ORIGINAL ARTICLE: EARLY PREGNANCY

Longitudinal changes in maternal serum concentrations of antimüllerian hormone in individual women during conception cycles and early pregnancy

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Objective: To study antimüllerian hormone (AMH) from gestation week 0–7.
Design: Longitudinal study of 85 pregnant women with AMH and reproductive hormones sampled during conception cycle and early pregnancy until week 7.
Setting: Fertility clinic.
Patient(s): Of 85 pregnant women, 69 had a singleton pregnancy, 1 a twin pregnancy, and 15 had a nonviable pregnancy (3 chemical pregnancies, 11 miscarriages, and 1 blighted ovum).
Intervention(s): None.
Main Outcome Measure(s): Relationship between AMH and gestation week, woman's age, body mass index (BMI), FSH dose, treatment modality, reproductive hormones, viability of pregnancies, and fetal gender.
Result(s): During the conception cycle, 86.1% of women had their maximum AMH at or before ovulation. The AMH level did not remain constant in viable pregnancies, but moved significantly away from baseline pregnancy level. In natural pregnancies the overall trend was for decreasing AMH level. In treatment pregnancies AMH level either consistently increased or decreased from gestation week 4 (time of first positive hCG) through to week 7. In contrast, the AMH level in nonviable pregnancies showed sporadic changes, both increasing and decreasing in the same individual from gestation weeks 4–7. The AMH level was negatively correlated with patient's age ($r = -0.507$) and P level ($r = -0.220$), but no other associations were observed with BMI, FSH dose, treatment modality, or fetal gender.
Conclusion(s): The AMH level peaked at or before ovulation in most women, trended down with natural pregnancies, and consistently increased or decreased in women with a viable pregnancy after therapy. Nonviable pregnancies showed erratic AMH patterns. Factors responsible for these different responses in pregnancy remain to be identified. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: AMH, conception cycle, early pregnancy

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Antimüllerian hormone (AMH) in women is produced by the granulosa cells (GCs) of small ovarian follicles and plays an essential role in ovarian folliculogenesis. Serum AMH concentrations decrease with advancing age and have been correlated with the number of antral follicles present in the ovary, such that low levels of AMH can often indicate low ovarian reserve, whereas a high level may indicate polycystic ovary syndrome (PCOS) (1). Changes of AMH with age (2, 3), in assessing ovarian reserve (4–6), and predicting ovarian responsiveness to gonadotrophin stimulation (7, 8) have been reported in detail.

Yet there are only a few studies of AMH levels during pregnancy and the

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findings have been inconsistent. In an early cross-sectional study (9), the AMH levels of a nonpregnant group and of women in different trimesters of pregnancy were summed and mean AMH determined. It was concluded that no significant changes occur to AMH levels during pregnancy. However, it is likely that the cross-sectional design of this study may have limited the ability to detect modest changes in AMH. Three other cross-sectional studies (10–12) have reported a decline in AMH levels in pregnancy and one of these studies also included a small subset of 15 women contributing longitudinal samples for all trimesters. A recent longitudinal study (13) followed 60 individual women, with sampling in each of the three trimesters. The earliest samples for this study were from the first antenatal visit (8–14 weeks gestation), and there was a significant reduction in AMH levels as the pregnancy progressed, returning to baseline levels after delivery. However, none of these studies were able to examine possible changes in AMH concentrations during the first few weeks of pregnancy in individual women. As such there is currently no published data that we are aware of documenting the longitudinal changes in AMH levels in very early pregnancy.

Our longitudinal study was undertaken to characterize changes in circulating AMH concentrations relative to ovulation and during subsequent early pregnancy. We collected blood from 85 women in the conception cycle and then in the subsequent period of pregnancy, monitoring until the time of a fetal heart on ultrasound scan at 7 weeks gestation or until the pregnancy failed.

MATERIALS AND METHODS

Patients

Ethical approval to undertake this research project was given by the Joondalup Health Campus Human Research Ethics Committee (Ethics Approval Number 1414) and the Edith Cowan University Human Research Ethics Committee (Ethics Approval Number 12077). Women who attended Fertility North consecutively for fertility treatment between October 2014 and January 2015 and agreed to participate were recruited after evidence of a positive pregnancy blood test 14 days after ovulation, namely if the serum hCG concentration was >25 IU/mL measured on a Centaur XP automated analyzer (Siemens Healthcare Pty. Ltd.). The 85 recruited pregnant women conceived either in an unstimulated monitoring cycle (n = 15), after low dose FSH stimulation with either intercourse (n = 13) or IUI (n = 7), a stimulated cycle (n = 25) for subsequent IVF or intracytoplasmic sperm injection (ICSI) treatment. Cycles after the transfer of frozen/thawed embryos (n = 25) were either stimulated with exogenous gonadotropins in the follicular phase (n = 23) or unstimulated (n = 2), although all these patients received exogenous steroid luteal support. Of all the pregnant women, 69 had a singleton pregnancy, 1 a twin pregnancy, and 15 had a nonviable pregnancy (3 chemical pregnancies, 11 miscarriages, and 1 blighted ovum). All women were nonsmokers and had a body mass index (BMI) <35.

Sample Collection and Processing

All dates were according to the gestational age relative to day 1 of the cycle in which conception occurred. Blood samples were collected from each woman at the following times: [1] Conception cycle. Blood was available for 43 women in week 0 (a baseline measurement on days 2–3 of the conception cycle) and from all women in week 2 (around the time of ovulation) and week 3 (midluteal phase); and [2] Pregnancy monitoring. Blood samples were collected in all 85 women from week 4, at the time of the serum positive pregnancy test, twice weekly until fetal heartbeat was seen by ultrasound (~week 7) or the pregnancy failed. If a woman had a nonviable pregnancy, blood samples were collected once weekly thereafter, until her serum hCG reached <5 IU/mL. The blood samples were stored frozen at −20°C until assayed for AMH.

AMH Assays and Quality Control

The precision of the Beckman Coulter AMH Gen II ELISA kit was assessed using commercial quality control material and pooled patient serum. The within-assay coefficient of variation (CV), determined by analyzing 20 replicates of 4 serum pools (5.4–94.9 pmol/L), was ≤10.5%. The between-assay CV of these pools, analyzed in each of the 23 separate assays, was ≤15.9%. The blood samples from each woman were thawed at room temperature and analyzed together on the same day in one batch to eliminate between-assay variability for each woman.

Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 22. The relationships between AMH, reproductive hormones, gestation week, age, and BMI during pregnancy were analyzed using bivariate correlations (two-tailed, Spearman's) to determine the correlation coefficient (r) and P value (significance). The relationship between FSH dosage (ovarian stimulation) and AMH levels at gestation week 4 were also investigated in this way. General linear models and repeated measures analysis of variance (ANOVA) were used to measure the relationships between AMH and gestation week. Differences were statistically significant when $P<.05$.

RESULTS

Patients and Basal AMH

The average age of all women (n = 85) in this study was 35.4 ± 0.5 years, and their serum AMH at week 4 ranged from 1.1–69.4 pmol/L, reflecting the wide range of ovarian reserve. Women with blood collected from week 0 were divided into four age groups (26–30 years, 31–35 years, 36–39 years, and ≥40 years), and the mean ± SEM AMH concentration for each group was 48.9 ± 15.9, 30.5 ± 6.4, 17.3 ± 2.6, and 12.9 ± 5.7 pmol/L, respectively. There was a significant difference in AMH levels between each age group ($P = .000$), and there was a significant negative correlation between first AMH measurement and age ($r = -0.507$, $P = .000$). The average woman's BMI was 24.7, ranging between 18.0 and 36.1. No significant correlation existed between patient BMI