have attained the maturity to respond to FSH but nevertheless contributed to an overall rise in MIS/AMH. Indeed, although women with PCOS have increased total numbers of antral follicles between 2- to 9-mm, the size distribution of these follicles is significantly shifted toward smaller antral (2- to 5-mm range) follicles, rather than the larger antral follicles (6- to 9 mm) of normoovulatory controls.[20] As a result, higher basal MIS/AMH levels in women with PCOS can reflect a true increase in the number of FSH-selectable follicles as in the case of normoovulatory women as well as a more severe degree of dysfunction in follicular development at the preantral stage. The culmination of these 2 factors renders basal MIS/AMH a poor correlate of ovarian response to COH in PCOS women.

Dynamic changes in MIS/AMH reflect the net shift in ovarian follicle distribution during folliculogenesis. Three types of follicles do not secrete MIS/AMH: (a) primordial, (b) atretic, and (c) large antrals selected by FSH for dominance. Although all other developing follicles between the primary and large antral stages secrete MIS/AMH, secondary and small antral follicles less than 5 mm have the highest secretion per follicle, based on immunohistochemical data.[6] Recruitment of primordial follicles to become primary follicles and the continual development of primary to secondary/small antral follicles both result in net increases in MIS/AMH. Atresia/apoptosis of developing follicles at any stage would result in a net decrease in MIS/AMH. Hence, an equilibrium between follicular development and follicular atresia would result in a dynamic MIS/AMH (ΔMIS/AMH) of zero. A positive ΔMIS/AMH reflects folliculogenesis in excess of atresia that would contribute to a larger cohort of FSH-selectable follicles and a heightened response to COH. Conversely, a negative ΔMIS/AMH reflects follicular atresia in excess of folliculogenesis and thus leading to diminished response to COH.

ΔMIS/AMH during pituitary suppression was evaluated for the first time in PCOS and normoovulatory women, although this change only achieved statistical significance in normoovulatory women. During pituitary gonadotropin suppression, FSH-dependent follicular growth is eliminated, and ΔMIS/AMH secretion represents gonadotropin independent folliculogenesis. An excess in follicular development over follicular atresia ultimately results in a greater pool of large antral follicles. Hence, ΔMIS/AMH during pituitary suppression would be expected to vary positively with ovarian response to COH. Indeed, ΔMIS/AMH not only correlated significantly with the number of mature oocytes retrieved in normoovulatory women but achieved the highest correlation coefficient compared with the static measures of MIS/AMH at baseline or after lupron suppression.

As discussed previously, the ability to use basal MIS/AMH as a marker for ovarian response to COH in PCOS is modulated by the degree of disruption or arrest of folliculogenesis. However, ΔMIS/AMH is a direct marker of folliculogenesis and therefore should not be significantly influenced by disruptions in folliculogenesis. Although an excess accumulation of preantral/small antral follicles would raise the MIS/AMH level without necessarily conferring a larger pool of FSH-selectable follicles, ΔMIS/AMH would nevertheless be zero if there was no net increase in follicular development or even negative if there was a net loss caused by atresia. In our study, ΔMIS/AMH was the only measure in PCOS that correlated with the number of mature oocytes retrieved after COH.

In conclusion, our data of dynamic changes in MIS/AMH provide new insight into the disruption of folliculogenesis in PCOS. Although MIS/AMH is a good predictor of the ovarian response to COH in normoovulatory women, this was not the case in PCOS. The arrest of follicular growth and the heterogeneity of FSH sensitivity among MIS/AMH-secreting follicles in PCOS render baseline measures of MIS/AMH a poor predictor of the gonadotropin response in PCOS.

REFERENCES:

1 de Vet A., Laven J.S., de Jong F.H., Themmen A.P., Fauser B.C.: Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 77. 357-362.2002; Abstract
2 Seifer D.B., MacLaughlin D.T., Christian B.P., Feng B., Shelden R.M.: Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril* 77. 468-471.2002; Abstract
3 van Rooij I.A., Broekmans F.J., te Velde E.R., et al: Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 17. 3065-3071.2002; Abstract
4 Nakhuda G.S., Chu M.C., Wang J.G., Sauer M.V., Lobo R.A.: Elevated serum mullerian-inhibiting substance may be a marker for ovarian hyperstimulation syndrome in normal women undergoing in vitro fertilization. *Fertil Steril* 85. 1541-1543.2006; Abstract
5 Visser J.A., Themmen A.P.: Anti-Mullerian hormone and folliculogenesis. *Mol Cell Endocrinol* 234. 81-86.2005; Abstract
6 Weenen C., Laven J.S., Von Bergh A.R., et al: Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 10. 77-83.2004; Abstract
7 Pigny P., Merlen E., Robert Y., et al: Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab* 88. 5957-5962.2003; Full Text
8 Hudson P.L., Dougas I., Donahoe P.K., et al: An immunoassay to detect human mullerian inhibiting substance in males and females during normal development. *J Clin Endocrinol Metab* 70. 16-22.1990; Abstract
9 Chu M.C., Carmina E., Wang J., Lobo R.A.: Mullerian-inhibiting substance reflects ovarian findings in women with polycystic ovary syndrome better than does inhibin B. *Fertil Steril* 84. 1685-1688.2005; Abstract
10 Piltonen T., Morin-Papunen L., Koivunen R., Perheentupa A., Ruokonen A., Tapanainen J.S.: Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. *Hum Reprod* 20. 1820-1826.2005; Abstract
11 Siow Y., Kives S., Hertweck P., Perlman S., Fallat M.E.: Serum mullerian-inhibiting substance levels in adolescent girls with normal menstrual cycles or with polycystic ovary syndrome. *Fertil Steril* 84. 938.2005; Abstract
12 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 81. 19.2004;
13 Cook C.L., Siow Y., Brenner A.G., Fallat M.E.: Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 77. 141-146.2002; Abstract
14 Eldar-Geva T., Margalioth E.J., Gal M., et al: Serum anti-Mullerian hormone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogenism. *Hum Reprod* 20. 1814-1819.2005; Abstract
15 Fallat M.E., Siow Y., Marra M., Cook C., Carrillo A.: Mullerian-inhibiting substance in follicular fluid and serum: a comparison of patients with tubal factor infertility, polycystic ovary syndrome, and endometriosis. *Fertil Steril* 67. 962-965.1997; Abstract
16 La Marca A., Orvieto R., Giulini S., Jasonni V.M., Volpe A., De Leo V.: Mullerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics. *Fertil Steril* 82. 970-972.2004; Abstract
17 Laven J.S., Mulders A.G., Visser J.A., Themmen A.P., De Jong F.H., Fauser B.C.: Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *J Clin Endocrinol Metab* 89. 318-323.2004; Full Text
18 Fanchin R., Schonauer L.M., Righini C., Guibourdenche J., Frydman R., Taieb J.: Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 18. 323-327.2003; Abstract
19 Webber L.J., Stubbs S., Stark J., et al: Formation and early development of follicles in the polycystic ovary. *Lancet* 362. 1017-1021.2003; Abstract
20 Jonard S., Robert Y., Cortet-Rudelli C., Pigny P., Decanter C., Dewailly D.: Ultrasound examination of polycystic ovaries: is it worth counting the follicles?. *Hum Reprod* 18. 598-603.2003; Abstract