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;106:1407–13. ©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 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.

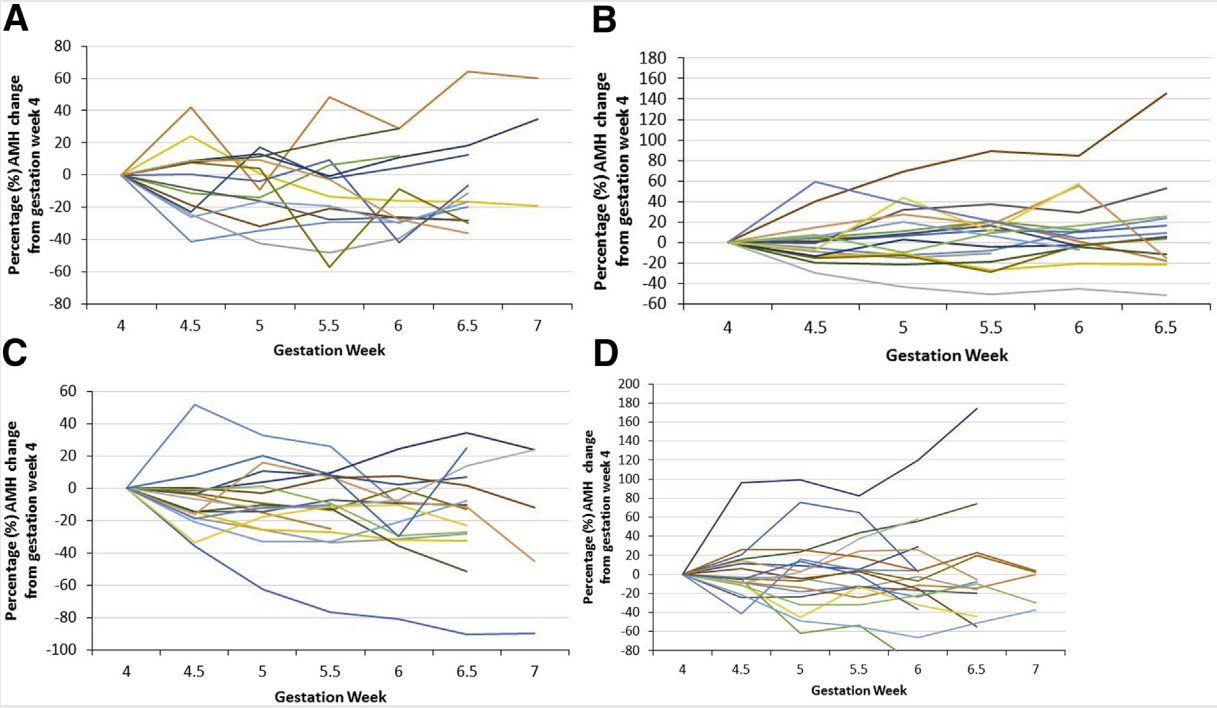
AMH Assays and Quality Control

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 $P<.05$.

FIGURE 1



Serum antimüllerian hormone (AMH) concentrations from gestation week 4 to the week of fetal heart detection by ultrasound of viable pregnancies conceived in (**A**) natural cycles, (**B**) ovulation induction or IUI cycles, (**C**) frozen ET cycles, or (**D**) IVF or intracytoplasmic sperm injection (ICSI) cycles.

Hamilton. AMH in conception cycles and early pregnancy. Fertil Steril 2016.

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 and their baseline AMH concentration ($r = -0.102$, $P = .537$), nor was there was any relationship between age and BMI ($r = -0.006$, $P = .972$).

AMH Changes during the Conception Cycle

When the AMH was analyzed parametrically for the cohort of 43 women who had blood collected at all three time points during the conception cycle, namely week 0 (days 2–3 of cycle), week 2 (around ovulation), and week 3 (midluteal phase), there was no significant change in AMH ($P>.05$). The mean ± SEM serum AMH was 25.9 ± 3.9 pmol/L at week 0, 29.3 ± 4.2 pmol/L at week 2, and 24.1 ± 3.5 pmol/L at week 3. However, examination of the time of peak AMH in each woman showed a pattern of change with peak AMH value occurring in 32.6% of women at week 0, 53.5% of women at week 2, and only 13.9% at week 3. The baseline AMH levels at week 0 were strongly correlated with both week 2 around ovulation ($r = 0.922$) and week 3 in the midluteal phase ($r = 0.927$).

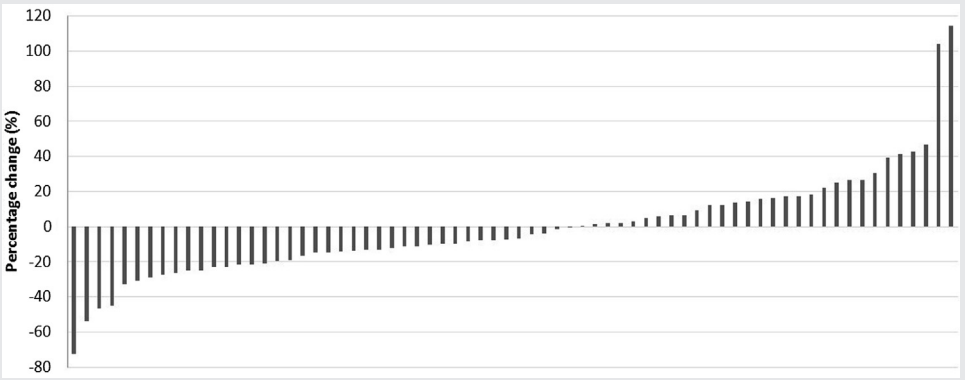
Treatment Modality and FSH Dose

There was a significant difference in absolute AMH levels between cycle types ($F = 4.377$, $P = .009$) reflecting the ovarian reserve selection criteria for the different treatment modalities. However, when the dose of exogenous FSH administered to women during their conception cycle (gestation weeks 0–2) was compared to the proportional AMH change in individual women in early pregnancy, there was no significant correlation ($r = 0.063$, $P = .568$).

AMH and Other Hormones

The concentrations during pregnancy of hCG, E_2, and P for viable pregnancies are shown in Supplemental Table 1. During gestation weeks 4–6.5, serum AMH concentration was negatively and significantly correlated to the concentrations of P ($r = -0.220$, $P = .000$), but not E_2 ($r = -0.023$, $P>.05$) or hCG ($r = 0.072$, $P>.05$).

FIGURE 2



Antimüllerian hormone concentrations expressed as the mean percentage change from gestation week 4 at time of the positive pregnancy test to the week when a fetal heart was detected by ultrasound (n = 70).

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AMH Changes in Viable Pregnancies

When considering the group as a whole, the circulating AMH concentrations were not significantly different between gestation weeks 4–6.5 for all viable pregnancies ($F = 1.302$, $P = .278$) as shown in Supplemental Figure 1. However, examination of the changes in individual women revealed that the AMH concentrations did not remain stable, but consistently changed and either increased or decreased in absolute concentrations (Fig. 1) or the mean change between gestation week 4 to detection of fetal heart (Fig. 2). In the 15 women with a viable pregnancy after an unstimulated monitoring cycle, 3 had AMH levels that increased and 12 decreased, whereas in the 19 women with ovarian stimulation for IVF/ICSI, there were 8 whose AMH increased and 11 that decreased, although this rate of occurrence between the two small groups was not statistically significant ($P = .32$). The largest increase in AMH levels during early pregnancy was seen in a woman where her AMH level increased by a mean of 114.1% in the short time period of 2.5 weeks, increasing from 40.6 pmol/L at gestation week 4 to 111.2 pmol/L in gestation week 6.5. In contrast, another woman had AMH levels decrease by a mean of 72.4%, decreasing from 6.6 pmol/L at gestation week 4 to 0.6 pmol/L at gestation week 6.5. When patients were divided into two subgroups, which had either a mean increase or decrease in AMH (AMH trend), there was a statistically significant difference between these groups ($P = .000$). Mean AMH variance between increasing or decreasing trend was almost seven times larger than the variance within the weeks, with a very strong effect size (Power = 0.996). The pattern between pregnancies that increased in AMH compared with those that decreased is shown in Table 1. Curiously, the group showing an increase in AMH during pregnancy had significantly lower AMH concentration in week 4, but this became significantly higher by week 5.5.

TABLE 1

Serum antimüllerian hormone concentrations (mean ± SEM; pmol/L) in 70 women with a viable pregnancy and showing either a progressive decrease or increase.

	Change in AMH	
Gestation week	**Decrease (n = 39)**	**Increase (n = 31)**
4	21.9 ± 2.9	18.7 ± 3.7[a]
4.5	19.5 ± 3.0	22.0 ± 3.9
5	17.8 ± 2.9	22.7 ± 3.7
5.5	17.4 ± 3.0	23.2 ± 3.8[a]
6	17.1 ± 3.2	23.3 ± 4.0[a]
6.5	17.1 ± 3.5	25.6 ± 4.5[a]

Note: AMH = antimüllerian hormone.
[a] $P < .05$ between the two groups at the same time point.

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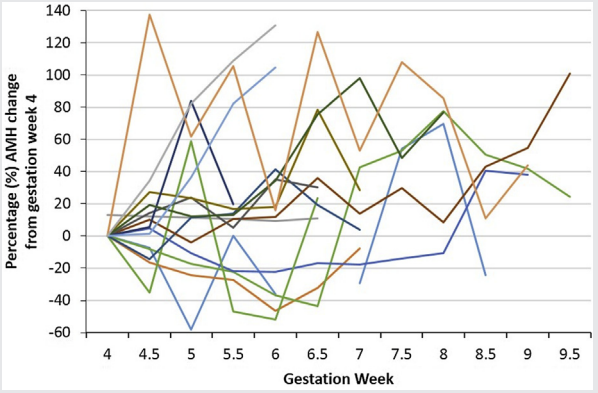
AMH Changes in Nonviable Pregnancies

There was no significant difference in age between women who had viable (37.0 ± 0.5 years) or nonviable (35 ± 1.3 years) pregnancies ($P = .214$). However, there were clear differences observed in the patterns of AMH change with nonviable pregnancies such that the women had inconsistent levels of AMH from time of positive pregnancy test (gestation week 4) to their final blood test (when hCG ≤5 IU/mL) as shown in Figure 3.

AMH and Fetal Gender

Of the 70 patients who achieved a successful pregnancy, 30 fetal or baby genders were identified at the time of data analysis, from either the 20-week ultrasounds or births, respectively. There was no relationship between the fetal/baby gender and the changes in the mother's AMH concentration during early pregnancy ($P = .809$). There was a decrease in mother's AMH levels in 54.5% (6/11) boys compared with 45.5% (5/11) that increased. Of the 19 girls, 12 (63.2%) had a decrease in the mother's AMH levels compared with 7 (36.8%) that increased.

FIGURE 3



Proportional change in serum antimüllerian hormone (AMH) relative to the concentration at the time of the positive pregnancy test (week 4) in nonviable pregnancies.

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DISCUSSION

This study documents the longitudinal changes occurring in AMH levels in individual women in the conception cycle before pregnancy and during the first few weeks of early pregnancy. Our data confirm the previously reported changes in AMH levels throughout the cycle, of a higher follicular/preovulatory AMH and lower luteal AMH level, when individual women are followed (14–17). We found an early trend of decreasing AMH level in most natural pregnancies and either consistently increasing or decreasing AMH levels in viable pregnancies achieved after fertility therapy. In contrast, nonviable pregnancies showed erratic changes in AMH levels. As such, this study provides novel information on the changes of AMH levels in viable and nonviable pregnancies, which suggests that very early changes to AMH levels in pregnancy are complex and not unidirectional.

The issue of whether or not AMH levels fluctuate during the menstrual cycle has been controversial. Some studies (18–20) have suggested that AMH levels do not vary significantly during the menstrual cycle. However, an increasing number of reports (14–17, 21), including the findings from our current study, support there being increased AMH levels in the late follicular/preovulatory phase with a decreased AMH in the luteal phase. Some of the different findings can be reconciled by considering the methodology and whether individual women's levels are followed (15), or whether group AMH values are summed and averaged (18–20, 22). In the present study, there were no significant differences in the group mean AMH levels between baseline, ovulation, and midluteal. However, when considering the pattern of change in individual women, AMH levels did peak at or before ovulation and decreased in the midluteal phase in 86.1% of the women.

During pregnancy, serum AMH levels in the first trimester have been reported by some studies (9, 13, 23) as comparable to nonpregnant levels. We had similar findings in the present study of no significant change in AMH from baseline (conception cycle) to gestation week 4, when all results were summed and mean values examined. However, when assessing longitudinal trends in individual women, different patterns of changing AMH levels were observed. Two well-designed studies (11, 13) report a decrease in AMH levels with advancing gestational age. Cross-sectional studies of AMH are limited by the fact that different women of the same age can show substantial variations in their AMH levels (9, 11, 24); however, the study conducted by Nelson et al. (13) was important in confirming longitudinal changes in the same cohort of pregnant women.

A remaining gap in our knowledge of AMH levels in pregnancy, however, has been how AMH changes in very early pregnancy and how this may differ in individual women. An important aspect of the present study was the ability to follow changes in AMH levels in individual women across early pregnancy. We showed that AMH levels in pregnant women when represented by average values do not change significantly in early pregnancy. However, when individual pregnancies are followed, a more complex pattern of AMH changes is observed. We found a trend for decreasing AMH levels in natural pregnancies, although 3 of 15 natural pregnancies had increasing AMH levels in the first few weeks of gestation. Viability of a natural pregnancy was not associated with increasing or decreasing AMH levels. The trend for decreasing AMH levels in natural pregnancies is similar to that reported by other investigators (11, 13). There is increasing evidence that adequate long-term gonadotropins are required for AMH (6, 25–27), and the suppression of gonadotropins that occurs during early pregnancy, and persists until postpartum, may in part explain the trend for reducing AMH levels noted in some of our early natural pregnancies and in pregnancies in general (11, 13).

In women achieving pregnancy after fertility treatments, AMH levels again did not remain stable but consistently changed, moving up or down in different women irrespective of baseline AMH and FSH stimulation. Consistently increasing or decreasing AMH levels was again not associated with viability of the pregnancy. It is likely there are other unidentified factors contributing to the increase in AMH levels observed during some pregnancies, but a decrease in other. In some respects these findings are not surprising as many of the previously accepted concepts about AMH, its physiological stability and independence of FSH levels are also being challenged. The fact that increasing or decreasing AMH level does not seem to affect the viability of the pregnancy implies that the AMH is acting as a marker or regulator of events in the ovary unrelated to pregnancy support provided by either the corpus luteum (CL) or exogenous steroids. That follicular growth and atresia occurs during the gestation period is known because follicles in all stages of development before the preovulatory stage can be observed throughout pregnancy (28).

The consistent upward or downward trend of AMH concentrations in viable pregnancies contrasted with the more erratic nature in nonviable pregnancies. There have been numerous studies that have assessed AMH levels in predicting an ongoing pregnancy, with conflicting results. Although age

is accepted as the best predictor of a successful (ongoing) pregnancy, some studies (29–32) report that ovarian reserve testing, such as antral follicle count or AMH level do not improve prediction, whereas other investigators note that levels of these markers can predict the risk of miscarriage (8, 33–36). Because this study observed notable differences in the patterns of AMH change between viable and nonviable pregnancies, it would be worthwhile to investigate this further in a larger longitudinal cohort.

The relationship between age and female fertility is well established (36–38), with AMH levels declining with advancing age. This study reconfirmed the strong correlation between age and AMH, by comparing the mean baseline AMH (day 2 of the menstrual cycle) to patient age. Women were assigned to four age categories (26–30, 31–35, 36–39, and $\geq$40 years) to demonstrate the significant decrease in mean AMH levels, despite the highly variable range of levels within each age group. High interindividual variability for AMH is not unexpected, as the hormone is widely used as a biomarker of ovarian reserve in women (39). There are many factors that have been shown to affect a woman's ovarian reserve, such as PCOS (40–42) and ethnicity (43). This present study reported no significant association between AMH and BMI, which is consistent with some studies (44, 45) and contradictory to others (46, 47) that have reported negative associations. Body mass index was not found to correlate with patient age, which is supported by a recent study (48) and a very large study (12), and therefore was excluded as a factor affecting the relationship between age and AMH.

Different fertility treatments involve various combinations and concentrations of medication, depending on the cycle type and individual response. Each woman has a tailor-made repertoire of drugs that was determined by their medical practitioner. This study found no significant correlations between the ovarian stimulation drug dosage and the trend in AMH levels, which is consistent with other findings (45, 49). Despite large differences in the combinations and dosages of patient medications, there were still women within each treatment group who exhibited an increase in AMH levels, whereas some women showed decreases. A recent report on the regulation of AMH by E_2 has shown that E_2 may either increase or decrease AMH synthesis depending on the type of E_2 receptor (alpha or beta) expressed (50). Differential effects of fertility treatments on E_2 receptors could be one of the possible mechanisms for the differences we observed and this warrants further investigation.

The present study found that there was no association between the gender of the fetus and the trend in AMH levels. As a male embryo develops, the Sertoli cells secrete around 1,000-fold higher AMH levels than in females, therefore it would have been interesting to note whether AMH crossed the placental barrier into the maternal blood to cause an increase in AMH levels if the fetus was male. However, comparison between gender and AMH levels proved insignificant. This result is consistent with other findings (51).

In conclusion, the longitudinal design of the present study enabled a direct comparison of AMH levels within the same individual during the time of conception and the very early weeks of pregnancy. This approach resulted in the description of unique changes in early pregnancy that may have otherwise remained unnoticed. Maternal serum AMH concentrations did not remain constant but varied during the conception cycle and early gestation. The AMH levels peaked at or before ovulation in most cases, and then consistently increased or decreased in these first few weeks of pregnancy in individual women with a viable pregnancy. Nonviable pregnancies showed early erratic AMH patterns, and factors responsible for these different responses in pregnancy remain to be identified.

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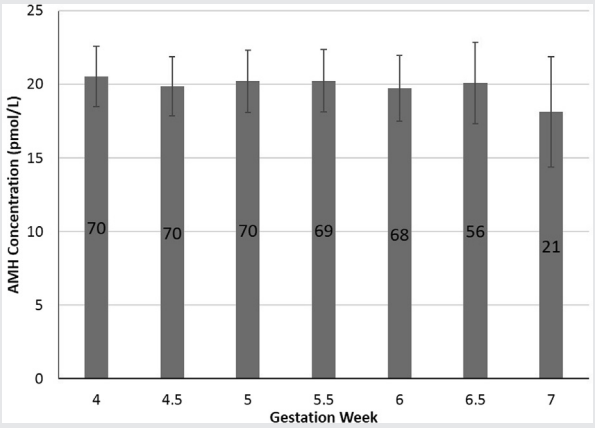
SUPPLEMENTAL FIGURE 1



Serum antimüllerian hormone (AMH) concentrations (mean ± SEM) from gestation week 4 to the week of fetal heart detection by ultrasound.

Hamilton. AMH in conception cycles and early pregnancy. Fertil Steril 2016.

SUPPLEMENTAL TABLE 1

Serum concentrations (mean ± SEM) of hCG, E_2, and P for viable pregnancies from gestation weeks 4–6.5 (n = 55).

Gestation week	hCG (IU/L)	E_2 (pmol/L)	P (nmol/L)
4	251.9 ± 30.1	1,205.9 ± 154.1	199.7 ± 27.5
4.5	1,384.1 ± 182.2	1,608.7 ± 227.3	198.6 ± 23.9
5	5,187.5 ± 565.4	1,979.3 ± 276.9	194.1 ± 21.2
5.5	13,852.5 ± 1,167.7	2,183.1 ± 311.3	187.2 ± 19.7
6	29,686.4 ± 2,183.5	2,338.3 ± 332.1	181.1 ± 18.9
6.5	53,844.4 ± 3,525.2	2,436.4 ± 299.5	177.0 ± 18.5

Hamilton. AMH in conception cycles and early pregnancy. Fertil Steril 2016.