Anti-Müllerian hormone plasma levels in spontaneous menstrual cycle and during treatment with FSH to induce ovulation

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BACKGROUND: Anti-Müllerian hormone (AMH) is member of the transforming growth factor-β superfamily of growth factors. AMH is detected in serum from women of reproductive age and its levels vary slightly with the menstrual cycle, reaching the peak value in the late follicular phase. The present study was undertaken to assess the effect of controlled ovarian stimulation on AMH secretion by the ovary in healthy women in order to obtain more insight into the relationship between this peptide and gonadal steroids. METHODS: Twenty-four normally cycling women attending the infertility clinic volunteered for this study and AMH was measured in blood samples obtained during both spontaneous and FSH-treated cycles. RESULTS: AMH plasma levels did not change significantly from day 2 to day 6 in spontaneous cycles. On the contrary, AMH levels decreased progressively from day 2 to day 6 in FSH-treated cycles. A significant positive correlation was found between the decrease in AMH and the increase in estradiol plasma levels in FSH-treated cycles and between basal AMH and the peak estradiol (E₂) during exogenous FSH administration. CONCLUSIONS: The present study demonstrated that AMH plasma levels did not change during the follicular phase of the menstrual cycle and that exogenous FSH administration is followed by a significant reduction in AMH levels which is probably secondary to the gonadotrophin effect on the process of follicular development.

Key words: Anti-Müllerian hormone/antral follicles/controlled ovarian stimulation/estradiol/FSH

Introduction

Anti-Müllerian hormone (AMH) is member of the transforming growth factor-β superfamily of growth factors (Cate et al., 1986). AMH expression is limited to testicular Sertoli cells and ovarian granulosa cells and occurs in a developmentally regulated, sexually dimorphic pattern. After birth, AMH levels in males are low, but rapidly rise to peak values by late infancy, then slowly decrease to the adult range at puberty (Josso et al., 1990; Lee et al., 1996). In females AMH is undetectable at birth, then increase to overlap with the male range at puberty (Josso et al., 1990). A relevant role for AMH in the regulation of follicle selection and maturation has been hypothesized (Baarends et al., 1995). Several studies in which AMH was absent or overexpressed indicated an inhibitory effect of AMH on growing follicles (Lyet et al., 1995; Durlinger et al., 1999).

AMH is detected in serum from women of reproductive age and its levels vary slightly with the menstrual cycle, reaching the peak value in the late follicular phase (Hudson et al., 1990; Josso et al., 1990; Cook et al., 2000).

Serum AMH levels have been shown to decrease over time in young normo-ovulatory women (de Vet et al., 2002), and to correlate with age, FSH and the number of antral follicles. Therefore, AMH might represent a sensitive marker for ovarian ageing (Fanchin et al., 2003a). Indeed, it has been shown that poor response during IVF, indicative of a diminished ovarian reserve (Beckers et al., 2002), is associated with reduced baseline serum AMH concentrations (Seifer et al., 2002). An inverse relationship has been observed between estradiol (E₂) and AMH plasma levels (Fanchin et al., 2003a), suggesting that E₂ may have a negative role on AMH production, or vice versa.

The present study was undertaken to assess the effect of controlled ovarian stimulation (COS) on AMH secretion by the ovary in healthy women in order to obtain more insight into the relationship between this peptide and gonadal steroids.

Materials and methods

Twenty-four normally cycling women attending the infertility clinic between March and September 2003 volunteered for this study and gave informed consent. All women had normal menstrual cycles (28–32 days) with normal FSH, LH, thyroid-stimulating hormone...
(TSH), prolactin (PRL), 17-hydroxyprogesterone (17-OHP), E2 and testosterone levels and normal luteal phase as assessed by progesterone measurement in the mid-luteal phase. Women were healthy and not taking any drug. Mean age was 28 years (range: 21–36) and the body mass index varied between 23 and 27 kg/m².

All women attended the clinic because of infertility due to male factor. Male infertility was diagnosed on the basis of abnormal findings in semen analysis according to the World Health Organization criteria: in brief, volume <2 ml, count <20 × 10⁸/ml, motility <50% and normal shape <30% were considered as abnormal. Semen analysis was performed at least twice with an interval of 3 months between tests. We performed intrauterine insemination for patients with male factor infertility if the total motile sperm count was ≥5 × 10⁸/ml (Dickey et al., 1999).

**Study protocol**

All women were investigated during two consecutive menstrual cycles, i.e. an untreated spontaneous cycle (first cycle) and a cycle treated with FSH (second cycle). Blood samples were obtained every other day until the day of the endogenous LH surge in the spontaneous cycles and until the day of hCG administration in the FSH cycles. All blood samples were stored at −20°C until assayed. None of the women became pregnant during the period of the study.

Human recombinant FSH (75 IU; Gonad F; Serono, Italy) was administered at a dose of two ampoules per day for 5 days, increasing by one ampoule per day until an ultrasonically detectable ovarian response was obtained. This effective daily dose was maintained until hCG administration. hCG (10 000 IU i.m., Profasi; Serono) was given when the diameter of the dominant follicle was ≥18 mm with not more than two follicles >16 mm.

Plasma FSH, LH, E₂, TSH, PRL, 17-OHP and testosterone concentrations were assayed by double-antibody radioimmunoassay using commercial kits from Radim (Italy) for FSH, LH, and TSH, from Sorin (Italy) for E₂ and testosterone, from Biodata (Italy) for PRL and 17-OHP. Samples were assayed in duplicate at two dilutions. Samples from a given subject were analysed for each hormone in the same assay to avoid inter-assay variation. Quality control pools at low, normal and high LH, FSH, E₂, PRL, LH, testosterone and 17-OHP concentrations were present in each assay. The detection limit of the assay was 0.20 IU/l for LH, 0.18 IU/l for FSH, 18 pmol/l for E₂, 277 pmol/l for testosterone, 0.2 mIU/l for TSH, 0.21 nmol/l for 17-OHP and 0.3 µg/l for PRL. Intra- and inter-assay variations were 7.8 and 8.2% for LH, 6.2 and 6.5% for FSH, 4 and 4.8% for 17-OHP, 4.2 and 4.9% for E₂, 3.4 and 4.6% for testosterone, 3.1 and 2.5% for TSH and 3.4 and 1.6% for PRL.

Serum AMH was measured using the AMH enzyme-linked immunosorbent assay kit (Immunotech, France). Briefly, 25 µl of each serum sample was incubated in duplicate on a polystyrene plaque pre-coated with a monoclonal anti-AMH antibody. After 1 h incubation, a second monoclonal anti-AMH antibody, coupled to biotin, was added, together with a streptavidin–horseradish peroxidase complex. After addition of TMB substrate, the resulting colour reaction was quantified using a MRX spectrophotometer at 450 nm. A preparation of purified recombinant human AMH was used to construct a standard curve. The limit of sensitivity of the assay was 0.7 pmol/l (0.1 ng/ml); inter- and intra-assay coefficients of variation were 8.7 and 5.3% respectively, for a serum AMH concentration of 35 pmol/l and 7.8%, and 4.9% for a serum AMH concentration of 1100 pmol/l. No cross-reaction was observed with pure transforming growth factor-β.

**Statistical analysis**

Results are expressed as means and SD. Comparisons between the groups were calculated by paired t-test if the data were normally distributed and by Mann–Whitney because of the small size of the groups. Correlations between different parameters were determined by using bivariate correlation statistics and are expressed as Spearman correlation coefficients. Statistical analysis was performed by the software Statsoft. Statistical significance was set at P < 0.05.

**Results**

In all women during the spontaneous cycle the LH surge was detected. In none of the women was an LH surge detected in the FSH cycles before the administration of hCG.

FSH and E₂ plasma levels in the follicular phase of spontaneous and FSH-treated cycles are shown in Figures 1 and 2. During FSH administration, FSH and E₂ plasma levels increased and the levels were significantly higher than in spontaneous cycles. Basal AMH levels on day 2 were similar in spontaneous and in FSH-treated cycles (1.7 ± 0.4 versus 1.8 ± 0.4 ng/ml). AMH plasma levels did not change significantly from day 2 to day 6 in spontaneous cycles. On
the contrary, AMH levels decreased progressively from day 2 to day 6 in FSH-treated cycles (Figure 3). The reduction was significant at days 4 and 6 as compared to basal values and to spontaneous cycles.

AMH plasma levels in the 4 days before LH surge (or injection of hCG in the FSH cycles) (day 0) are shown in Figure 4. In spontaneous cycles, no modifications in AMH levels were observed. A progressively significant reduction in AMH levels was found in FSH-treated cycles. *P < 0.05 2 days before hCG and the day of hCG versus 4 days before hCG administration. Values are mean ± SD.

**Figure 3.** Concentrations of anti-Müllerian hormone (AMH) during the follicular phase (days 2, 4 and 6 of the cycle) of spontaneous cycles (○) and FSH-treated cycles (●). During FSH administration, AMH plasma levels significantly decreased at days 4 and 6. *P < 0.05 day 4 and day 6 versus day 2. Values are mean ± SD.

**Figure 4.** Concentrations of anti-Müllerian hormone (AMH) during the late follicular phase of spontaneous cycles (○) and FSH-treated cycles (●). The data were normalized to the day of onset of LH surge (or hCG injection) (day 0). In spontaneous cycles, no modifications in AMH levels were observed. A progressively significant reduction in AMH levels was found in FSH-treated cycles. *P < 0.05 2 days before hCG and the day of hCG versus 4 days before hCG administration. Values are mean ± SD.

A significant positive correlation (r = 0.62; P < 0.05) was found between the decrease in AMH and the increase in E2 plasma levels in FSH-treated cycles (data not showed). Moreover, a positive correlation (r = 0.43; P < 0.05) was found between basal AMH and the peak E2 during exogenous FSH administration (data not showed).

**Discussion**

In the present study it has been shown that AMH plasma levels do not change significantly during the follicular phase of the menstrual cycle. Alternatively a significant decrease in AMH levels was observed in FSH-treated cycles. A recent study investigated the dynamics of AMH levels during COS (Fanchin et al., 2003b) and found a significant decrease in AMH levels during FSH administration. Our study is the first to compare AMH dynamics in spontaneous and FSH-treated cycles in the same patient.

AMH is a homodimeric disulphide-linked glycoprotein, strongly expressed in Sertoli cells from testicular differentiation up to puberty and to a much lesser degree in granulosa cells from birth up to menopause. In the adult rat ovary, AMH can be detected in the growing follicles but disappears before ovulation (Ueno et al., 1989). In cultured rat granulosa cells, exogenous AMH inhibits aromatase synthesis (di Clemente et al., 1994) and inhibits the proliferation of granulosa luteal cells (Kim et al., 1992; Seifer et al., 1993). AMH and its receptor mRNA are highly expressed in granulosa cells of mainly preantral and small antral follicles (Hirobe et al., 1992; Baarends et al., 1995). Hence a relevant role for AMH in the regulation of follicle selection or maturation has been hypothesized (Baarends et al., 1995).

The reduction in AMH levels observed during FSH administration may be due to a negative role of FSH on AMH secretion. It is well established that FSH up-regulates testicular AMH gene expression in adults (Lukas Croisier et al., 2003), whereas Baarends et al. (1995) previously reported that FSH may down-regulate the AMH protein and AMH type II receptor expression in adult rat ovaries. Alternatively the reduction in AMH levels could be due to the supraphysiological increase in E2 levels observed when exogenous FSH is administered. Indeed E2 has been implicated in the down-regulation of AMH and AMH type II receptor mRNA in the ovary (Baarends et al., 1995). Moreover the decrease in AMH in the FSH-treated group might be the result of a growth stimulation by FSH of the follicles that enlarge, therefore losing their AMH expression.

In a recent study the modifications in AMH levels during COS were investigated (Fanchin et al., 2003b). Serum AMH levels declined progressively during COS, and throughout COS, serum AMH levels correlated positively with the number of small but not large antral follicles, and with inhibin B serum levels. The authors concluded that serum AMH levels decline gradually during multiple follicular maturation, probably reflecting the marked reduction in the number of small antral follicles due to COS, and confirming the scarce AMH expression by larger follicles. These findings have been confirmed by our study. We also found no correlation between AMH and FSH or E2 levels during the spontaneous menstrual cycle and this permits to hypothesize that the decrease in AMH levels during FSH administration is probably due to the exogenous gonadotrophin effect on follicular growth. However, several studies indicated that AMH is not detected from the small antral follicle stage onward (Hirobe et al., 1992; Baarends et al., 1995) and that follicles showing signs of atresia also have decreased or no AMH expression. A recent study has shown that the highest level of AMH expression was present in the granulosa cells of secondary, preantral and small antral follicles ≤ 4 mm in diameter.
In larger (4–8 mm) antral follicles, AMH expression gradually disappeared (Weenen et al., 2004). Moreover, early follicle growth in humans appears to be independent of stimulation by gonadotrophins. Follicles appear to become dependent on FSH only at a later developmental stage. In the human, follicle development to antral stage continues throughout life until depletion of follicles at the approach of menopause, even in the presence of conditions under which endogenous gonadotrophin release is substantially diminished (Richardson and Nelson, 1990). Such conditions include during pre-puberty, pregnancy, and steroid contraceptive use. In addition, follicle growth to early antral stage has been described in women with absent gonadotrophin secretion, due either to hypophyseectomy or to hypothalamic/pituitary failure (Schoot et al., 1994). Hence, the reduction in AMH levels during exogenous gonadotrophin administration may be explained on the basis of a direct or indirect effect of FSH on AMH synthesis and secretion and/or atresia of small non-recruited antral follicles. In conclusion, the present study demonstrated that AMH plasma levels did not change during the follicular phase of the menstrual cycle and that exogenous FSH administration is followed by a significant reduction in AMH levels which is probably secondary to the gonadotrophin effect on the process of follicular development.

References


