**Reference ranges of AMH in early pregnancy: the Generation R Study, a population-based prospective cohort study**

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**Running title:** AMH and placental biomarkers in early pregnancy

**Keywords:** Ovarian reserve, placental biomarker, nomogram, first trimester, human Choriogonadotrophin (hCG), soluble FMS-Like Tyrosine kinase-1 (sFLT), Placental Growth Factor (PLGF).

**Tweetabel abstract:**

Association between higher AMH in early pregnancy and placental biomarkers suggests a better placental development.

**Abstract**

**Objective:** The objective of this study is to establish maternal reference values of AMH in a fertile multi-ethnic urban pregnant population and to evaluate the effect of gestational age.

**Design:** This study was embedded in the Generation R Study, an ongoing population-

based prospective cohort study from early pregnancy onwards.

**Setting:** City of Rotterdam, the Netherlands, out of hospital setting.

**Patients:** In 5806 women serum AMH levels were determined in early pregnancy (median 13.5 weeks; 95% range 10.5-17.2).

**Intervention(s):** None.

**Main outcome measures:** Maternal AMH levels in early pregnancy and its association with placental biomarkers, including human Chorionic Gonadotrophin (hCG), soluble FMS-Like Tyrosine kinase-1 (sFLT), and Placental Growth Factor (PLGF).

**Results:** Anomogram of AMH in early pregnancy was developed. Serum AMH levels showed a decline with advancing gestational age. Higher AMH levels were associated with a higher level of the first trimester placental biomarkers hCG and sFLT. This last association was predominantly mediated by hCG. AMH levels were negatively associated with PLGF levels.

**Conclusion:** In this large study we show that AMH levels in the first trimester decrease with advancing gestational age. The association between AMH and the placental biomarkers hCG, sFLT and PLGF suggests a better placental development with a lower vascular resistance in mothers with higher AMH levels. Hence AMH might be useful in predicting adverse pregnancy outcome due to impaired placental development.

**Keywords:** Ovarian reserve, placental biomarker, nomogram, first trimester, human Choriogonadotrophin (hCG), soluble FMS-Like Tyrosine kinase-1 (sFLT), Placental Growth Factor (PLGF).

**Introduction**

Anti‐Müllerian hormone (AMH) is a glycoprotein from the transforming growth factor‐beta family and is produced by granulosa cells of antral and preantral follicles 1, 2. AMH plays an important role in ovarian function and folliculogenesis and is believed to be the best biomarker of so called ovarian reserve 1, 3. AMH has basically three different functions in the human ovary. First, it inhibits the recruitment of follicles from the primordial follicle pool. Second, it inhibits follicle stimulating hormone (FSH)-induced aromatase activity thereby increasing intra-ovarian androgen concentrations with a consequent decrease in estrogen levels. Finally, AMH decreases the individual sensitivity to FSH of large antral follicles, thereby inhibiting the selection of the dominant follicle 1.

The ovary-specific expression pattern in granulosa cells of growing non-selected follicles makes AMH an ideal indirect marker for the size of the ovarian follicle pool 2. Serum AMH is believed to be the best marker of the ovarian reserve 1, 4. It is widely used as a predictor of ovarian response in controlled ovarian stimulation and to predict age‐at‐menopause as well as primary ovarian insufficiency 5-7. Besides the role of AMH as a predictor of menopause and response to ovarian stimulation its role is more and more being explored in different fields, including the association with pregnancy complications and outcomes 8-10.

Studies suggest that AMH levels can fluctuate substantially during the menstrual cycle 11-14. A few studies have described AMH levels during pregnancy, with conflicting results 15-18. Some studies conclude that AMH levels remain stable throughout pregnancy 16-18, whilst others reported a more dynamic role for AMH with a decrease with advancing gestational age 15, 18-20.

Early pregnancy is characterized by a complex interplay between placental biomarkers and steroid hormones. Placental biomarkers such as soluble fms-like tyrosine kinase-1 (sFLT), human chorionic gonadotrophin (hCG) and placental growth factor (PLGF) are known to be important representatives of placental (dys)function 21. These biomarkers have been associated with pregnancy complications such as pre-eclampsia, SGA and preterm birth 22-24. Some studies also suggest that decreased preconception AMH levels might be correlated with adverse pregnancy outcome 8, 25, 26. The interplay between AMH and placental function, reflected by these biomarkers, could therefore be of interest 22.

Considering the controversy about AMH concentrations during pregnancy and the possible correlation between AMH and adverse pregnancy outcome, we studied AMH levels in a large prospective cohort of almost 6000 pregnant women. We aimed to establish reference intervals for serum AMH and to evaluate the effect of gestational age on AMH serum levels. We also studied the association between serum AMH and sFLT, hCG and PLGF.

**Materials and methods**

***Study design and population***

This study was embedded in the Generation R Study, an ongoing population-based prospective cohort study. The design and the cohort of the Generation R study have been extensively described elsewhere 27. It is conducted in Rotterdam, the second largest city in the Netherlands, and eligible women were all those who were resident in the study area and expected to deliver between April 2002 and January 2006. In total 8976 pregnant women were enrolled of which 63.2% in the early pregnancy period. For the present study only women of whom first trimester blood samples were available were included for the current study (Figure 1).

Gestational age was established using data from the first ultrasound examination 28. Information on possible determinants (sociodemographic factors, life style habits and obstetrical history) was obtained from questionnaires. Sociodemographic factors included information on age, educational level and ethnicity. Ethnic background was derived from the country of birth of the woman herself and her parents. For this study we divided the women in two groups ‘Caucasian’ and ‘other ethnicities’ 29. Educational level was assessed by the highest completed education and classified into three categories: 1) primary education; 2) secondary education; and 3) university or college 30. Life style habits included [body mass index](https://www.sciencedirect.com/topics/medicine-and-dentistry/body-mass-index) (BMI; in kg/m2; calculated from length and weight measured at enrolment) and smoking. Obstetrical history included information on parity and fertility treatment.

***Hormonal assays***

AMH levels were measured in blood samples drawn at inclusion in the study in early pregnancy. The venous samples were taken by research nurses and stored at room temperature before being transported to the regional laboratory for processing and storage for future studies (STAR-MDC). Processing was planned to finish within a maximum of three hours after venous puncture. The samples were centrifuged and thereafter stored at −80°C 31.

AMH measurements were preformed using the AnshLabs pico AMH ELISA (AnshLabs, Webster, Tx, USA). All measurements were performed according to standard procedures between January 2018 and February 2020. The samples were thawed and measured on the same day. Loss of signal for AMH due to prolonged storage at -80° was deemed negligible given our experience with in-house used quality control materials 32. During the study, kit controls as well as pooled serum controls were used to assure accuracy. Coefficients of variation were 2.9% at 0.3 ng/mL and 6.2% at 0.1 ng/mL, respectively, for kit controls. For pooled serum controls, coefficients of variation were 5.4% at 0.8 ng/mL and 7.1% at 0.2 ng/mL, respectively.

Information was available for the following first trimester biomarkers: hCG, PLGF and sFLT. hCG was analyzed in serum using a solid-phase two-site chemiluminescent immunometric assay, calibrated against WHO 3rd IS 75/537, on an Immulite 2000 XPi system (Siemens Healthcare Diagnostics, Deerfield, IL, USA) 33. The interassay coefficient of variation was 8.0, 6.3 and 5.1 % at the concentration of 9.7, 53.1 and 821.5 IU/L, respectively 34. PLGF and sFLT were analyzed in plasma, using an immune-electrochemiluminescence assay on the Architect System. The between-run coefficients of variation for PLGF were 4.7% at 24 pg/mL and 3.8% at 113 pg/mL. The coefficients for sFLT were 2.8% at 5.5 ng/mL and 2.3% at 34.0 ng/mL 35, 36.

***Statistical analyses***

Non-parametric specific reference ranges (RR) were determined by the 2.5th-97.5th percentiles for each year of maternal age. The model-based AMH reference ranges for maternal age were created using Generalized Additive Models for Location, Size and Shape (GAMLSS). These specific statistical tools enable flexible, (semi) parametric, reference range calculations while accounting for skewness and kurtosis of the data during the modelling process. We used 2 cubic splines for maternal age, 3 cubic splines for sigma variation and a Box Cox t family distribution (after sensitivity analyses using Akaike Information Criterion and worm plots) in order to achieve the best fit 37. Subsequently, age specific Z-scores and 2.5th, 50th and 97.5th values were derived from the model. We applied the same technique for model-based AMH reference ranges for gestational age. We used two cubic splines for gestational age, no cubic splines for sigma variation and also a BCT distribution in order to achieve the best fit. Next, associations between AMH and several first trimester biomarkers (hCG, PLGF, and sFLT) were analyzed using multivariate linear regression analyses. Since levels of these biomarkers significantly changed during gestation, we constructed hCG, PLGF and sFLT, gestational-age adjusted standardized Multiple of the Median (MoM) scores, which we used in these analyses. MoM scores >3.0 were excluded from these analyses. The models were adjusted for maternal age, smoking, BMI, education level, maternal ethnicity (Caucasian and ‘other ethnicities’), parity and fetal sex. Multivariable linear regression analyses were performed utilizing three restricted cubic splines for hCG, PLGF and sFLT, maternal age and BMI. Mediation analyses were additionally performed for hCG, PLGF and sFLT. Standardized direct and unstandardized indirect effects were computed for each of 5000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles. IBM Statistical Package of Social Sciences version 24.0 for Windows (SPSS Incl., Chicago, IL, USA) and rms library in R statistical package, version 3.03 were used for statistical analyses.

**Results**

***AMH levels during early pregnancy***

Descriptive characteristics of the study population are shown in Table 1. The included women (n=5806) had a median gestational age of 13.4 weeks (range 10.5-17.2) and had a mean (SD) age of 29.6 (±5.1) years. Of the included women, 60.4% were Caucasian and the median BMI at intake was >24 kg/m2 (90% range 19.2 – 33.4). Of all women 36.6% had overweight or were obese. Most women (60.2%) were pregnant of their first child and a minority (1.3%) achieved pregnancy through assisted reproductive technologies (ART) (Table 1).

Population-based, maternal age specific reference ranges for AMH in pregnancy are shown in Table 2. In addition, model based reference centile curves are depicted in Figure 2. Serum AMH levels seemed to remain rather constant until the age of 25 years. After 25 years of age we observed a steady decline. Throughout gestation we observed a decline in serum AMH between 8 and 12 weeks of gestation (Table 3 and Figure 3).

***Association of serum AMH levels with markers of placental function***

Next, we analyzed the association of serum AMH levels with markers of placental function. Table 4 shows the decline in AMH serum levels during early pregnancy coinciding with an increase in hCG, PLGF and sFLT.

Over the full spectrum, there was a significant positive association between AMH levels and hCG (P < 0.0001) as well as sFLT (P < 0.05). Higher AMH levels were associated with higher hCG and sFLT levels. On the contrary, AMH was negatively associated with PLGF levels (P < 0.01). (Figure 4).

We used mediation analyses to examine the mediation impact of hCG on the relationship between placental biomarkers (sFLT and PLGF) and AMH. We identified that the relationship between sFLT and AMH was fully mediated by hCG (Figure S1). The relationship between PLGF and AMH was not mediated by hCG (Figure S2).

**Discussion**

**Main Findings**

In this large cohort study, a nomogram of AMH serum concentrations during the first trimester of pregnancy was developed, demonstrating that serum AMH levels decrease already early in the first trimester of pregnancy. Finally, this decrease in AMH levels seems to be associated with a significant decrease in PLGF and an increase in hCG and sFLT levels.

**Strengths and limitations**

A major strength of this prospective cohort study is the large sample size and the long term follow up. To the best of our knowledge this is the largest study in the field addressing AMH serum concentrations in the first trimester of pregnancy. In this study AMH was measured in the population at different gestational age. We demonstrated a suppression of AMH levels already early in the first trimester. A possible limitation is the absence of an AMH measurement before pregnancy.

**Interpretation**

Our results confirm the results of other studies, showing that AMH levels decline during the first trimester of pregnancy. Most studies reported a decline of AMH levels from the late first trimester of pregnancy onwards 20, 38, 39. Others observed a decline earlier in pregnancy, between 7 and 14 weeks of gestation 40. The analyzed AMH levels in those studies were not adjusted for maternal age, smoking, BMI, education level, maternal ethnicity, parity or fetal sex. The decrease in AMH levels during pregnancy is probably due to the suppression of the hypothalamic pituitary gonadal axis which leads to a change in follicle dynamics and a decrease in AMH levels 41. Indeed Durlinger et al. showed that suppression of the hypothalamic pituitary ovarian axis using a GnRH antagonist in mice leads to different follicle class distribution and a different AMH expression. Due to low FSH levels, the growth of the small follicles is slower and the granulosa cell mass seems to be less resulting in lower AMH levels 41. Moreover, combined oral contraceptive pill use suppresses the hypothalamic pituitary-ovarian axis through an increase in negative feedback and thereby inhibits FSH and LH release from the pituitary preventing dominant follicle selection causes similar changes in AMH output 42, 43. During pregnancy the gonadotropin dependent stages of folliculogenesis are also inhibited. Indeed, the ovary seems to be suppressed in pregnancy mimicking the prepubertal quiescent state 44. Hence, AMH serum concentrations decrease from the first trimester of pregnancy onwards due severely depressed FSH as well as LH levels caused by high serum levels of estrogen and progestogens originating from the corpus luteum and later on from the placenta. Indeed, Koniger et al. found an AMH decline during pregnancy followed by a rapid increase of AMH to near pre-pregnancy levels within a few days after delivery 20.

Different other underlying mechanisms of AMH suppression in pregnancy have been explored including the influence of fetal sex and maternal BMI. Stojsin-Carter et al. found a trend that fetal-sex was linked with differences in maternal AMH levels in cattle. That might be driven by a decrease in maternal AMH production coupled with sex-dependent fetal AMH production 45. In a large study performed in a healthy general female population, AMH was negatively related to BMI, the relationship was age dependent. AMH levels decreased and BMI increased with age. The correlation between AMH and BMI was secondary to the stronger relationship of the two variables with age 46. Part of the observed reduction in AMH levels during pregnancy could also be explained by the pregnancy-associated hemodilution and increased plasma-protein binding 47.

The rapid increase in AMH levels post-partum suggests a physiological cross-talk between the corpus luteum and later on via the placenta (through sex steroid feedback) on the one hand and the ovary (through reduced secondary cyclic recruitment of follicles) on the other hand resulting in suppressed AMH serum levels during pregnancy 20. Moreover, since the menstrual cycle is not restored immediately after delivery and during the puerperium it also suggests that placental factors might play a role in suppressing AMH levels during pregnancy. Placental biomarkers cross-talk with other organs, such as the thyroid, pituitary and the ovary. hCG causes the so called “luteal rescue” and a subsequent increase in estrogen and progesterone production in the corpus luteum until the luteo-placental shift takes place 48. Hence it is the indirect driver of the suppression of the GnRH pulse generator during pregnancy and contributes in that way to the decrease in pituitary gonadotrophins causing a decrease in AMH.

[Fetal growth](https://www.sciencedirect.com/topics/medicine-and-dentistry/fetus-growth) is dependent on adequate development of the placenta 49. Korevaar et al. showed that a higher hCG MoM was associated with a higher placental weight 50. Other important placental biomarkers, associated with placental (dys)fynction, are sFLT and PLGF. Impaired [angiogenesis](https://www.sciencedirect.com/topics/medicine-and-dentistry/angiogenesis) and vasculogenesis in [early pregnancy](https://www.sciencedirect.com/topics/medicine-and-dentistry/first-trimester-pregnancy) compromises [placental and embryonic development](https://www.sciencedirect.com/topics/medicine-and-dentistry/placenta-development) 49. sFLT is an anti-angiogenic factor that binds to free circulating [vascular endothelial growth factor](https://www.sciencedirect.com/topics/medicine-and-dentistry/vasculotropin) and PLGF, thereby inhibiting [blood vessel](https://www.sciencedirect.com/topics/medicine-and-dentistry/vascular-bundle) growth 22. PLGF is the most abundantly regulated angiogenic factor in first trimester decidua 51. In other studies it has been demonstrated that higher sFLT levels in early pregnancy are associated with lower placental vascular resistance leading to higher placental weight as well as birth weight 22. The positive association, we observed, between AMH, hCG and sFLT was fully mediated by hCG.

The negative association between AMH, hCG and PLGF was not mediated by hCG. Upregulation of PLGF leads to the activation of an inflammatory state, with a subsequent release of different cytokines. These cytokines can modulate the cells of the immune system and could therefore interfere with adequate vascular development of the placenta 52. PLGF supports the early events of implantation and placental development. Upregulation of PLGF leads to the activation of an inflammatory state, with a subsequent release of different cytokines. These cytokines can modulate the cells of the immune system and could therefore interfere with adequate vascular development of the placenta 52.

Taken together the significant association between AMH and the placental biomarkers sFLT mediated by hCG suggests that higher AMH levels are coinciding with a lower vascular resistance in the early placental bed. Similarly, higher AMH levels are associated with lower PLGF levels and this might prevent the release of cytokines that interfere with proper placental development. Hence AMH might be useful in predicting adverse pregnancy outcome due to impaired placental development. Moreover, studies that assess AMH levels pre-pregnancy, during gestation, and postpartum may help to better understand the mechanism of how the ovary might influence the placenta and vice versa and how this interaction impacts on follicular recruitment during pregnancy and after delivery. Prospective pregnancy studies that evaluate maternal and pregnancy outcomes in addition to other biomarkers in pregnancy are important to better understand how AMH is related to maternal and fetal outcomes of pregnancy.

**Conclusion**

AMH levels in pregnancy decrease with advancing gestational age. The underlying mechanism may be due to the cross-talk with placental biomarkers. AMH is significantly associated with placental biomarkers as hCG and PLGF. The significant association between AMH and sFLT was mediated by hCG. Those biomarkers are correlated with placental development. Therefore, they are potential candidates for predicting adverse pregnancy outcomes.

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**Disclosure of interest**

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**Contribution to authorship**

As part of the Generation R study research project, this study was planned and designed by JL, ES, SS, YL and RD. SB and BL preformed all the laboratory tests. SV RD, AA and SS preformed the statistical analysis. RD, SS, YL, TK and JL interpreted the data and wrote the manuscript. All authors contributed substantially to revisions of the manuscript and approved the final version.

**Details of Ethical approval**

The study was approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam, the Netherlands (MEC 198.782/2001/31). Written informed consent was obtained from all participants.

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**Figure legends**

**Figure 1.** Population for current study – AMH and pregnancy

**Figure 2.** Maternal age specific reference ranges for AMH

Maternal age (years) specific reference ranges for AMH levels (µg/L). AMH reference values were calculated according to a (semi) parametric 2.5th-97.5th percentiles by GAMLSS modeling in a population-based approach. Colored lines depict the maternal age specific centiles for AMH levels.

**Figure 3** Gestational age specific reference ranges for AMH

Gestational age (weeks) specific reference ranges for AMH levels (µg/L). AMH reference values were calculated according to a (semi) parametric 2.5th-97.5th percentiles by GAMLSS modeling in a population-based approach. Colored lines depict the maternal age specific centiles for AMH levels.

**Figure 4.** Associations of AMH with hCG, sFLT and PLGF

Graphs show the significant associations between maternal levels of hCG, sFLT and levels of AMH with 95% confidence interval. Analyses were performed on gestational age specific Z-scores for AMH and gestational age specific Multiple of the Median (MoM) scores for hCG and sFLT. MoM scores >3.0 were excluded from the analyses. Analyses were additionally adjusted for maternal age, educational level, ethnicity, parity, BMI, smoking and fetal sex. Analyses were performed using linear regression analyses utilizing two restricted cubic splines for hCG, *P< 0.001*. Analyses were performed using linear regression for sFLT,  *P 0.02.*

Graphs show the significant associations between maternal levels of PLGF levels of AMH with 95% confidence interval. Analyses were performed on gestational age specific Z-scores for AMH and gestational age specific Multiple of the Median (MoM) scores for PLGF. MoM scores >3.0 were excluded from the analyses. Analyses were additionally adjusted for maternal age, educational level, ethnicity, parity, BMI, smoking and fetal sex. Analyses were performed using linear regression for PLGF, *P=0.01.*

**Figure S1** Relationship between sFLT and AMH as mediated by hCG

The total effect (c) is significant. The direct effect (c’) of sFLT on AMH was not significant and fully mediated by hCG. \* P value< 0.05.

**Figure S2** Relationship between PLGF and AMH as mediated by hCG

The total effect (c) is significant. The direct effect (c’) of PLGF on AMH is significant and therefore not mediated by hCG. \* P value< 0.05.

**Table 1.** Baseline characteristics (n = 5806)

|  |  |
| --- | --- |
| **Outcome** | **Women** |
| Gestational age at blood sampling, median (90% range), (weeks) | 13.4 (10.5; 17.2) |
| *<8.00*  *8.01-10.00*  *10.01-12.00*  *12.01-14.00*  *>14.00* | 0.5  2.6  17.3  41.7  37.9 |
| Age mother at enrollment, mean (SD), (years) | 29.6 (5.1) |
| *<25* | 20.0 |
| *25-30* | 28.6 |
| *30-35* | 37.9 |
| *>35* | 13.4 |
| Ethnicity, % |  |
| *Caucasian*  *Non-Caucasian* | 60.4  39.6 |
| Education level, % |  |
| *Primary education* | 10.3 |
| *Secondary education* | 46.1 |
| *University or college* | 43.6 |
| BMI at intake, median (90% range), (kg/m2) | 23.6 (19.2-33.4) |
| *<25kgm2* | 63.4 |
| *25-30kg/m2* | 24.7 |
| *>30kg/m2* | 11.9 |
| Smoking |  |
| *Never smoked* | 71.5 |
| *Smoked until pregnancy* | 9.6 |
| *Continued smoking in pregnancy* | 18.9 |
| Pregnant % |  |
| *Spontaneously* | 98.7 |
| *ART* | 1.3 |
| Parity |  |
| *Nulliparous* | 60.2 |
| *Para-1* | 27.4 |
| *Para-2 or more* | 12.4 |

Abbreviations: BMI, body mass index. Values are valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

**Table 2.** Maternal age specific reference ranges for AMH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age (years) | n | Median | 2.5th | 97.5th |
| <16 | 4 | 2.952 | 0.723 | 8.215 |
| 16 | 7 | 2.920 | 0.710 | 8.162 |
| 17 | 30 | 2.888 | 0.696 | 8.109 |
| 18 | 58 | 2.856 | 0.683 | 8.055 |
| 19 | 107 | 2.825 | 0.669 | 8.000 |
| 20 | 127 | 2.792 | 0.655 | 7.947 |
| 21 | 167 | 2.757 | 0.640 | 7.896 |
| 22 | 191 | 2.720 | 0.622 | 7.848 |
| 23 | 224 | 2.680 | 0.602 | 7.803 |
| 24 | 244 | 2.635 | 0.579 | 7.760 |
| 25 | 280 | 2.586 | 0.553 | 7.719 |
| 26 | 291 | 2.531 | 0.523 | 7.682 |
| 27 | 291 | 2.470 | 0.490 | 7.648 |
| 28 | 382 | 2.403 | 0.455 | 7.608 |
| 29 | 416 | 2.329 | 0.419 | 7.553 |
| 30 | 487 | 2.246 | 0.383 | 7.467 |
| 31 | 504 | 2.153 | 0.348 | 7.340 |
| 32 | 464 | 2.051 | 0.312 | 7.174 |
| 33 | 407 | 1.942 | 0.278 | 6.979 |
| 34 | 341 | 1.826 | 0.243 | 6.760 |
| 35 | 259 | 1.705 | 0.210 | 6.522 |
| 36 | 167 | 1.581 | 0.178 | 6.263 |
| 37 | 121 | 1.455 | 0.149 | 5.983 |
| 38 | 94 | 1.327 | 0.122 | 5.685 |
| 39 | 59 | 1.202 | 0.098 | 5.373 |
| 40 | 30 | 1.079 | 0.077 | 5.047 |
| 41 | 20 | 0.960 | 0.059 | 4.706 |
| 42 | 17 | 0.845 | 0.045 | 4.346 |
| 43 | 7 | 0.732 | 0.033 | 3.957 |
| >43 | 3 | 0.621 | 0.024 | 3.529 |

AMH reference ranges (µg/L) were calculated according to a population-based approach in the whole study population per maternal age category (years [yrs]).

**Table 3.** Gestational age specific reference ranges for AMH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gestational  Age (wks) | n | Median | 2.5th | 97.5th |
| 5 | 3 | 3.809 | 0.643 | 11.809 |
| 6 | 4 | 3.592 | 0.593 | 11.236 |
| 7 | 10 | 3.373 | 0.544 | 10.657 |
| 8 | 34 | 3.152 | 0.497 | 10.038 |
| 9 | 79 | 2.930 | 0.451 | 9.418 |
| 10 | 167 | 2.712 | 0.408 | 8.798 |
| 11 | 453 | 2.505 | 0.367 | 8.202 |
| 12 | 1258 | 2.325 | 0.333 | 7.684 |
| 13 | 1173 | 2.186 | 0.305 | 7.298 |
| 14 | 900 | 2.094 | 0.285 | 7.054 |
| 15 | 670 | 2.037 | 0.270 | 6.925 |
| 16 | 502 | 1.989 | 0.256 | 6.829 |
| 17 | 388 | 1.946 | 0.244 | 6.744 |
| 18 | 159 | 1.898 | 0.232 | 6.419 |

AMH reference ranges (µg/L) were calculated according to a population-based approach in the whole study population per gestational age category (weeks gestation [wks]).

**Table 4.** AMH and placental biomarkers according to gestational age

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Weeks of gestation** | **4.0-6.0** | **6.1-8.0** | **8.1-10.0** | **10.1-12.0** | **12.1-14.0** | **14.1-16.0** | **16.1-18.0** |  |
| **n** | **4** | **29** | **163** | **1088** | **2452** | **1350** | **777** | ***P values*** |
| AMH (**ug**/**L)** | 2.87 | 3.51 | 2.89 | 2.27 | 2.11 | 1.96 | 1.93 | <0.005 |
|  | (1.56-3.10) | (0.48-8.70) | (0.70-7.93) | (0.53-6.64) | (0.42-5.88) | (0.37-5.73) | (0.42-5.65) |  |
|  |  |  |  |  |  |  |  |  |
| hCG (IU/L) | 3659 | 60887 | 75533 | 58234 | 49844 | 33525 | 23410 | <0.005 |
|  | (455-8077) | (22716-137849) | (33133-129909) | (25731-106628) | (23379-94075) | (14324-72545) | (8154-52436) |  |
|  |  |  |  |  |  |  |  |  |
| PLGF (pg/ml) | 12.30 | 14.00 | 19.85 | 28.30 | 37.20 | 67.80 | 113.50 | <0.005 |
|  | (8.80-13.30) | (8.10-500.30) | (12.20-33.24) | (15.14-58.00) | (19.16-87.90) | (30.10-163.40) | (49.10-252.69) |  |
|  |  |  |  |  |  |  |  |  |
| sFLT (ng/ml) | 0.21 | 3.99 | 5.18 | 5.08 | 5.07 | 5.25 | 5.17 | <0.005 |
|  | (0.12-0.36) | (1.13-14.26) | (2.45-12.08) | (2.38-11.27) | (2.29-11.84) | (2.14-12.78) | (2.05-13.05) |  |
|  |  |  |  |  |  |  |  |  |

Abbreviations: hCG, human chorionic gonadotropin; PLGF, placental growth factor; sFLT, soluble fms-like tyrosine kinase-1.Values are medians (90% range) for continuous

variables with a skewed distribution. Presented values are not imputed. Differences between different groups of gestation were tested through one-way ANOVA.