Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women

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BACKGROUND: Anti-Müllerian hormone (AMH), secreted by the granulosa cells of preantral and small antral follicles, has been described as a potential marker of the ovarian reserve. The aim of this prospective study is to investigate the variations of AMH during the menstrual cycle in a young selected population of normo-ovulatory women and to analyse the correlation with other cyclic hormones. METHODS: Twenty healthy volunteers from 19 to 35 years old, with regular menstrual cycles (26–31 days), normal ovulation (day 10–16), normal hormonal profile and normal body mass index (18–26 kg/m²) were recruited. AMH, inhibin B, LH, FSH, estradiol and progesterone were measured on days 3, 7, 10, 11, 12, 13, 14, 15, 16, 18, 21 and 25 of a spontaneous cycle. RESULTS: AMH serum levels, either expressed by cycle day or aligned according to the ovulation day, did not show any significant variations during the menstrual cycle. CONCLUSIONS: No significant fluctuation of the AMH level during the menstrual cycle was observed. Therefore, this hormone is particularly interesting for clinical evaluation of the ovarian reserve as it may be used at any time during the cycle.

Keywords: anti-Müllerian hormone; MIS; menstrual cycle; ovarian function

Introduction

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance (MIS), is a homodimeric glycoprotein hormone belonging to the large family of transforming growth factors-β (TGF-β) such as activins, inhibins or others (Cate et al., 1986). AMH is well known for its essential role during the fetal sex differentiation process (Josso et al., 1993). In women, AMH is expressed uniquely by the ovary in the granulosa cells. AMH expression begins in the primary follicles, gradually increasing then peaking in preantral and antral follicles of maximum of 4 mm. After this threshold, the expression decreases, becoming undetectable when the follicles reach a diameter of 8 mm (Weenen et al., 2004). AMH is therefore expressed during the two critical regulatory steps of folliculogenesis: the initial recruitment and the cyclic selection for follicular dominance (McGee et al., 2000). Moreover, in mice, AMH has been shown to be involved in the regulation of the initial follicular recruitment and decreases the responsiveness of growing follicles to FSH (Durlinger et al., 1999, 2001).

The AMH-specific expression at the early follicular stage and its function in the regulation of the follicular growth, suggest that AMH may be a very sensitive marker of the ovarian reserve. Indeed, an excellent correlation between antral follicular count (AFC) by ultrasound and serum AMH levels during the early follicular phase has been observed (de Vet et al., 2002). Furthermore, AMH serum level is negatively correlated with age. Serum AMH levels, measured twice in a group of women between two visits at an interval ranging from 1.1 to 7.3 years, decreased earlier, with aging, than other classical ovarian markers such as FSH and inhibin B (de Vet et al., 2002).

As a marker of the ovarian reserve, evaluation of the potential variation of serum AMH levels during a normal cycle is essential. The specific expression of AMH by mainly small growing follicles suggests that AMH serum levels should remain steady during the menstrual cycle. The possibility of performing serum AMH measurements at any time during the cycle will be an additional advantage compared to other ovarian reserve markers. Cook et al. (2000) first compared AMH levels among 20 volunteers at three different times during the cycle: at the beginning (day 3), during ovulation and during the mid-luteal phase. They found a small but significant increase of AMH serum levels during ovulation. Others, using two AMH samplings among 56 patients when
performing an FSH challenge test prior to IVF treatment, showed that AMH serum levels during early follicular and mid-luteal phase were similar (Eldar-Geva et al., 2005). Recently, further studies have confirmed the absence of fluctuation of serum AMH levels during the cycle (La Marca et al., 2006; Hehenkamp et al., 2006).

In light of this, we have, in a prospective study, evaluated the precise fluctuation of AMH serum levels during the menstrual cycle in a population of selected young normo-ovulatory women.

Materials and Methods

Population

Non-pregnant women between 18 and 35 years old without hormonal contraception for at least three months were recruited between June 2005 and March 2006. Firstly, all volunteers were invited to complete a questionnaire concerning their medical and gynaecological history. A preliminary blood test was performed to exclude endocrinological disorders. Fasting glucose, C-peptide, delta-4-androstenedione, sex hormone-binding globulin (SHBG), testosterone, prolactin and TSH were assayed. Only volunteers with regular menstrual cycles (26–31 days), with body mass index (BMI) between 18 and 26 kg/m², without hormonal therapy and without endocrinological or metabolic pathologies were included in the analysis.

The study was performed during one menstrual cycle, considering the first day of menstruation as the first day of the cycle (D1). AMH, inhibin B, LH, FSH, estradiol (E₂) and progesterone were measured on days 3, 7, 10, 11, 12, 13, 14, 15, 16, 18, 21 and 25 of the cycle. All blood tests were performed in the morning and the volunteers were allowed to eat before the test. Ovulation day (L0) was defined as the day following the LH peak. Women were considered ‘normo-ovulatory’ if the LH peak occurred between D10 and D16. This study received the approbation of the local ethic committee and all the volunteers signed a written inform consent.

Hormonal assays

The hormone assays were obtained by a 10 ml venous puncture blood sample. The samples were centrifuged within 2 h and sera were stored at −20°C. Serum AMH and inhibin B levels were measured by enzyme immunoassay using commercially available kits (MIS/AMH Elisa and Inhibin B; OBI, Diagnostic Systems Laboratories). The AMH and inhibin B functional detection limits were 0.1 ng/ml and 31 pg/ml, respectively. For AMH, the intra-assay and inter-assay coefficients of variation were <5 and 8%, respectively. For inhibin B, the intra- and inter-assay coefficients of variation were both <7%. Serum levels of FSH, LH, E₂ and progesterone were determined using an automatic electro-chemiluminiscent technique (Model E170, Roche, Mannheim, Germany). The sensitivity and inter-assay coefficient of variations were, respectively, 1 mU/ml and <4% for FSH, 1 mU/ml and <3% for LH, 0.02 ng/ml and <4% for E₂ and 0.25 ng/ml and <5% for progesterone.

Statistical analysis

Analysis of variance with repeated measures and age as a covariable was used to study the stability of AMH during the menstrual cycle (General linear model). Correlations between the different hormone values were evaluated using the Spearman product moment of correlation. All the statistical evaluations were performed using SPSS 13.0 for Windows 2000. The results are expressed as mean ± SD.

Results

Seventy-eight women were recruited for the study between June 2005 and March 2006. After the inclusion visit, 37 volunteers were enrolled and were tested during a natural cycle. Two women abandoned the study and 15 were subsequently excluded due to a late ovulation day (mean ovulation day: 20.7 ± 1.6). No difference in AMH levels was observed between women who ovulated between day 10 and day 16, and those who ovulated after day 16 (data not shown).

Therefore, our analysis was carried out on the remaining 20 volunteers (Fig. 1). Their mean age was 26.2 ± 4.2 years, mean BMI was 21.1 ± 2.1 kg/m² and mean cycle duration was 28.1 ± 1.9 days. Ovulation occurred at day 14 (range from day 10 to day 16). The fluctuations of serum AMH levels from day to day during the menstrual cycle were not significant in this group of young and normo-ovulatory women (P = 0.408, Fig. 2).

To refine our analysis, we compared serum levels of AMH aligned according to the ovulation day and again no statistical differences in the level of AMH between days were found (P = 0.408, Fig. 3).

Individual means of AMH range from 0.4–5.3 ng/ml (mean: 2.4 ± 1.1 ng/ml). The mean intra variation coefficient was 14%.

The relationship between AMH levels and the women’s age or BMI was studied using an analysis of variance for repeated measures with age and BMI as covariables. In this group of normo-ovulatory women, neither age nor the BMI seemed to influence the AMH levels, although the two patients aged of 33 and 35 years had a lower mean AMH levels compared to...
the mean of the group (1.2±0.1 and 0.39±0.09 ng/ml, respectively, compared to 2.4±1.1 ng/ml for the group). A correlation between AMH and inhibin B levels at day 3 ($r = 0.473, P = 0.04$) was however detected.

**Discussion**

In the present study, we reported no significant fluctuations of AMH serum levels during menstrual cycle in a selected population of normo-ovulatory women. The absence of fluctuation of AMH serum levels could be explained by the AMH expression profile. The pool of growing follicles secreting the highest amount of AMH (<4 mm) is dependant on the follicular recruitment rate, which is associated with the follicular pool and not with the cyclic phase. This pool of growing follicles is constantly renewed according to the follicular reserve, making the AMH serum level constant during a spontaneous cycle. Furthermore, the level of AMH produced by the dominant follicle during cyclic recruitment is negligible and, thus, does not influence the serum levels, which only reflect the small growing follicular pool. This stability of AMH serum levels during the menstrual cycle illustrates that AMH acts as a paracrine factor secreted by the ovary and regulates non-cyclic events within the ovary.

Ovarian aging is characterized by a progressive reduction of the follicular reserve constituted mainly by the primordial follicles (Gougeon et al., 1996). The AMH serum level is reflected by the pool of growing follicles and correlates with the number of primordial follicles. Consequently, in women, AMH serum levels rise slowly reaching a maximum level at puberty, then progressively decreases with age. An association between the number of follicular structures at biopsy and AMH levels has also been previously described in premature ovarian failure patients (Meduri et al., 2007). Moreover, AMH is strongly correlated to the AFC realized by ultrasound, also reflecting the number of growing follicles (de Vet et al., 2002). Until now, the AFC has proved to be the best indicator for ovarian reserve.
of ovarian reserve (Scheffer et al., 2003). However, AMH levels, as measured during spontaneous cycle, are also now considered to be sensitive indicators of the ovarian response to gonadotrophins during IVF cycle (van Rooij et al., 2002).

The AFC measurement requires a transvaginal sonographic exam during the beginning of the follicular phase and moreover this exam is operator-dependant. Others markers of the ovarian reserve include FSH and inhibin B serum levels. Both these tests must be performed at the beginning of the menstrual cycle to be relevant, due to their significant variations during the cycle. Furthermore, the FSH and inhibin B serum levels are closely related one to the other and their modification appears relatively late in the process of ovarian ageing (de Vet et al., 2002). Indeed, the FSH serum levels begin to rise when the production of inhibin B decreases because of the reduction of growing follicles; this occurs relatively late in the sequence of events associated with the ovarian senescence.

In our study, we have not observed any correlation between AMH and FSH serum levels at day 3, probably due to the size of our sample and the small distribution of age that was allowed for the purpose of this study. However, a correlation was observed between AMH and inhibin B serum levels at day 3.

Due to the cycle dependence of the available ovarian reserve markers, a new objective test reflecting the pool of growing follicles independently from gonadotrophins should be useful in clinical practice. We demonstrate here that serum AMH level has the advantage of being cycle independent.

However, our results contradict the previous data describing a small but significant increase of AMH serum level in the late follicular phase (Cook et al., 2000). Indeed, even by aligning our results to the ovulation day (12 measurements per patients), no significant difference was observed. In the previous study, only three dosages were taken and ovulation was determined no significant difference was observed. In the previous study, our results to the ovulation day (12 measurements per patients), Vet et al. appears relatively late in the process of ovarian ageing (de Vet et al., 2006; La Marca et al., 2000). Further-

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