## Prenatal screening in twin pregnancies

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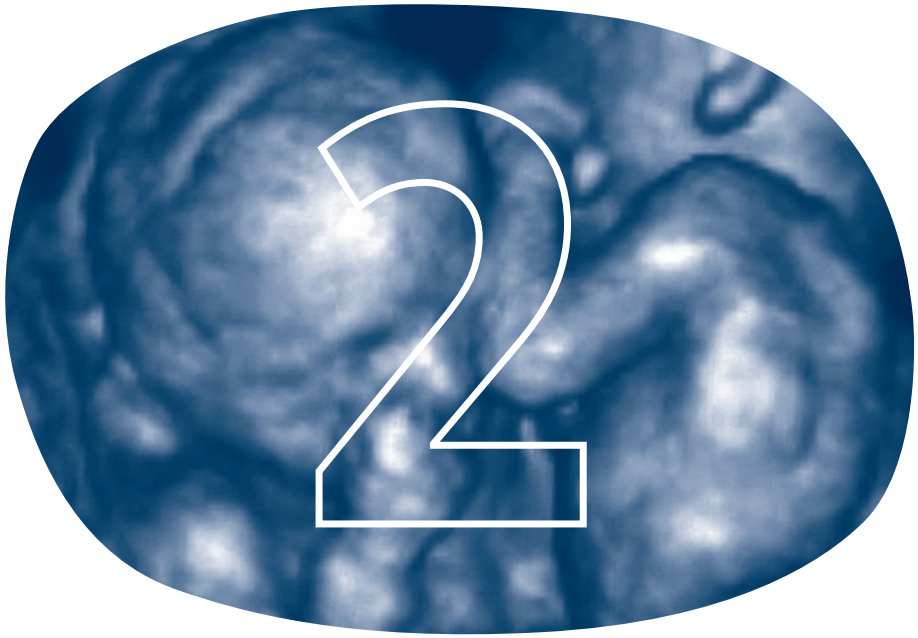



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# Early first trimester free β-hCG and PAPP-A serum distributions in monochorionic and dichorionic twins

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# Abstract

### . . . Objective
In the Netherlands, prenatal screening for trisomy 21 in the first trimester of pregnancy for singletons is conducted through a combined test based on maternal age, nuchal translucency measurement and maternal serum free β-hCG and PAPP-A. In our clinic risk calculations in twins are currently based on the NT of both fetuses instead of the combined test. In this study we looked at differences in early first trimester free β-hCG and PAPP-A between mono- and dichorionic twins.

### . . . Methods
A total of 202 twin pregnant women participated in the study and agreed to donate first trimester blood for research.

### . . . Results
The data of 200 euploid twins were used for setting up reference values for free β-hCG and PAPP-A. Trisomy 21 was identified in the two remaining pregnancies. In all twins the overall median weight-corrected MoM was 1.99 for free β-hCG, and 2.14 for PAPP-A. Monochorionic twins have a significantly lower free β-hCG weight-corrected MoM (1.53 vs 2.11; Mann-Whitney U, p=0.002) and a significantly lower PAPP-A weight-corrected MoM (1.59 vs 2.40; Mann-Whitney U, p=0.003) compared to dichorionic.

### . . . Conclusion
This study strengthened the need to make a distinction between mono- and dichorionic twins for the risk calculation in Down syndrome screening as biochemical markers are significantly lower in monochorionic twins than in dichorionic.




# Introduction

The importance of distinguishing between singletons and multiple pregnancies for prenatal screening has been stated by several authors [1;2].
The incidence of multiple births has increased since the introduction of assisted reproductive technology. Moreover, the increase in maternal age at conception contributes to the higher frequency of twin pregnancies. Screen positive test results are more common in twin pregnancies since advanced maternal age increases the risk of a pregnancy affected by Down syndrome [3-5].
Down syndrome screening in twins is more complex than in singletons. Both nuchal translucency (NT) measurement, i.e. 'fetus specific', and maternal biochemical markers, which are 'pregnancy specific', have to be taken into consideration. Previous studies have concentrated on setting reference values for free β-hCG and PAPP-A in unaffected twins [5-8]. Only recently has it been suggested that there are differences in biochemical marker distribution between monochorionic and dichorionic twins [9].
In the Netherlands prenatal screening for Down syndrome in singleton pregnancies is conducted through the first trimester combined test. The combined test calculates the risk for Down syndrome based on maternal age, fetal NT measurement and maternal serum free β-hCG and PAPP-A levels. Currently in our clinic, risk calculations in twins are based on NT-measurements of each individual fetus instead of the combined test. In this prospective study reference values for first biochemical markers and possible differences between monochorionic and dichorionic twins were investigated.

# Patients and Methods

Women pregnant with twins were enrolled in this study from 2004 to 2007. An ultrasound was performed between 11 and 14 weeks' gestation. The crown-rump length (CRL) was measured and NT was assessed in each twin if the CRL was between 45 and 79 mm according to Fetal Medicine Foundation (FMF) standards. Chorionicity was classified as monochorionic if there was a single placental mass in the absence of the λ-sign at the inter-twin membrane-placental junction, and dichorionic if there was a single placenta mass separate placentas mass with λ-sign present [10;11]. Risk assessment for Down syndrome was calculated using maternal age and NT measurements only. Women with a screen-positive test result (risk cut-off 1:200) were counselled concerning the risk for trisomy 21 and the risk of invasive testing. These women then either opted for, or refrained from invasive diagnostics. Fetal chromosomal status was determined by chorionic villus sampling or amniocentesis.
Maternal serum free β-hCG and PAPP-A levels were measured between 9+0 and 13+6 weeks of gestation using the Delfia Xpress (PerkinElmer, Turku, Finland). Coefficients of variation (CV) for PAPP-A were 3.2% at 480 and 2274 mU/L and for free β-hCG CV's of 3.0 and 3.8% were observed at 26.0 and 176.9 µg/L respectively (n=160).

In order to correct for gestational variation of analytes, all parameters are expressed as the weight-corrected Multiple of the Median (MoM) for unaffected singleton pregnancies. Gestational age at serum withdrawal was indicated by the requesting gynaecologist based on first day of last menstrual period and early first trimester dating scan.
The study was approved by the Ethics Committee of the VU university medical center Amsterdam. Pregnancy outcome was evaluated by questionnaires and delivery room records.

### . . . Statistical analysis

Patient characteristics of mono- and dichorionic twins are presented as mean ± SD and percentages (%), and tested for significance with independent t-tests and chi-square tests. Differences in weight-corrected MoMs of free β-hCG and PAPP-A between mono- and dichorionic twins were assessed for significance with nonparametric Mann-Whitney U test on the nontransformed MoMs and an independent t-test of the logtransformed MoMs. A multiple linear regression analysis was conducted to assess whether the relationship between gestational age and the concentration of free β-hCG and PAPP-A was different for mono- and dichorionic twins. The concentration free β-hCG or PAPP-A was used as the dependent variable. A dummy variable indicating the chorionicity (mono- or dichorionic), gestational age in days and the interaction term between chorionicity and gestational age were used as independent variables. A higher-order term of gestational age in days was included to obtain the best fitting curves for each biochemical marker in relation to gestational age. To account for possible confounding, the variables that initially differed between mono- and dichorionic twins were added as independent variables in the regression model. The results of this analysis are graphically displayed and contrasted with the PerkinElmer equation for singletons.
Statistical analyses were performed using SPSS version 15.0. p-Values <0.05 were considered significant. A logistic transformation was performed prior to the analysis on the non-normally distributed concentrations of free β-hCG and PAPP-A and the weight-corrected MoM free β-hCG and PAPP-A.

## Results

A total of 202 twin pregnancies with known fetal outcome were enrolled. Chorionicity was known in all cases: 37 monochorionic (18%) and 165 dichorionic (82%).
Table 1 and 2 summarize the characteristics of the group. No differences between mono- and dichorionic twins were found for maternal age and ethnicity. A higher percentage of mothers who smoked was seen in the monochorionic twin group.
Trisomy 21 was identified in 3 fetuses in 2 pregnancies. The data of 200 euploid twins were used for calculating the medians for the weight-corrected MoM free β-hCG and PAPP-A.
In monochorionic twins, free β-hCG concentrations ranged from 24-201 µg/L and PAPP-A ranged from 220-12700 mU/L compared with 20-448 µg/L and 250-17680 mU/L for dichorionic twins. Table 3 shows the medians for the weight-corrected MoM free β-hCG and PAPP-



**Table 1** Patients' characteristics

| | All twins (n 202) | Euploid Dichorionic (n 163) | Euploid Monochorionic (n 37) | p-value * =<0.05 |
|---|---|---|---|---|
| Mean maternal age (years) | 34.4 ± 3.9 | 34.6 ± 3.5 | 33.2 ± 5.2 | 0.144 |
| Ethnicity (%) | | | | |
| > Caucasian | 97.5 | 98.2 | 94.6 | |
| > Non-Caucasian | 2.5 | 1.8 | 5.4 | 0.502 |
| Smoking (%) | | | | |
| > no | 95.1 | 96.9 | 86.5 | |
| > yes | 4.9 | 3.1 | 13.5 | 0.02* |
| Conception (%) | | | | |
| > naturally conceived | 70.3 | 65.0 | 94.6 | |
| > ART (IVF and ICSI) | 29.7 | 35.0 | 5.4 | |
| Mean crown-rump length (mm) | 62.2 | 62.4 | 61.3 | 0.283 |
| Median nuchal translucency (mm) | 1.3 | 1.3 | 1.3 | 0.355 |

**Table 2** Gestational age (days) distributions at serum withdrawal. Mean gestational age at withdrawal is 81 days

| Gestational age in days | All twins (n 202) | Dichorionic (n 165) | Monochorionic (n 37) |
|---|---|---|---|
| 63-69 | 31 | 29 | 2 |
| 70-76 | 35 | 30 | 5 |
| 77-83 | 43 | 34 | 9 |
| 84-90 | 65 | 51 | 14 |
| 91-97 | 28 | 21 | 7 |

**Table 3** First trimester medians of weight-corrected MoM free β-hCG and PAPP-A for euploid mono-and dichorionic twins

| | Euploid twins (n 200) | Dichorionic (n 163) | Monochorionic (n 37) |
|---|---|---|---|
| Median weight-corrected MoM free β-hCG | 1.99 | 2.11 | 1.53 |
| Median weight-corrected MoM PAPP-A | 2.14 | 2.40 | 1.59 |

A for both mono- and dichorionic twins. The median weight-corrected MoM free β-hCG was significantly lower in monochorionic than in dichorionic twins (1.53 vs 2.11; Mann-Whitney U, p=0.002). The median weight-corrected MoM PAPP-A was also significantly lower in monochorionic twins than in dichorionic (1.59 vs 2.40; Mann-Whitney U, p=0.003). Also, an independent t-test on the logtransformed MoMs demonstrated that both free β-hCG (p=0.001, 95%CI: 0.13-0.51) and PAPP-A (p=0.001, 95%CI: 0.13-0.55) were significantly lower in monochorionic twins compared to dichorionic twins.
A subgroup analysis between samples taken before and after 12 weeks' gestation demonstrated that samples taken before 12 weeks had a significant lower weight-corrected MoM free β-hCG in monochorionic twins compared with dichorionic (Mann-Whitney U, p=0.001), but samples taken after 12 weeks did not show this difference (Mann-Whitney U, p=0.183). Pregnancies conceived with assisted reproductive techniques (ART: IVF and ICSI) versus naturally conceived pregnancies showed a higher weight-corrected MoM free β-hCG (2.18 vs 1.91) and a lower PAPP-A (2.02 vs 2.16), although this was not significantly different (Mann-Whitney U, p=0.060 and p=0.397).

For singletons, the Lifecycle PerkinElmer equation based on the VU University Medical Center Amsterdam (VUMC) medians of biochemical markers was used as reference, namely for free β-hCG: $y = 193.588 - 2.406 * x + 0.00690 * x^2$ and for PAPP-A: $y = 10 \text{ ^}(-0.952 + 0.0724 * x - 0.0002657 * x^2)$ where x refers to gestational age in days. For twins, the multiple regression analysis on the log-transformed free β-hCG and PAPP-A concentrations revealed that the interaction term between gestational age and chorionicity was not significant. The regression coefficient of the dummy variable indicating chorionicity was significant for both outcomes (free β-hCG: -0.330, p<0.05; PAPP-A: -0.393, p<0.05). After correction for smoking the difference between mono- and dichorionic twins remained (free β-hCG: β corrected for smoking -0.337, p<0.05; PAPP-A: β corrected for smoking -0.341, p<0.05). There was a negative relationship in time between free β-hCG and gestational age (β = -0.027, p<0.05) and a positive relationship in time for PAPP-A (β = 0.082, p<0.05). Higher order terms did not improve the fit of the equation for the log concentration free β-hCG/PAPP-A to gestational age. This implies that the relationship between the log-concentration and gestational age can be described by two separate parallel regression lines for mono- and dichorionic twins. See Table 4 for the separate equations for the relation between the log concentrations of free β-hCG and PAPP-A with gestational age for all twins, mono- and dichorionic.

**Table 4** Separate regression characteristics for the relationship between the log concentration of free β-hCG and PAPP-A with gestational age for all twins, mono-and dichorionic

| | | All twins | Dichorionic | Monochorionic |
|---|---|---|---|---|
| Log free β-hCG | Constant | 6.826 | 6.726 | 6.396 |
| Log free β-hCG | β (GA-days) | -0.029 | -0.027 | -0.027 |
| Log PAPP-A | Constant | 1.607 | 1.490 | 1.097 |
| Log PAPP-A | β (GA-days) | 0.079 | 0.082 | 0.082 |



**Figure 1 –** Predicted free β-hCG concentrations time course in the first trimester of pregnancy for singletons, twins, mono-and dichorionic twins

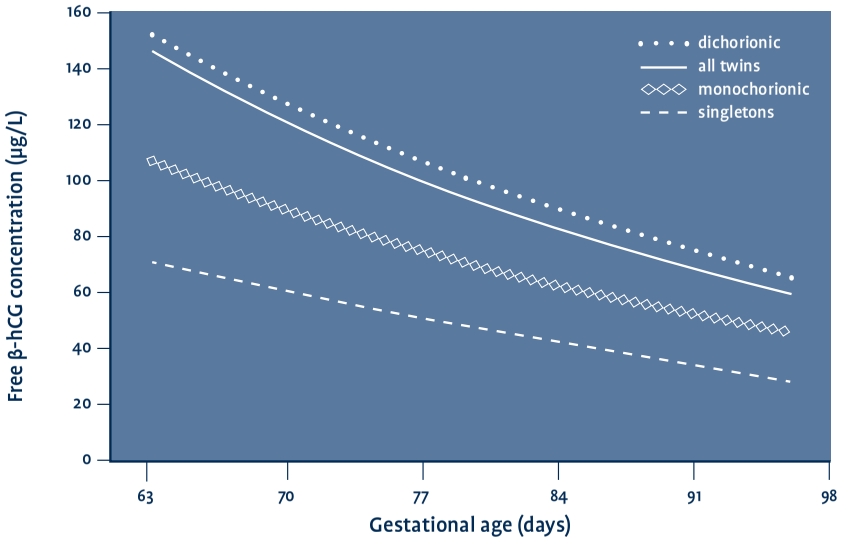


**Figure 2** Predicted PAPP-A concentrations time course in the first trimester of pregnancy for singletons, twins, mono-and dichorionic twins

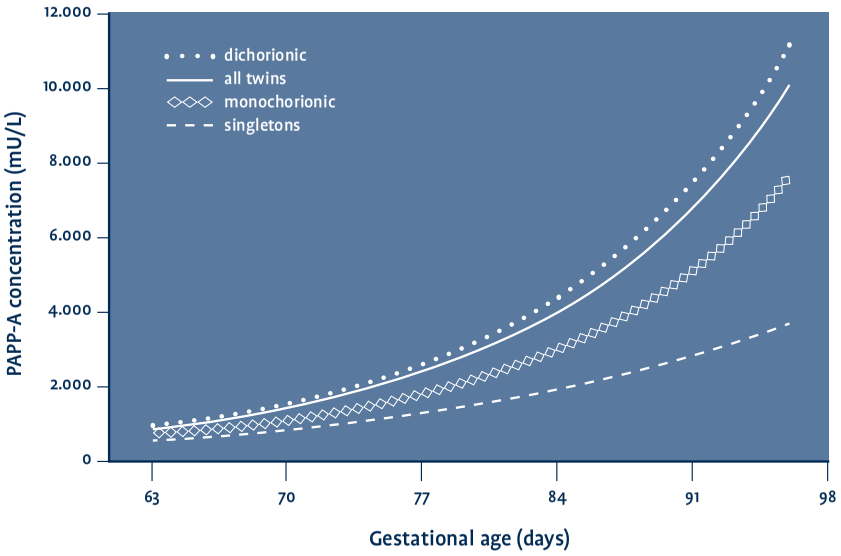

Figure 1 and 2 graphically display the relationship between the retransformed exponential of the log, the predicted concentration of free β-hCG (Figure 1) and PAPP-A (Figure 2) and the gestational age in days for singletons, all twins, mono- and dichorionic twins.

Trisomy 21 was identified in 3 fetuses in both dichorionic pregnancies. For Case I, fetuses were discordant: the affected fetus had NT 4.8 mm and CRL 57 mm; whereas the unaffected fetus had NT 1.4 mm and CRL 54 mm. Free β-hCG concentration at 10+0 weeks' gestation was 160 µg/L, weight-corrected MoM 2.90, and concentration PAPP-A 1155 mU/L, weight-corrected MoM 2.02. Case II was detected with amniocentesis with concordant fetuses. Free β-hCG concentration at 10+0 weeks' gestation was 42.5 µg/L, weight-corrected MoM 0.79 and concentration PAPP-A 762.25 mU/L, weight-corrected MoM 1.38.

# Discussion

In this paper we investigated whether twin chorionicity should be incorporated into prenatal screening for trisomy 21. It is important to assess zygosity and chorionicity in twins: zygosity determines whether the fetuses are concordant (both affected) or discordant (only one affected) for a certain anomaly. Zygosity cannot be determined by ultrasound. Chorionicity, which refers to the type of placentation, can be accurately determined by first trimester ultrasound examining the number of placental sites, the thickness of the inter-twin membrane, the lambda (λ) and T signs [10;11].
The sensitivity of NT measurements in twins is similar to singletons however, since increased NT is more frequently seen in euploid monochorionic twins the specificity is lower [12]. An increased NT in both singleton and in multiple pregnancies is associated with chromosomal abnormalities and structural abnormalities such as cardiac defects and adverse pregnancy outcome [4;13]. In monochorionic twins discordance in NT thickness is associated with subsequent development of Twin-to-Twin Transfusion syndrome (TTTS) [14;15]. Our study supports this hypothesis: three cases of early TTTS had an increased NT (>3.5mm). The use of individual fetal nuchal translucency measurements allows a 'fetus specific' risk calculation for trisomy 21 in twins. Moreover, NT identifies the specific fetus at risk, and parents can decide upon further testing of either one fetus or both [16].
Interpretation of biochemical marker in twins who are discordant for anomalies is difficult as altered serum levels from the affected fetus may be masked by the unaffected co-twin. Determination of the individual contribution of each fetus to the analyte level is not possible. MoM values of serum markers in unaffected twin pregnancies are approximately doubled compared with singletons. Weight-corrected MoM free β-hCG 1.99 and PAPP-A 2.14 reported in our study are supported by others [5-9;17].



In our study, MoM values were not corrected for ethnicity, smoking and conception mode. The studied population was predominantly Caucasian (Table 1). Ethnicity influences biochemical marker distributions, especially in Afro-Caribbean and Asian women show higher PAPP-A and free β-hCG levels [18]. Given the lack of ethnic diversity in our population, MoM values were not corrected. Smoking, in particular, reduces PAPP-A levels and free β-hCG to a lesser extent [19]. Only a small percentage of the studied group was smokers (Table 1). We demonstrated that differences in first trimester biochemical markers found between mono- and dichorionic twins were persistent, even after correction for smoking. ART (IVF and ICSI) versus naturally conceived pregnancies showed no significant differences, although there was a tendency for the weight-corrected MoM free β-hCG to be elevated and PAPP-A decreased. The studies of Orlandi et al. and Gonce et al. also found no significant differences in ART versus naturally conceived twin pregnancies [8;20].
Early data by Spencer et al. (2001) found no statistically significant difference for the median weight-corrected MoM free β-hCG and PAPP-A between mono- and dichorionic twins, although PAPP-A was lower in monochorionic fetuses [21]. This suggestion of no impact of chorionicity on first trimester biochemical markers has been confirmed by others [7;8;22]. Recently Spencer et al. (2008) have reported significant lower corrected MoM PAPP-A in monochorionic twins (1.76 vs 2.04), whereas for corrected MoM free β-hCG only a tendency for lower levels has been found [9]. Our study demonstrates that monochorionic twins have a significant lower weight-corrected MoM free β-hCG (1.53 vs 2.11) and PAPP-A (1.59 vs 2.40) than dichorionic twins. Gonce et al. (2005) previously described a reduction in overall MoM free β-hCG in early first trimester samples [8]. Subgroup analysis demonstrated that weight-corrected MoM free β-hCG are significantly reduced in monochorionic compared with dichorionic twins in samples taken before 12 weeks' gestation and not different taken after 12 weeks' gestation. This latter finding corroborates the findings of Spencer et al. (2008), as samples are taken at more advanced gestational age in their study and no significant differences between mono- and dichorionic twins in corrected MoM free β-hCG were found [9].

Finding different biochemical marker distributions in mono- and dichorionic twins is not surprising, since there have been suggestions that maternal serum analyte levels are proportional to the number of fetoplacental units [5]. Johnson et al. demonstrated that hormone concentrations correlated with the number of placental tissues, which, since monochorionic twins share one placenta, support the findings from our study [23].
Differences in biochemical marker distributions between mono- and dichorionic twins in the second trimester have already been described, however, in this study significantly higher median MoM free β-hCG were found in monochorionic compared with dichorionic pregnancies [24].

The combination of NT and biochemistry is advised for the risk calculation of trisomy 21 in twins. Data are currently not sufficient to evaluate biochemical distributions for affected Down syndrome twin pregnancies (both concordant and discordant). The two cases described in this study can be added to data already reported concerning Down syndrome affected twins.

Test results for the first trimester combined test in twins are generally better than the sole use of NT for both fetuses. Detection rates between 75% and 80% have been described using the first trimester combined test [5;6]. The addition of biochemistry to the risk calculation helps to reduce the false positive rate [8;25]. This study strengthened the need to make a distinction between mono- and dichorionic twins as biochemical markers are significantly lower in monochorionic twins than in dichorionic.



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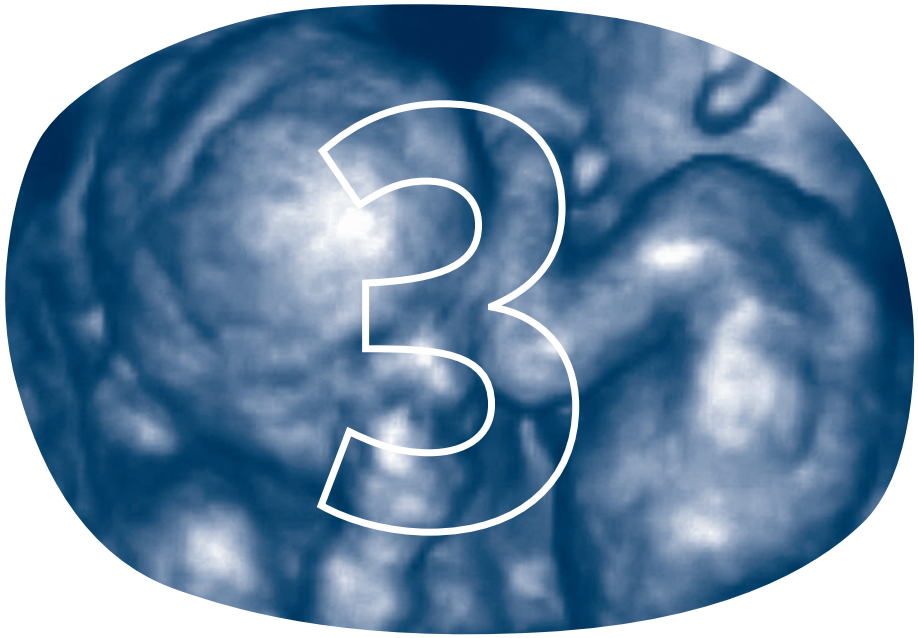




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# Processing effects and storage conditions on A Disintegrin and Metalloprotease (ADAM12s), a maternal serum marker for adverse pregnancy outcome

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