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Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance

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Objective: To examine Mullerian Inhibiting Substance (MIS) as an emerging diagnostic marker of ovarian function.

Design: Medline review of published studies pertaining to the role of MIS in assessing ovarian aging, predicting response to ovulation induction in preparation for in vitro fertilization, assessing risk of developing ovarian hyperstimulation (OHSS) before ovulation induction, and diagnosis of polycystic ovarian disease (PCOS).

Result(s): The majority of published studies to date support a role for MIS as a marker of ovarian reserve. Specific cut-off values are dependent upon the particular assay used. Mullerian Inhibiting Substance may offer value in assessing risk of OHSS and diagnosis of PCOS.

Conclusion(s): Potential advantages of MIS compared with other conventional markers of ovarian reserve include: 1) MIS is the earliest marker to change with age; 2) it has the least intercycle variability; 3) it has the least intracycle variability; and 4) it may be informative if randomly obtained during the cycle. Widespread clinical use of MIS may await the availability of an international standard for MIS so that results using different assays may be reliably compared. (Fertil Steril® 2007;88:539–46. ©2007 by American Society for Reproductive Medicine.)

Key Words: MIS, AMH, ovarian reserve, ovarian aging, IVF, PCOS, OHSS

The present review examines Mullerian Inhibiting Substance (MIS), also known as antimullerian hormone (AMH), as an emerging diagnostic marker of ovarian function. Mullerian Inhibiting Substance is a homodimeric glycoprotein growth factor belonging to the TGF-beta family (1, 2). It is a large glycoprotein with a molecular weight of 140 kDa and is four times larger than LH or FSH. The gene for MIS/AMH is found on chromosome 19 p13.3 (3). The biologic activity of this growth factor requires interaction with two plasma membrane serine-threonine kinase receptors termed type I and type II. The type II receptor is believed to be responsible for ligand binding, and the type I heteromer is the signaling receptor

via cross-phosphorylation of the receptors as well as other substrates.

The existence of MIS/AMH was first proposed in 1947 by Professor Alfred Jost who showed that a component of testes other than testosterone, which he called a *mullerian l'hormone inhibitrice*, was responsible for destroying the mullerian ducts in developing male embryos (4, 5). It was not for nearly another 20 years that two independent research laboratories began efforts to purify the protein. The Donahoe group (6) and, for a time, Dr. Josso's laboratory (7) called the protein mullerian-inhibiting substance, as it was named earlier by Dr. Jost. Later Josso's laboratory coined the name antimullerian hormone, which is widely in use today. In this article we will use MIS and AMH interchangeably.

The clinical significance of MIS/AMH has for decades been limited to its critical role in fetal sexual development. However, owing to a combination of both basic and clinical research advances within the last 15 years, MIS/AMH has emerged to have increasing relevance with regard to ovarian function. Some of these basic advances have included noting that the MIS/AMH gene has a sexually dimorphic pattern of expression (1, 2) and the discovery of MIS/AMH synthesis by human granulosa cells (8). In addition, a number of investigators developed tests to measure MIS/AMH in biologic fluids, including serum. Initial tests were radioimmunoassays

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(9, 10) using polyclonal antibodies raised to MIS/AMH purified from bovine testes. These tests were very useful in many research settings, but they were of limited value in the clinical arena, because they did not measure the human protein with the precision necessary to be of great value. The development of highly specific and sensitive ELISAs to measure MIS/AMH in human biologic fluids (11–13) set the stage for studies of the protein in a variety of clinically important settings. Cloning the human gene in the mid 1980s, first by Cate et al. (14), made it possible to produce sufficient recombinant human protein to design highly specific and sensitive monoclonal antibody-based ELISAs (11–13). Several variants of these assays were commercialized in Europe and the United States and marketed by Immunotech, Beckman-Coulter, and Diagnostic Systems Laboratories. Numerous reports studying serum MIS/AMH levels in ambiguous genitalia, gynecologic oncology (1–2), and assisted reproduction, as well as other areas, began to document the usefulness of including serum MIS/AMH measurements in patient management decisions.

In this review we will examine the role MIS/AMH contributes to assessing ovarian aging, predicting response to ovulation induction in preparation for in vitro fertilization (IVF), assessing risk of developing ovarian hyperstimulation syndrome (OHSS), and diagnosis and response to treatment for polycystic ovarian disease (PCOS; Table 1). Despite the fact that the data summarized here used either of the commercial ELISAs or a research assay (12), there is strong qualitative agreement among the results. The absolute level of MIS/AMH varies, however, because there is no standard reference compound for the protein, and assay sensitivities vary as well. To preserve continuity of data in longitudinal studies and until such time as a protein standard exists, it is suggested that investigators select the ELISA assay that best suits their needs and use it exclusively for their studies.

GONADAL ORIGINS OF MIS/AMH

In the male, MIS/AMH is produced initially by Sertoli cells during fetal sex differentiation, resulting in complete regression of the mullerian ducts (1, 2), and its synthesis continues throughout life. In the female, granulosa cells from primary and preantral follicles produce MIS only after birth. MIS/AMH synthesis ceases with menopause. It is not detectable in the serum at birth, and serum MIS/AMH is reliably measured as reproductive potential is reached (11–13). One of the roles for MIS/AMH after birth appears to be inhibition of steroidogenesis in testes (15, 16) and ovaries (17). In ovaries, another function is to inhibit initial follicle recruitment as well as FSH-dependent growth and selection of preantral and small antral follicles (18). Mullerian Inhibiting Substance was first demonstrated to be present in the follicular fluid of women undergoing IVF, suggesting that it has an autocrine role in follicular maturation (8). Human antral follicles measuring less than 6 mm express the greatest amount of MIS/AMH, whereas follicles 8 mm or greater, theca cells, and atretic follicles have virtually undetectable levels (19, 20). Therefore, MIS/AMH is believed to impede the transi-

TABLE 1

Potential roles of serum MIS/AMH in gynecology.

1. Assessing ovarian reserve
 - General population
 - Infertile population before undergoing ovulation induction
 - Before and after cancer therapy
2. Assessing the risk of ovarian hyperstimulation before ovulation induction
3. Diagnosis and surveillance of PCOS therapy
4. Surveillance of granulosa cell tumor
5. Ambiguous genitalia

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tion from primordial follicles into growing antral follicles in both mice and humans. In women, MIS/AMH has not yet been shown to have biologic action outside the adult human ovaries, although MIS type II receptors have been detected in adult tissues of mullerian origin (21, 22).

CLINICAL STUDIES OF OVARIAN AGING AND OVARIAN RESERVE

Clinical research examining serum MIS/AMH as a marker of ovarian function and/or reserve was initiated after the initial discovery in 1999 that MIS/AMH-null mice are born with a normal number of follicles but have an accelerated decline in their primordial pool (23). Clinical investigators that followed this discovery have examined serum MIS/AMH in a variety of study designs, using different subject populations and several types of ELISA assays. There are a few studies that have investigated serum MIS/AMH in the general population of fertile women, but the vast majority of clinical studies have been in women with a known history of infertility who are undergoing ovulation induction in preparation for assisted reproduction. The following discussion focuses upon clinical studies in assessing changing ovarian function with natural aging in both basal and stimulated conditions, i.e., ovulation induction in preparation for IVF.

In 2002, de Vet et al. (24) prospectively followed 41 normal ovulatory premenopausal women (mean age 29 years) over a mean of 2.6 years (range 1–7 years.) They noted a 38% decline in mean serum day 3 MIS/AMH levels from 2.1 ng/mL to 1.3 ng/mL despite an absence of an interval change in number of antral follicles, serum FSH, or inhibin B. Van Rooij et al. (25) in a prospective longitudinal study of 81 healthy volunteers with proven fertility (mean age 39.6 years) over an interval of 4 years noted mean day 3 serum MIS/AMH to decline 58% (1.2 ng/mL to 0.5 ng/mL). Mullerian Inhibiting Substance demonstrated the most consistent change with antral follicle count (AFC) and was the only marker to demonstrate a mean longitudinal decline over time compared with serum FSH, inhibin B, and E₂.

These two prospective longitudinal studies from the Netherlands (24, 25) examining interval changes in women from the general population demonstrate consistent findings of a decrease in serum MIS/AMH over time. In both studies, interval changes in more conventional markers, i.e., serum FSH, inhibin B, and E₂ or AFC, did not demonstrate as remarkable a change as MIS/AMH over the same period. Both studies support the concept of a temporal sequence of serial change in early follicular ovarian biomarkers with aging. The earliest change begins with a decline in serum MIS/AMH, followed by a decline in inhibin B, followed by a later rise in serum FSH. While it is clear that this sequential serial change occurs in early follicular serum markers, the precise timetable for these changes remains to be described.

Tremellen et al. (26) from Australia examined cross-sectional changes in early follicular (days 3–5) MIS/AMH levels among 238 women from a general infertility population. They found that mean MIS/AMH levels remained relatively stable, between 2.0 and 2.5 ng/mL, from 18 to 29 years of age, followed by a decline to 1 ng/mL by age 37. Despite a 50% decline in MIS/AMH levels between 29 and 37 years of age, they noted minimal changes in FSH over the same time interval. Postmenopausal or postoophorectomy women have undetectable serum MIS/AMH levels (11–13). Van Rooij et al. (27) examined ovarian markers of ovarian reserve, i.e., FSH, inhibin B, E₂, MIS/AMH, and AFC, in 81 volunteer normally cycling women between ages 25 and 46 who visited at two time points with a mean interval of 4 years. In 14 women, their cycle became irregular at the time of their second visit. Mullerian Inhibiting Substance, AFC, and age demonstrated the greatest predictive accuracy for the onset of an irregular cycle. A receiver operating characteristic (ROC) curve analysis showed area under the curve (ROC_{AUC}) values of 0.87, 0.80, and 0.82, respectively. After adjusting for age, only MIS/AMH and inhibin B were significantly associated with cycle irregularity and the onset of the menopausal transition within 4 years (27).

Most recently, Freeman et al. (28) followed a cohort of healthy late reproductive age (mean age 45.8 years) obese (body mass index [BMI] ≥ 30 kg/m²) and nonobese women over 8 years and noted a significant inverse association between MIS/AMH levels and BMI. Obese women had 65% lower mean MIS/AMH levels than nonobese women. Although the investigators pointed out that the underlying mechanism responsible for the relationship between obesity and MIS/AMH is not clear, they speculated that obesity might be associated with decreased ovarian reserve and/or with follicular dysfunction.

Unlike conventional early follicular phase markers such as serum FSH, inhibin B, and E₂, MIS/AMH serum levels appear to be independent of gonadotropin secretion. Investigators have demonstrated negligible variation of MIS/AMH in response to GnRH agonist (29, 30) or pregnancy (31). Consistent with these findings is the minimal variation of MIS/AMH throughout the menstrual cycle as confirmed by four clinical studies (32–35). Fanchin et al. (36) demonstrated that MIS/AMH has the greatest reproducibility with the least

variation between cycles of any of the ovarian serum markers, including FSH, inhibin B, or E₂. Because the collection of serum samples at exact times in the early follicular phase is rarely feasible, the relative minimal intra- and intercycle variation of MIS/AMH offers its potential utility as a unique untimed single marker of ovarian reserve.

Seifer et al. (37), on behalf of the Women's Interagency HIV Study, recently demonstrated that MIS/AMH values from early follicular and other random abnormal cycle phases taken 6 weeks before and 3–6 months after early follicular (days 2–5) index samples were highly correlated with each other ($r > 0.93$; $P < .0001$). Collectively, these studies (29–37) suggest that MIS/AMH may offer a simplified method of assessing ovarian function without regard to menstrual timing. Most recently, La Marca et al. (38) demonstrated that an MIS/AMH serum sample on any day of the menstrual cycle predicted ovarian response in IVF. Data from 48 women (28 during the follicular phase and 20 during the luteal phase) revealed lower MIS/AMH levels associated with increased cycle cancellation rates and fewer retrieved oocytes (i.e., less than 4). Using an MIS/AMH threshold value of 0.5 ng/mL, the sensitivity was 85% and specificity 82.3% in predicting poor response. The findings of this initial study are promising, and additional prospective clinical studies examining the accuracy of a single random serum MIS/AMH in predicting ovarian reserve are expected in the next several years.

The concomitant reduction in serum MIS/AMH with age, as noted in these studies, can be directly tied to specific clinical outcomes when examined in the context of IVF. The first publication noting an association between early follicular serum MIS/AMH and ovarian response was by Seifer et al. in 2002 (39). A retrospective analysis of 107 women undergoing ovulation induction in preparation for IVF demonstrated a 2.5-fold greater mean serum concentration of MIS/AMH (2.5 ng/mL) if 11 or more oocytes were retrieved compared with women who had six or fewer oocytes retrieved (1.0 ng/mL). The mean age of women in that study was 35 years old. Thus, higher day 3 mean serum MIS/AMH concentrations were shown to be associated with greater number of retrieved oocytes.

This work was corroborated by a prospective study by van Rooij et al. (29), who noted serum MIS/AMH levels to be highly correlated with number of antral follicles and number of retrieved oocytes in 130 women, mean age 34 years, undergoing ovulation induction in preparation for IVF. An ROC analysis showed MIS/AMH and AFC to have similar predictive value for a poor response of fewer than four oocytes or cycle cancellation after ovulation induction for IVF. The investigators concluded that a poor response in IVF, indicative of diminished ovarian reserve, is associated with reduced baseline basal serum MIS/AMH.

Fanchin et al. (40) followed with a prospective study comparing early follicular phase serum MIS/AMH with that of FSH, inhibin B, and E₂ in 75 women, mean age 34 years, undergoing ovulation induction for IVF. Using high-resolution

ultrasound they found little correlation between concentrations of E₂, inhibin B ($r = 0.29$), or FSH ($r = -0.29$) and AFC. However, a highly significant correlation was noted between MIS/AMH and number of antral follicles ($r = 0.74$; $P < .0001$). The authors suggested that MIS/AMH may reflect ovarian follicular status better than conventional hormone markers, namely FSH, inhibin B, or E₂. Muttukrishna et al., in both retrospective (41) and prospective (42) studies, noted MIS/AMH to be the single most useful marker with the greatest association with follicle number and number of eggs retrieved (42) as well as a predictor of poor response using a cut-off of 0.2 ng/mL based on ROC analysis (41).

In another study, Fanchin et al. (30) demonstrated that during ovulation induction there is a decline in the concentration of MIS/AMH, in contrast to rising E₂ and inhibin B, which parallels the decreasing number of preantral follicles growing to become larger preovulatory follicles. This is consistent with previous immunostaining of the human ovary that illustrated that the greatest production of MIS/AMH is from granulosa cells within small preantral follicles 2–6 mm in diameter, with less production within follicles greater than 6–8 mm (20). This is also consistent with a more recent study demonstrating that MIS/AMH concentrations are two to three times higher in the follicular fluid of 3–8 mm small follicles compared with MIS/AMH levels in the follicular fluid of large preovulatory follicles (43).

Four studies have examined MIS/AMH as a predictor of pregnancy. Two studies support an association between MIS/AMH and pregnancy, and two studies do not but do support an association with ovarian response. Hazout et al. (44) retrospectively analyzed 109 consecutive day 3 serum samples from women younger than 42 years of age, mean age 33, who were undergoing ovulation induction for IVF. Mean serum values for clinical pregnancy (cardiac activity by transvaginal ultrasound) were greater than two times those of women who did not become pregnant (2.4 ng/mL vs. 1.1 ng/mL, respectively). Multivariate regression analyses demonstrated that day 3 serum MIS/AMH had the greatest independent contribution to predicting pregnancy. Other IVF outcomes, such as number of retrieved mature oocytes and number and quality of embryos obtained, were also associated with higher MIS/AMH serum levels despite similar day 3 FSH, inhibin B, and E₂ levels.

Eldar-Geva et al. (45) prospectively studied 56 women less than 38 years old (mean age 30 years) with normal day 3 FSH levels (<10 IU/L), measuring serum MIS/AMH, E₂, and inhibin B before and 24 hours after administration of 300 IU recombinant FSH on cycle day 3–4 and during the luteal phase. They noted that the only predictor for ongoing pregnancy was follicular- or luteal-phase MIS/AMH.

Penarrubia et al. (46) retrospectively examined 80 women, mean age 35 years, undergoing their first IVF/ICSI cycle to assess if day 3 basal serum MIS/AMH and/or MIS/AMH levels on the fifth day of gonadotropin therapy predicted ovarian response and pregnancy. In this study basal and day

5 serum MIS/AMH concentrations were significantly lower in the canceled than in the cycling group. An ROC analysis showed that MIS/AMH on day 5 of gonadotropin therapy was a better predictor of ovarian response than basal MIS/AMH. However, MIS/AMH was not useful in the prediction of pregnancy.

Ficiocioglu et al. (47) prospectively studied 50 women undergoing IVF. Mean serum MIS/AMH levels of women (average age 31 years) with five or more retrieved oocytes were higher than those with fewer than five eggs. The number of mature oocytes, AFC, and maximum E₂ levels were greater in women with high day 3 MIS/AMH levels despite similar ages and day 3 FSH and E₂. The most sensitive and specific indicator of retrieved oocytes was MIS/AMH, followed by number of antral follicles followed by age. However, statistical analysis did not support MIS/AMH as predictive of pregnancy defined by a positive beta-hCG.

Although those two studies do not demonstrate that MIS/AMH is predictive of pregnancy (46, 47), there are two studies showing better quality of retrieved oocytes (48) and higher-quality embryos for transfer (49) associated with MIS/AMH serum levels. Ebner et al. (48) prospectively examined oocytes from 141 women undergoing ICSI and noted higher serum MIS/AMH levels associated with better oocyte appearance. Oocytes were assessed for anomalies that included central granulation of the cytoplasm, refractile bodies, dark incorporations, vacuoles, aggregation of smooth endoplasmic reticulum, and perivitelline space granularity. Cycle cancellation rate was correlated with MIS/AMH levels and best-quality oocytes were associated with median MIS/AMH values (1.66–4.52 ng/mL) compared with those women with MIS/AMH levels less than 1.66 ng/mL or greater than 4.52 ng/mL. Fertilization and cleavage to blastocyst stage were not influenced by MIS/AMH levels.

Silberstein et al. (49) found embryos of better morphology and cleavage in women with mean MIS/AMH levels greater than 2.7 ng/mL than below this threshold. However, it is noted that MIS/AMH was sampled on the day of hCG administration and not in early follicular phase. Most recently, Smeenk et al. (50) using a multivariate regression analyses, noted that day 3 serum MIS/AMH was predictive of number of oocytes and embryos but not predictive of embryo quality or pregnancy rates.

Another potential use for serum MIS/AMH as a marker of ovarian reserve in addition to naturally aging and healthy women with infertility will be for women undergoing cancer treatment. As survival rates continue to improve and the prevalence of cancer increase, the assessment of reproductive function before and after treatment for various cancers becomes increasingly relevant. Investigators from Edinburgh have published two clinical studies in women treated for cancer. Bath et al. (51) demonstrated reduced serum MIS/AMH levels but not reduced inhibin B levels in young women treated for childhood cancer while still retaining ovarian

function despite chemotherapy. Anderson et al. (52) assessed markers of ovarian reserve in premenopausal women who underwent chemotherapy or gonadotropin suppressive therapy for breast cancer. Changes in serum MIS/AMH levels were demonstrated to show evidence of gonadotoxicity during chemotherapy more dramatically than E₂ or inhibin B. These data suggest that MIS/AMH may be a marker of ovarian injury, potentially allowing assessment of gonadotoxicity of different chemotherapy regimens.

CLINICAL STUDIES OF OVARIAN HYPERSTIMULATION SYNDROME AND POLYCYSTIC OVARY SYNDROME

The association of a dose-response relationship existing between serum MIS/AMH levels and ovarian response to ovulation induction raises the natural question of whether an exaggerated response to ovulation induction might result from high basal MIS/AMH levels. In other words, could elevated serum MIS/AMH be associated with a greater probability of developing OHSS as a complication of ovulation induction? Tremellen et al. (26) in a subgroup analysis of 16 patients with 18 or more retrieved oocytes noted a trend but not a statistically significant difference ($P=.052$) in mean day 3–5 MIS/AMH levels between those who hyperstimulated and those who did not. However, Nakhuda et al. (53) noted that baseline serum MIS/AMH levels in women with OHSS was six times higher than in those with a normal response to gonadotropins (3.6 ng/mL vs. 0.63 ng/mL, respectively; $P<.004$). That report suggests that MIS/AMH could potentially be a marker for determining before ovulation induction women at risk for developing OHSS.

Another clinical situation that has been associated with elevated serum MIS/AMH levels is PCOS. Fallat et al. (54) first noted in 1997 that women with PCOS had higher serum and follicular fluid MIS/AMH levels than women with tubal factor or endometriosis who were undergoing retrieval for IVF. The same group of investigators (55) prospectively compared 27 women with PCOS with 20 women with normal menstrual cycles and noted higher serum MIS/AMH levels in women with PCOS.

Pigny et al. (56) examined serum MIS/AMH in 59 women with PCOS and 45 women with normal menstrual cycles. They reported a twofold increase in serum MIS/AMH levels in the women with PCOS. They further speculated that increased MIS/AMH tone within the cohort would lead to the follicular arrest of PCOS, by inhibiting FSH at the time of selection. The same group of investigators (57) compared the serum MIS/AMH of 73 women meeting the Rotterdam criteria for PCOS with that of 96 women with normal menses. They reported a threefold higher concentration of serum MIS/AMH in those with PCOS. Furthermore, they suggested that serum MIS/AMH levels could be substituted for follicle count as a diagnostic criterion in the Rotterdam definition of PCOS when accurate sonographic data are not available. Studies by Laven et al. (58) and Chu et al. (59), comparing women with PCOS with normo-ovulatory women, corroborate

the findings of the studies by the Fallat (54, 55) and Pigny (56, 57) groups.

Elevated serum MIS/AMH levels in PCOS is apparently not restricted to adult women. Adolescent girls (ages 12–18 years) with PCOS have elevated serum MIS/AMH values when compared to control subjects (60). Those data suggest that PCOS shows evidence of altered folliculogenesis during adolescence, which is associated with elevated serum MIS/AMH levels. Furthermore, girls (ages 4–7 years) born to women with PCOS have elevated serum MIS/AMH values compared with control subjects (61), suggesting that altered folliculogenesis in PCOS may have a genetic component.

Two additional clinical studies demonstrated that metformin reduces serum MIS/AMH levels in women with PCOS (62, 63), thus further supporting an association between MIS/AMH and the pathogenesis of PCOS. Interestingly, some of the studies (63, 64) suggest that women with PCOS may have a prolonged reproductive life span, because their serum MIS/AMH remains detectable beyond normally cycling women of similar age.

A better understanding of the association between MIS/AMH and PCOS will undoubtedly evolve from a combination of clinical and basic research. Most recently it was demonstrated that MIS/AMH production per granulosa cell is up to 75 times greater in granulosa cells from anovulatory women with PCOS than in granulosa from women with normal ovaries (65). Therefore, it may be hypothesized that small follicles from women with PCOS contain granulosa cells that produce an overabundance of MIS/AMH which acts in a paracrine fashion to inhibit folliculogenesis from progressing from primordial follicles to primary follicles and from small preantral follicles to antral follicles. If such an understanding of PCOS is proven to be correct, there may be an opportunity to manufacture an MIS/AMH antagonist for the treatment of PCOS.

CONCLUSION

Since 2002, there have been both retrospective and prospective studies examining the possible utility of early follicular serum MIS/AMH as a marker of ovarian reserve. Investigators from ten countries in four continents have examined a variety of patient populations, using a variety of study designs assessing a variety of outcomes as surrogate parameters of ovarian reserve. These outcomes have included level of maximum E₂ rise, AFC, number of oocytes, quality of oocytes, and embryo quality, as well as implantation, pregnancy, and cancellation rates.

The majority of published studies to date support a role for MIS/AMH as a marker of ovarian reserve. Cut-off values reported in the literature are dependent upon the particular MIS/AMH standard protein in the assay used for these clinical studies. Agreement on absolute values for circulating MIS/AMH might be possible with the development of an international reference compound. When relevant, research

TABLE 2**Factors that may influence serum MIS/AMH concentration.**

- A. Factors that decrease MIS/AMH
 Increasing age
 Increasing body mass index
 Administration of gonadotropins
 Administration of chemotherapy or radiation
 Oophorectomy
- B. Factors that increase MIS/AMH
 PCOS
- C. Factors that do not influence MIS/AMH
 Day of menstrual cycle
 GnRH agonists
 Oral contraceptives
 Pregnancy

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investigators could normalize their results using such a protein preparation, but at present there is no commercially available material. As the MIS/AMH assays have evolved with improved iterations, i.e., greater sensitivity and less time to perform, the absolute values may vary but the trends remain consistent. Few studies have applied ROC analysis, but of those that have (29, 46, 47) two support MIS/AMH as predictive of number of retrieved oocytes (29, 47). Because pregnancy is an outcome impacted by additional non-ovarian factors such as male and endometrial influences, it is unlikely that any single marker will be sufficient to predict pregnancy. However, several studies demonstrate diminished ovarian reserve reflected by lower serum MIS/AMH levels despite unchanged early follicular markers such as E₂, FSH, or inhibin B (26, 44, 47).

Potential advantages of MIS/AMH compared with other conventional markers of ovarian reserve include: 1) it is the earliest marker to change with age; 2) it has the least inter-cycle variability; 3) it has the least intracycle variability; and 4) it offers the potential for being informative if randomly obtained during the cycle. An awareness of which factors may influence serum MIS/AMH concentrations is useful and is summarized in Table 2. It is expected that as the MIS/AMH assay becomes more refined there will be additional published studies examining the precision of using the measurement of this protein as a single random predictor of IVF outcomes, as well as to its use in combination with more conventional markers. Unique reference ranges for women with PCOS versus normally cycling women would offer additional value.

Widespread clinical use of the MIS/AMH ELISA may await the availability of an international standard for MIS/AMH so that results generated using different assays may be reliably compared from study to study. Having a reference standard would also increase the probability of developing specific cut-off values for clinical use.

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