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Anti-Müllerian hormone concentrations in maternal serum during pregnancy

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BACKGROUND: In females, anti-Müllerian hormone (AMH) is expressed only by the ovary. AMH is secreted by the granulosa cells of ovarian follicles and appears to regulate early follicle development. 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. This study investigated serum AMH levels throughout gestation and after delivery in healthy pregnant women. METHODS: This cross-sectional study recruited pregnant women and healthy non-pregnant women, 84 in total. AMH, FSH and E_2 were measured in the follicular phase, in the three trimesters of pregnancy and in early puerperium. RESULTS: Estradiol and FSH levels followed the expected patterns during gestation. During the follicular phase of the menstrual cycle AMH levels were 1.9 ± 0.5 ng/ml. In the three trimesters of pregnancy and in early puerperium AMH levels were: 2.1 ± 0.56, 2.4 ± 0.64, 1.95 ± 0.6 and 2.05 ± 0.55 ng/ml respectively. No significant modifications were found in AMH levels during pregnancy and in the early puerperium. CONCLUSIONS: This study has obtained information on AMH and on the possible relationship with FSH. We hypothesize that the profile of the new marker of ovarian activity AMH may indicate that initial non-cyclic ovarian follicular activity during pregnancy is not abolished. Moreover FSH, does not seem to play a direct role on AMH synthesis and secretion.

Key words: anti-Müllerian hormone/early puerperium/estradiol/FSH/pregnancy

Introduction

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance or factor, a member of the transforming growth factor-β (TGF-β) family, is essentially involved in the regression of Müllerian ducts in the male fetus, the initial step of organogenesis of the male genital tract. AMH is a homodimeric disulphide-linked glycoprotein with a molecular weight of 140 kDa (Di Clemente *et al.*, 2003)

In contrast to other members of the family, which are widely expressed and have a varied repertoire of biological activities depending on the cellular context, AMH is expressed only in the gonads and exerts inhibitory effects on the development and function of reproductive organs. The most striking effect of AMH is its capacity to induce regression of the Müllerian ducts, the anlage of the female internal reproductive organs. In the absence of AMH, Müllerian ducts of both sexes develop into uterus, Fallopian tubes and the upper part of the vagina (Munsterberg and Lovell-Badge, 1991). In females, AMH is expressed by the ovary (Hirobe *et al.*, 1994). AMH is secreted by the granulosa cells of ovarian follicles (Vigier *et al.*, 1984) and appears to regulate early follicle development (Durlinger *et al.*, 2002). AMH seems to affect the transition from resting primordial follicles into growing follicles (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. In a recent study, a group of women was studied twice and the interval between the two visits ranged from 1.1 to 7.3 years. A reduction in mean AMH levels of ~38% was observed (de Vet *et al.*, 2002). Therefore, AMH might represent a sensitive marker for ovarian ageing (de Vet *et al.*, 2002; Fanchin *et al.*, 2003; Laven *et al.*, 2004).

Currently there are no data on AMH modifications during human pregnancy. The present study evaluated serum AMH levels throughout gestation and after delivery in healthy pregnant women. Moreover, pregnancy offers the opportunity to study the relationship between AMH and FSH.

Materials and methods

This cross-sectional study was carried out in 84 women. The study was approved by the Hospital Ethical Committees and each participating woman provided informed consent. It was decided, *a priori*, to study four groups of women. Group A, control non-pregnant healthy women; group B, women in the last part of the first trimester of pregnancy (weeks 9–11); group C, women in the last part of the second trimester (weeks 21–23); group D, women in the last part of the third trimester (weeks 36–38); and group E women between 48 and 72 h after delivery. Non-pregnant women were nulliparae healthy women, not seeking pregnancy and with BMI in the normal range (mean ± SD: 23 ± 1.5 kg/m^2). All singleton pregnant women recruited in the study were primigravidae. Women with a known history of thyroid dysfunction, diabetes mellitus or any personal or familial immune disease were excluded from the study. Each woman was assessed only once.

The groups analysed were (respectively: number of women and range of age): A, as internal controls $n = 15$, 18–32 years, without ongoing pregnancy; B, $n = 27$, 19–35 years, studied in the first trimester of pregnancy; C, $n = 21$, 17–34 years, studied in the second trimester; D, $n = 13$, 20–37 years, studied in the third trimester; E, $n = 8$, 20–35 years, studied in the early puerperium.

A medical questionnaire and blood samples were obtained for AMH, FSH and E_2 determination. Blood samples were obtained between 09:00 and 11:00 after a light breakfast. Plasma FSH and E_2 levels were assayed by double-antibody radioimmunoassay using commercial kits from Radim (Italy) for FSH and from Sorin (Italy) for E_2. 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 FSH and E_2 concentrations were present in each assay. The detection limit of the assay was 0.18 IU/l for FSH and 5 pg/ml for E_2. Intra- and inter-assay variations were 6.2 and 6.5% for FSH and 4.2 and 4.9% for E_2.

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 an 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.

Results are expressed as means and SD. Comparisons between the two groups were calculated by analysis of variance and by Mann–Whitney *U*-test. 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 $P < 0.05$. On the basis of our results we calculated that 7500 patients should be recruited to obtain a significant difference (alpha 90%) in AMH levels during the three trimesters.

Results

FSH and E_2 levels (Figures 1 and 2) followed the expected patterns during gestation. A significant increase in estradiol was observed during all trimesters of pregnancy. Three days after delivery, estradiol levels were similar to those observed during the follicular phase of women of reproductive age. FSH serum levels were constantly and significantly reduced to values closed to the detection limit throughout pregnancy and during the early puerperium.

During the follicular phase of the menstrual cycle, AMH levels were 1.9 ± 0.5 ng/ml (median 2, range 0.4–2.8). In the three trimesters of pregnancy and in early puerperium, AMH levels were: 2.1 ± 0.56 ng/ml (median 2.1, range 0.9–3), 2.4 ± 0.64 ng/ml (median 2.4, range 1.2–4), 1.95 ± 0.6 ng/ml (median 2, range 0.2–3) and 2.05 ± 0.55 ng/ml (median 2, range 1–2.9) respectively (Figure 3).

No significant modifications were found in AMH levels during pregnancy. A trend toward a reduction in the third trimester was observed (not statistically significant). In all three trimesters, no gender differences in the AMH levels were observed (data not shown). In the early puerperium, AMH serum levels were similar to those observed during pregnancy. No correlations were found between E_2, FSH and AMH serum levels in participants of the study.

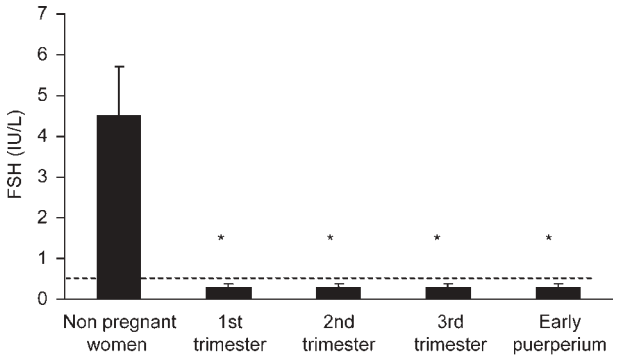

Figure 1. Serum FSH levels in controls, throughout pregnancy and in early puerperium. Values are mean ± SD; *$P < 0.05$.

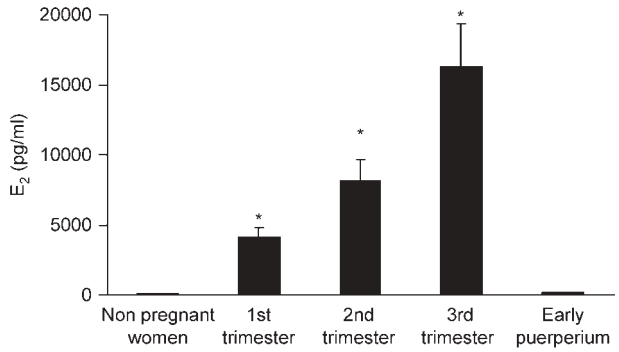

Figure 2. Serum estradiol (E_2) levels in controls, throughout pregnancy and in early puerperium. Values are mean ± SD; *$P < 0.05$.

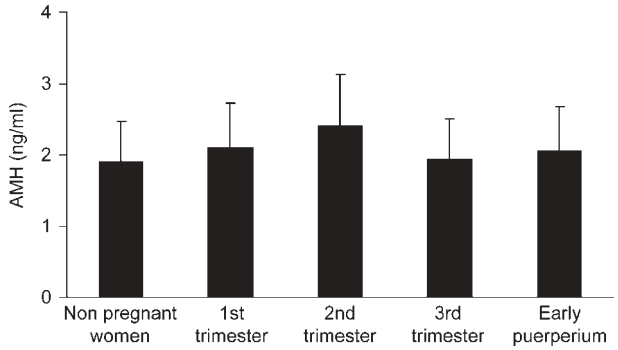

Figure 3. Serum anti-Müllerian hormone (AMH) levels in controls, throughout pregnancy and in early puerperium. Values are mean ± SD.

Discussion

The present study provides baseline information on AMH profile throughout normal pregnancy and in the early puerperium.

Maternal circulating AMH levels are similar to those found in the follicular phase of non-pregnant women. No significant changes were found 2–3 days after the delivery. The lower but not statistically significant levels of AMH during the third trimester and during the puerperium might be due to small differences in age between the five groups. Indeed it has been demonstrated that AMH levels decreased over time in normo-ovulatory women (de Vet *et al.*, 2002; Laven *et al.*, 2004).

Our results may indicate that placenta does not produce and secrete AMH in the maternal circulation. Indeed it has been shown that AMH is expressed only in the gonads (Teixeira *et al.*, 2001). However, appropriate studies on possible placental AMH production are needed. Until then it cannot be excluded that both the ovary and placenta contribute to AMH levels found in pregnant women. Recently it has been reported that there might be an estrogen-responsive element in the promoter area of the AMH gene (Chen *et al.*, 2003). Since estrogen levels are much higher during pregnancy than in the normal cycle, estrogens might therefore inhibit expression of the AMH during pregnancy. The lack of AMH decline after delivery argues against the placental production and the effect of high levels of estradiol on AMH gene expression.

The findings of our study suggest that initial follicle recruitment is not abolished during pregnancy. It is thought that prolonged elevation of circulating progesterone during pregnancy may reduce follicular recruitment. Early studies in pregnant mice indicated that fewer follicles start growth per unit time with a consequent conservation of follicle reserve in ageing animals (Lapolt *et al.*, 1998).

Epidemiological studies indicate that women with increased parity show a delay in menopausal onset (Whelan *et al.*, 1990; Cramer *et al.*, 1995). However, considering that AMH is solely produced by granulosa cells of preantral and small antral follicles (Durlinger *et al.*, 2002), we should conclude that initial non-cycle recruitment of follicles is not abolished during pregnancy.

Furthermore, our study provided information regarding the relationship between FSH and AMH. Studies conducted in rats have shown that FSH inhibits AMH secretion (Baarends *et al.*, 1995) and, as reported by other independent studies, a physiological link between FSH and AMH has been proposed (Seifer *et al.*, 2002; Van Rooij *et al.*, 2002; Fanchin *et al.*, 2003). We found that the deep suppression of pituitary FSH secretion observed during pregnancy is not followed by modification in AMH serum levels. This observation should exclude a direct role of FSH on AMH production. Probably the relationship between FSH and AMH found in non-pregnant women is consequent to their mutual correlation with a third parameter (the early antral follicular count). However, pregnancy is a complex situation that could limit the interpretation of data.

The present study on AMH serum levels during pregnancy has obtained information on ovarian activity and on the possible relationship between FSH and AMH.

We hypothesize that the profile of the new marker of ovarian activity, AMH, may indicate that initial non-cyclic ovarian follicular activity during pregnancy is not abolished. Moreover, FSH does not seem to play a direct role on AMH synthesis and secretion.

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