Placenta function in twins

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June 4, 2018

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TITLE:
Placenta function in twins

THEME:
Statistical Modelling and Analysis

PROJECT PERIOD:
From: February, 1.
To: June, 4.

PROJECT GROUP:
Rikke Ehlers Sand

SUPERVISOR:
Jakob Gulddahl Rasmussen

CIRCULATION:
Online in the University's project library.

NUMBER OF PAGES: 41

ABSTRACT:

The aim of this report is to create a statistical model that can help answer the question whether twins tend to suffer from placental dysfunction more often than singletons or if twins by some genetic conditions have smaller normal weight than singletons. To answer this question, data from Aalborg University Hospital was used. The data was of hierarchical nature and composed of two levels - one for each individual mother and one for each individual fetus. In order to incorporate this nature mixed effects models was used. During this report theory about mixed effects models will be presented. Multiple models will be fitted and tested to make sure all model assumptions are met. Among seven models the model with lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) will be chosen as the one that models the data best. Based on this model it is seen that the estimated fetal weigth for twins at a given placental T2* value is significantly different from singletons which could indicate that twins have a smaller normal weight than singletons. Hence, there might be a need for new reference curves when assessing the size of twin fetuses and thereby the placenta function.

Preface

This report was written during the 10th semester of mathematics and statistics at the Department of Mathematical Sciences at Aalborg University. It is written by Rikke Ehlers Sand.

The report will go into theory about mixed effects models which will be used to model estimated fetal weight for singletons and twins. The requirements for reading this report is the curriculum of the semesters of the BSc in Mathematics at AAU.

Citation and referencing stile used in this report is based on Vancouver. Citations made on the right side of a period refer to all above until previous citation. Citations made on the left side of a period refer only to the sentence where it appears.

To mark the end of an exampel a triangle (Δ) will be used.

I would like to thank my supervisor Jakob Gulddahl Rasmussen for the help during the project and Anne Sørensen and Ditte Nymark Hansen from Aalborg University Hospital for providing the data.

Contents

1 | Introduction

The placenta plays a key role when it comes to fetal development and maternal health. The placenta is responsible for nutrient supply and regulation of respiratory gases. Additionally, it acts as a immunologic barrier between the fetus and the mother, protecting the fetus from toxic and waste products. [4, 5, 20] However, in some cases the placenta fails to meet the requirements from the fetus as a result of a dysfunctional placenta [17]. Placenta dysfunction can lead to fetal growth restriction (FGR) which is associated with reduced oxygen supply to the fetus causing it to fail at reaching its genetic growth potential [7, 8]. FGR is associated with approximately 50% of all stillbirths. Diagnosing FGR is complicated as different measures of placental dysfunction have been associated with increased risk for adverse pregnancy outcomes. Furthermore, developing tests is complicated by the difficulty in timing the measurements as the placenta changes with increasing gestational age. [21] The diagnosis of FGR is further complicated by the difficulty in separating the normal small fetuses from the truly growth-restricted ones. [17] This diagnostic complication is also prevalent when assessing the size of twin fetuses. Today when assessing whether a fetus is able to reach its genetic growth potential, a twin is compared to a normal singleton, which might be misleading, as twins tend to have a smaller birth weight. Therefore, there is a need to examine whether twins more often suffers from dysfunctional placentas or if twins by some genetic conditions have a smaller normal weight than singletons. As an attempt to separate the fetuses with FGR from the normal small fetuses, a study by Sinding et al. (2016) found that placental MRI transverse relaxation time, T2*, could be used as a marker of dysfunctional placenta [7]. The T2* value can be used to assess the oxygenation in the placenta and it is obtained though a MRI scan. This way the amount of deoxyhemoglobin present in the tissue can be assessed. A high amount of deoxyhemoglobin results in a low signal seen on the scan. During hyperoxia the amount of deoxyhemoglobin found in the tissue is reduced which leads to a decrease in the estimated T2* value. Hence if the placenta T2* value is low it indicates placenta dysfunction. [10]

The aim of this study is to provide more knowledge about placental function in twins by using linear mixed effects models to model the estimated fetal weight for singletons and twins. We want to compare the size of the fetus with placental T2* for twins and singletons. It is hypothesized that if the estimated fetal weights are the same for both twins and singletons at a given T2* value then the placenta function is the same too, hence the same reference curves can be used for both singletons and twins. If the weight for twins is smaller, then

twins have a smaller normal weight than singletons, hence there is a need for new reference curves for twins.

Previous work

Multiple studies have been conducted to examine both the fetal- and birth weight for twins, and linear mixed effects models have been used in a number of these.
A pilot study by Ye Shen (2014) was conducted to investigate if maternal oral infections were associated with twin birth weights [22]. Two models, with different objectives, were fitted using linear mixed effects models. No significant results were found, but the results suggested that maternal oral health could be associated with birth weight of twin neonates.
A study by Sushimta Shivkumar (2011) aimed at providing ultrasound-based, in utero, fetal weight references for each gestational age for a twin population [19]. Modified mixed effects models was used to model fetal growth in twins using serial ultrasound measurements of fetal weight adjusted for sex and chorionicity. It was found that fetal weight in twins was consistently lower than singletons over the course of pregnancy when compared to other published fetal weight references.
The study by Shivkumar was only modeling twins in contrast to this study where both twins and singletons are included. Furthermore, this study will focus on placenta T2* values relative to fetus size, rather than ultrasound-measurements.

2 | Initial Data Analysis

The data used in this study was provided by Anne Sørensen, Ditte Nymark Hansen and their research team from Aalborg University Hospital.

In this study 154 pregnant women were followed during their pregnancy. All women were pregnant with either singleton or dichorionic twins (Each twin had its own placenta). The study was developed to look for potential differences in estimated fetal weights for twins and singletons, in order to improve the screening for placenta dysfunction.
During the experiment the women were scanned in an MRI scan twice at different gestational ages. Based on these scans estimated fetal weights were calculated. In addition, a placenta T2* value was noted along with gestational age at both ultrasound and MRI.

2.1 Correlation patterns

In order to explore the different correlations in data, different plots were made.
One would expect that twins from the same mother would be correlated, why scatterplots for fetus 1 and fetus 2 were made. One scatterplot showed birth weight zscores for fetus 1 against fetus 2 (See Figure 2.1), while another showed placental T2* values for two twins (See figure 2.2).

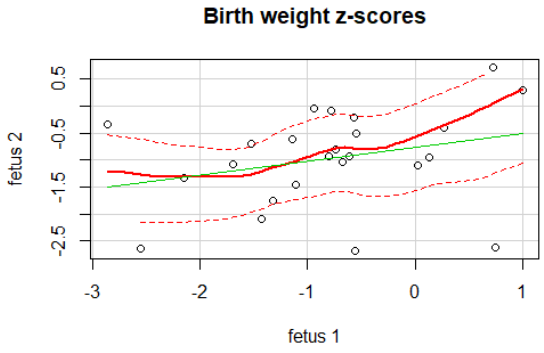

Figure 2.1: Birth weight z-scores for fetus 1 and fetus 2 plotted against each other. The shape of the point cloud indicates weak correlation

The scatterplots in figure 2.1 implies a weak correlation between two fetuses from the same mother. This corresponds to findings in previous studies [19, 22].
Additionally, a scatterplot for T2* values from fetus 1 and fetus 2, calculated at different scans, were assessed (See Figure 2.2).

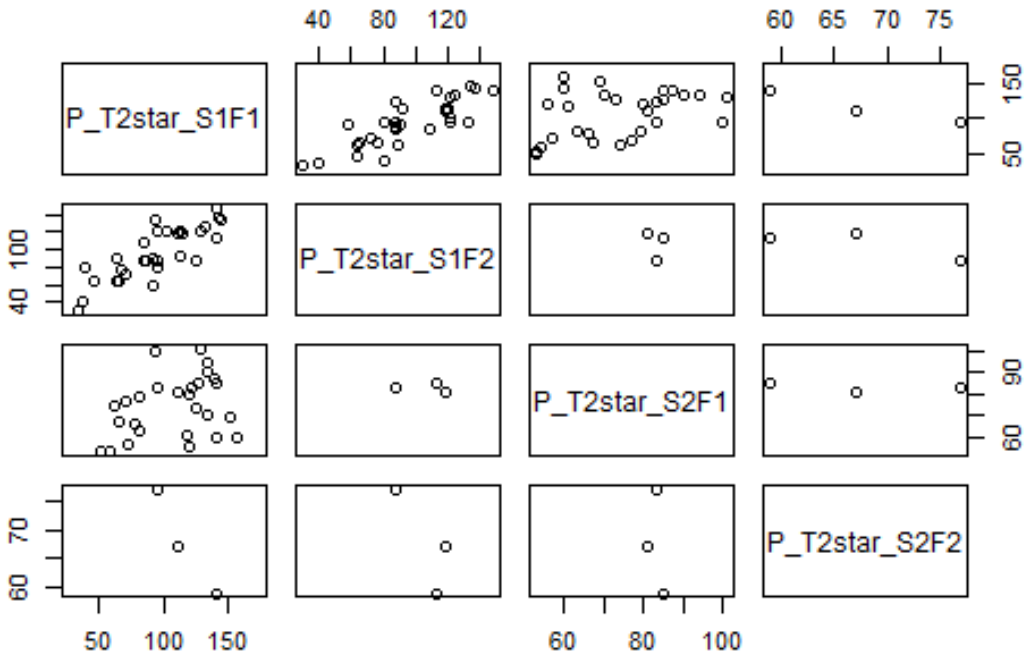

Figure 2.2: T2* values for two twin fetuses which were calculated using results from different scans. It is seen that the placenta T2* value from twins from the same mother are correlated. In contrast, the correlation between different scans for the same fetus is more weak

As expected the plot indicated a correlation between the T2* values for twins from the same mother. In contrast, the correlation between different scans for the same fetus was found to be more weak.
In order to adjust for this correlation, linear mixed effects could be used.

Before fitting the models, data underwent some cleaning. Initially, the data consisted of 154 observations of 40 variables. Each observation represented a mother, who had either singleton or twins fetuses. The composition of data was changed in order to provide ID numbers to each fetus. Simultaneously, variables consisting of information regarding fetus one or two were combined into one variable. E.g. birth weight for fetus one (`BW_F1`) and birth weight for fetus two (`BW_F2`) were combined into one variable (`Birth_weight`) which

then contained information about fetus weights for both fetuses. Due two high percentage of missing values the variables `Normalmateriale`, `Proteinuria_g` and `Blood_pressure` were removed from the data set. Additionally, information about whether the twin mothers were pregnant with dichorionic or monochorionic twins were excluded, since all were dichorionic and hence all observations were the same. Furthermore, informations from first and second scan was combined into one variable which then had double length. In order to provide variables of equal length, some observations were included twice. Finally, the data set consisted of 362 observations of 16 variables.

2.1.1 Missing values

Data from the healthcare sector is often associated with some degree of missing data. Therefore, to get an overview of the amount of missing data, the observed values were plotted against the missing values using the command `missmap` in `R`. The plot is seen in Figure 2.3

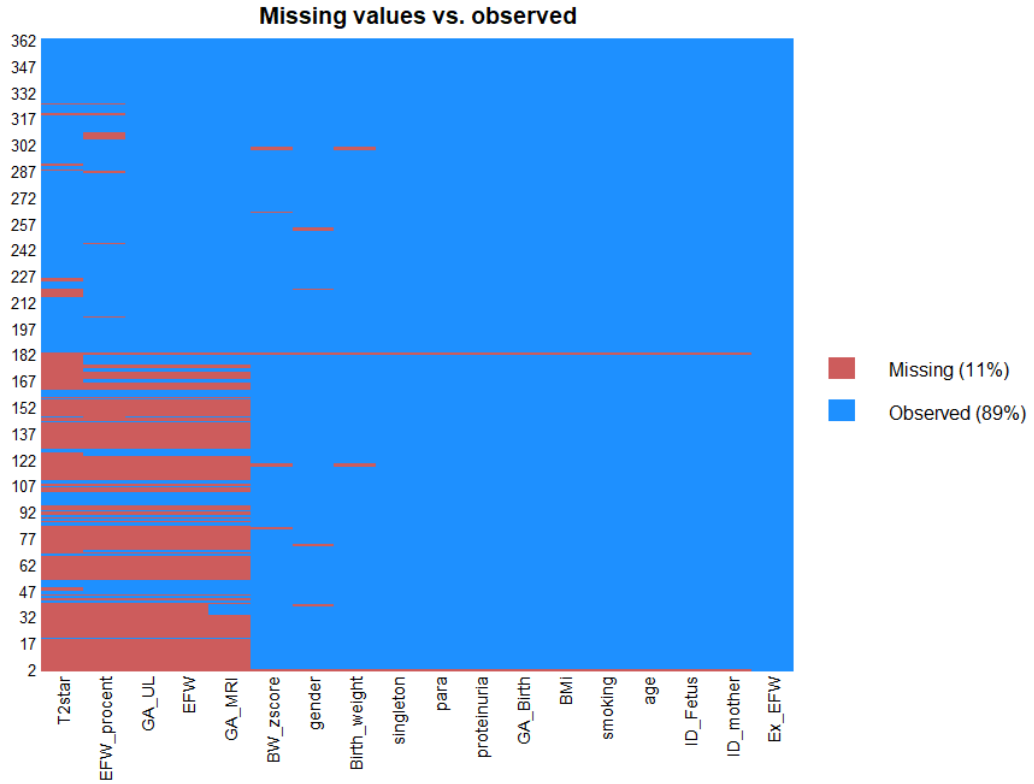

Figure 2.3: Missing values plotted against the observed in order to get an overview of the amount of missing values

As seen in Figure 2.3 some of the variables included missing values. Therefore, the data was tested to see if the data was missing completely at random (MCAR). If data is missing based on the MCAR mechanism it indicates that the probability that data is missing is not

related to any data neither observed or missing. Hence the probability that a given observation is missing is the same for all units. This assumption was tested using the command `LittleMCAR` in `R`. The test indicated that data was MCAR, which implies that missing values can be ignored without leading to incorrect conclusions or misinterpretation of the data. [9] Therefore, all missing values were excluded. This resulted in a data set consisting of 190 observations of 16 variables. The variables are listed in the following:

Details about the variables

- `ID_mother` - unique ID number for each mother
- `ID_fetus` - unique ID number for each fetus
- `singleton` - singleton with 0=no and 1=yes
- `smoking` - the mother's smoking habits with 0=never, 1=now, 2=former
- `BMI` - BMI of the mother calculated using the standard formula. Values ranging from 16.5-32.7
- `proteinuria` - protein in the urine from the mother with 0=no and 1=yes
- `para` - number of births prior to this pregnancy. Values ranging from 0-4
- `age` - mother's age in years ranging from 19 to 40 years of age
- `GA_birth` - gestational age at delivery.
- `gender` - the sex of the fetus with 0=boy and 1=girl
- `Birth_weight` - birth weight of the fetus ranging from 0-4700 g
- `BW_zscore` - birth weight, z-score for the fetus. Values ranging from -5.02-2.26
- `EFW` - estimated fetal weight for the fetus at first and second scan in gram ranging from 1-3609
- `GA_MRI` - gestational age at MRI for the fetus at first and second scan ranging from 15.6-70
- `T2star` - T2* value calculated based on MR-scan of placenta at first and second scan ranging 0-182
- `Ex_EFW` - explanatory variable for estimated fetus weight with 1= first scan and 2= second scan.

3 | Mixed effects models

The data used in this study is of hierarchical nature and is composed of two levels; one level for each mother, a second level for fetuses because of longitudinal measurements (see figure 3.1). Because of this nature, data is expected to be correlated, which was also suggested in section 2.1. Therefore, mixed effect models could be relevant, as it allows a wide variety of correlation patterns to be explicitly modeled [15]. In fact, we are capable of modeling dependencies between observations within and between groups [11].
Mixed effects model includes both fixed- and random effects, which will be described in the following. The theory in this chapter is based on [11] unless stated otherwise.

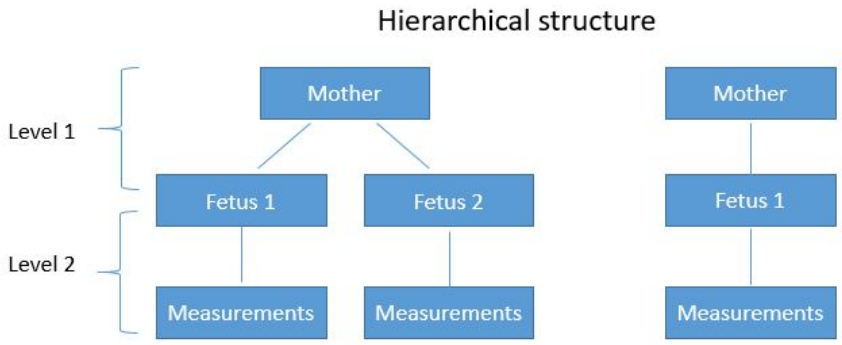

Figure 3.1: The hierarchical structure in data is composed of two levels; mother and fetuses. This leads to a model of estimated fetal weight that contains measures for individual fetuses as well as measures for each mother within which the fetuses are grouped

3.1 Fixed effects models

Models with fixed parameters are called fixed effects models. This implies that the model parameters are fixed hence non-random quantities. Variables as sex and ethnicity does not change and hence have fixed effects. Age changes at a constant rate over time and is also a fixed effect. The general definition of a fixed effects models is presented in Definition 3.1.

Definition 3.1 (Fixed effects model)
Consider the linear unobserved effects model for N observations and T time periods

$$Y_{it} = X_{it}\beta + \mu_i + \varepsilon_{it} \quad \text{for} \quad i = 1, \ldots, N \text{ and } t = 1, \ldots, T \tag{3.1}$$

where Y_{it} is the dependent variable observed for individual i, X_{it} is the $1 \times K$ design matrix, β is a $K \times 1$ parameter vector, μ_i is the unobserved time-invariant individual effect and ε_{it} is the error term. [12]

Fixed effects models are characterized as models, which focus on in-group action while between-group action is assumed to be due to random error. This implies that a typical parameterization for fixed effects models consist of a parameter specific for each group, μ_i. [3, 11]
Variables which are not fixed but random and unpredictable are random effects. For fixed models these random effects are treated as non-random or fixed. As a consequence between-group variation is not modeled.

3.1.1 Example

The data included measures from 81 fetuses. The aim was to model the estimated fetus weight. If the aim was to model the estimated fetus weight for these specific fetuses, a fixed effects model could be used. A fixed model is then

$$y_i = \beta_0 + \beta_1 a_i + \beta_2 g_i + \varepsilon_i \tag{3.2}$$

where y_i is the estimated fetus weight for fetus i, a_i is the age effect and g_i is the gender effect, which are both observed fixed effects. Additionally, β is the model parameters which are to be estimated. The error term ε represent the deviations from our predictions due to random factors which we cannot control experimentally. The error are assumed to be independent $N(0, \sigma^2)$-distributed.
For a data set of six observations including six mothers with singletons, this could be written in matrix terms as

$$\underbrace{\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix}}_{y} = \underbrace{\begin{bmatrix} 1 & a_1 & g_1 \\ 1 & a_2 & g_2 \\ 1 & a_3 & g_3 \\ 1 & a_4 & g_4 \\ 1 & a_5 & g_5 \\ 1 & a_6 & g_6 \end{bmatrix}}_{X} \underbrace{\begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{bmatrix}}_{\beta} + \underbrace{\begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \\ \varepsilon_4 \\ \varepsilon_5 \\ \varepsilon_6 \end{bmatrix}}_{\varepsilon} \tag{3.3}$$

The variance is σ^2

$$\mathrm{Var}(y) = \sigma^2 I = \begin{bmatrix} \sigma^2 & 0 & 0 & 0 & 0 & 0 \\ 0 & \sigma^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma^2 & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & \sigma^2 & 0 \\ 0 & 0 & 0 & 0 & 0 & \sigma^2 \end{bmatrix}$$

Δ

The model in (3.2) does not take between-groups interactions in to consideration, which could be relevant when modeling data from twin fetuses. In order to model the correlation between two twin siblings a random effects model could be used.

3.2 Random effects models

Random effects models are used e.g. when modeling individual groups for which the selection from a large population occur randomly. For random effects models the levels are considered as an outcome of picking a number of groups randomly from a large population where only between-group variation within the population is of interest, var$[\mu_i]$.

Definition 3.2 (One-way Model with Random Effects)
Consider the random variables $Y_{ij}, i = 1, 2, \ldots, k; j = 1, 2, \ldots, n_i$ with k representing the number of groups and n_i the number of observations in group i.
A one-way random effects models for Y_{ij} is a model such that

$$Y_{ij} = \mu + U_i + \varepsilon_{ij} \tag{3.4}$$

with $U_i \sim N(0, \tau^2)$ and $\varepsilon_{ij} \sim N(0, \sigma^2)$, and where ε_i and ε_j are mutually independent for $i \neq j$, U_i, U_j are mutually independent for $i \neq j$, and further are U_i independent of ε_j.
We shall put

$$N = \sum_{i=1}^{k} n_i$$

When all groups are the same size, $n_i = n$, we shall say that the model is balanced.

Note, that Equation (3.4) can be rewritten, by transforming μ to linear predictor $X\beta$, as

$$Y = X\beta + U + \varepsilon \tag{3.5}$$

with X is a vector of 1's, $\beta = \mu, U = (U_1, U_2, \ldots, U_k)^T$ and $\varepsilon = (\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_k)^T$

3.2.1 Example

In Example 3.1.1 we were only interested in the individual fetuses. If we consider the fetuses as a sample of fetuses, we need to use a random effects model where the random effect from each mother is modeled explicitly. A random effect model could be formulated as

$$y_i = \mu + U_i + \varepsilon_i \tag{3.6}$$

where y_i is the estimated fetus weight from fetus i, μ is the average of the estimated fetus weight for the entire population and U_i is the random effect for each mother.
For a dataset of six observations representing three mothers with two twins, this could be written in matrix terms as

$$\underbrace{\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix}}_{Y} = \underbrace{\begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \end{bmatrix}}_{X} \underbrace{\begin{bmatrix} \mu \end{bmatrix}}_{\beta} + \underbrace{\begin{bmatrix} U_1 \\ U_1 \\ U_2 \\ U_2 \\ U_3 \\ U_3 \end{bmatrix}}_{U} \tag{3.7}$$

The variance of y is given as

$$\mathrm{Var}(y) = \begin{bmatrix} \tau^2 & \tau^2 & 0 & 0 & 0 & 0 \\ \tau^2 & \tau^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & \tau^2 & \tau^2 & 0 & 0 \\ 0 & 0 & \tau^2 & \tau^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & \tau^2 & \tau^2 \\ 0 & 0 & 0 & 0 & \tau^2 & \tau^2 \end{bmatrix}$$

Δ

In order to incorporate both in-group and between-group information in the model a mixed effect model could be used.

3.3 General Linear Mixed Effects Models

Models using both fixed and random effects in the same analysis are referred to as mixed effects models. Mixed models are a powerful tool when modeling correlated data, and describes dependence between- and within groups by assuming that there exist one or more latent variables for each group of data. Since these latent variables are assumed to be random, these are thought of as random effects. The linear mixed effects model is defined in Definition 3.3.

Definition 3.3 (Linear Mixed Effects Model)
Let X and Z denote known matrices. Let $\varepsilon \sim N(0, \Sigma)$ and $U \sim N(0, \Psi)$ be independent. Then a mixed general linear model is

$$Y_{ij} = X_{ij}\beta_j + Z_{ik}U_k + \varepsilon \tag{3.8}$$

where $i = 1, 2 \dots k$, $j = 1, 2, \dots n_i$ with k representing the number of groups and n_i the number of observations in group i. The parameters β are called fixed effects and quantities U are called random effects.

The fixed effects parameters tell how the population means differ between any set of treatments, and hence only influences the mean of y. The random effects parameters represent the general variability among subject or other units, and hence only influences the variance of y. [15]
Note, the model in Equation (3.8) can be written on matrix vector form as

$$Y = X\beta + ZU + \varepsilon \tag{3.9}$$

where $\beta = (\beta_1, \beta_2, \dots, \beta_j)^T$, $U = (U_1, \dots, U_k)^T$ and $\varepsilon = (\varepsilon_{11}, \varepsilon_{12}, \dots, \varepsilon_{km})^T$, additionally X is a $N \times j$ and Z is a $N \times k$ matrix. The i, j'th element Z is 1 if y_{ij} belongs to the i'th group, otherwise it is zero.

3.3.1 Example

The mixed effects model allows us to combine the fixed effects model and the random model into one. Hence by using example 3.1.1 and 3.2.1 we can formulate a mixed effects model as

$$y_i = \beta_0 + \beta_1 a_i + \beta_2 g_i + Z_i U_{1i} + Z_i U_{2i} + \varepsilon_i$$

where y_i is the estimated fetus weight for fetus i, a_i is the age effect and g_i is the gender effect, which are both observed fixed effects. Additionally, β is the fixed model parameters which are to be estimated. The random model parameters are denoted, U, and are distributed as $U \sim N(0, \Psi)$. The random effect from the mothers is U_{1i} with distribution $U_{1i} \sim N(0, \tau_m^2)$ and for each fetus U_{2i} with distribution $U_{2i} \sim N(0, \tau_f^2)$. The error term ε represent the deviations from our predictions due to random factors which we cannot control experimentally. The error are assumed to be independent $N(0, \sigma^2)$-distributed.
For six observations, representing three mothers with two twin fetuses, this can be written

in matrix terms as:

$$\underbrace{\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix}}_{y} = \underbrace{\begin{bmatrix} 1 & a_1 & g_1 \\ 1 & a_1 & g_2 \\ 1 & a_2 & g_3 \\ 1 & a_2 & g_4 \\ 1 & a_3 & g_5 \\ 1 & a_3 & g_6 \end{bmatrix}}_{X} \underbrace{\begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{bmatrix}}_{\beta} + \underbrace{\begin{bmatrix} 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}}_{Z} \underbrace{\begin{bmatrix} U_{11} \\ U_{12} \\ U_{13} \\ U_{21} \\ U_{22} \\ U_{23} \\ U_{24} \\ U_{25} \\ U_{26} \end{bmatrix}}_{U} \tag{3.10}$$

The variance for y is
$\mathrm{Var}(y) = \Sigma + Z\Psi Z^T =$

$$\begin{bmatrix} \sigma^2+\tau_m^2+\tau_f^2 & \tau_m^2 & 0 & 0 & 0 & 0 \\ \tau_m^2 & \sigma^2+\tau_m^2+\tau_f^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma^2+\tau_m^2+\tau_f^2 & \tau_m^2 & 0 & 0 \\ 0 & 0 & \tau_m^2 & \sigma^2+\tau_m^2+\tau_f^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & \sigma^2+\tau_m^2+\tau_f^2 & \tau_m^2 \\ 0 & 0 & 0 & 0 & \tau_m^2 & \sigma^2+\tau_m^2+\tau_f^2 \end{bmatrix}$$

Δ

3.4 Parameter Estimation

The parameter estimation of fixed effects and variance parameters in mixed models will be introduced in the following.

It follows from the independence between the random effects U and the error term ε that the dispersion matrix can be expressed as

$$D\left[\begin{pmatrix} \varepsilon \\ U \end{pmatrix}\right] = \begin{bmatrix} \Sigma & 0 \\ 0 & \Psi \end{bmatrix}, \tag{3.11}$$

where Σ and Ψ are matrices with the variance from U and ε in the diagonal, respectively. The linear mixed effects model in equation (3.8) can also be expressed as a hierarchical model where $Y|U = u$ is a general linear model with linear predictor $\eta = X\beta + Zu$ and $U \sim N(0, \Psi)$ hence

$$U \sim N(0, \Psi)$$
$$Y|U = u \sim N(X\beta + Zu, \Sigma)$$

The model follows a multivariate normal distribution, hence the probability density functions are

$$f_U(u;\psi) = \frac{1}{(\sqrt{2\pi})^q|\Psi|^{1/2}} \exp\left[-\frac{1}{2}u^T\Psi^{-1}u\right] \text{ for } u \in \mathbb{R}^q \tag{3.12}$$

$$f_{Y|u}(y,\beta) = \frac{1}{(\sqrt{2\pi})^N|\Sigma|^{1/2}} \exp\left[-\frac{1}{2}(y - X\beta - Zu)^T\Sigma^{-1}(y - X\beta - Zu)\right] \tag{3.13}$$

for $y \in \mathbb{R}^N$
It follows from Definition 3.3 that the marginal distribution of Y, which is normally distributed, can be expressed as

$$E[Y] = X\beta \tag{3.14}$$

$$D[Y] = \Sigma + Z\Psi Z^T =: V \tag{3.15}$$

In order to estimate the fixed model parameters the log-likelihood is introduced.

3.4.1 Estimation of Fixed Effects

In order to find the maximum likelihood estimate of the mean value parameter, the log-likelihood was found. In general, the likelihood function is constructed based on the probability distribution of the observed data. However, since the random effects, U, are not observed the joint distribution of y and U cannot be used. Instead the maximum likelihood estimation is based on the marginal distribution of y [2]. Using the mean value and variance from equation (3.14) and (3.15) respectively, the log-likelihood (apart from an additive constant) becomes

$$\ell(\beta,\psi;y) = -\frac{1}{2}\log|V| - \frac{1}{2}(y - X\beta)^T V^{-1}(y - X\beta) \tag{3.16}$$

The score function can be used to obtain the estimates of the mean value parameters. By setting the score function equal to zero and solving the equation one obtains the maximum likelihood estimate. The score function represent the derivatives of the log-likelihood function from (3.16).
The score function for the parameter set β is given as

$$\ell'_\beta(\beta;y) = J^T\ell'_\mu(\mu(\beta);y)$$

where J is the Jacobian $J = \frac{\partial \mu}{\partial \beta}$ and $\mu(\beta) = X\beta$ is the mean value function.
Then the score function with respect to β for a fixed value ψ becomes

$$\begin{aligned}
\ell'_\beta(\beta;\psi) &= \left[\frac{\partial \mu}{\partial \beta}\right]^T \frac{\partial}{\partial \mu}\ell_\mu(\mu(\beta);\psi) \\
&= X^T\left[-\frac{1}{2}\left(\frac{\partial (y-X\beta)^T V^{-1}(y-X\beta)}{\partial X\beta}\right)\right] \\
&= X^T\left[-\frac{1}{2}\left(-2V^{-1}(y-X\beta)\right)\right] \\
&= X^T[V^{-1}(y-X\beta)] \\
&= X^T[V^{-1}y - V^{-1}X\beta]
\end{aligned}$$

For a fixed ψ the estimate of β is found as a solution to

$$X^T V^{-1} y = (X^T V^{-1} X)\beta \tag{3.17}$$

If X has full rank then the solution is uniquely given by $\beta = (X^T V^{-1} X)^{-1} X^T V^{-1} y$.

The observed Fisher information matrix for β is

$$I(\hat{\beta}) = \frac{\partial}{\partial \beta}(X^T V^{-1} X)\beta = X^T V^{-1} X$$

The equation (3.17) is also known as the weighted least squares.
To measure the accuracy obtained in determining the parameters the dispersion matrix for $\hat{\beta}$ is found. This is the inverse of the Fisher information matrix and hence

$$\mathrm{Var}[\hat{\beta}] = (X^T V^{-1} X)^{-1}$$

The solution of Equation (3.17) might depend on unknown variance parameters ψ. Therefore, the profile log-likelihood for the variance parameter, ψ, is introduced. The profile log-likelihood is

$$\ell(\psi) = -\frac{1}{2}\log|V| - \frac{1}{2}(Y - X\hat{\beta})^T V^{-1}(Y - X\hat{\beta}) \tag{3.18}$$

To determine the estimates for the variance parameters ψ the profile likelihood from Equation (3.18) needs modification. The modified profile log-likelihood attempt to improve some of the less satisfying properties of the profile likelihood caused by the fact that the profile likelihood is not directly based on the probability function. The aim of the modified profile likelihood is obtain approximations which are closer to the ones from marginal or conditional inference The modified profile log-likelihood is [6]

$$\begin{aligned}
\ell_m(\psi) &= \ell(\psi) - \frac{1}{2}\log|I(\hat{\beta})| \\
&= -\frac{1}{2}\log|V| - \frac{1}{2}(Y - X\hat{\beta})^T V^{-1}(Y - X\hat{\beta}) - \frac{1}{2}\log|X^T V^{-1} X|
\end{aligned} \tag{3.19}$$

If $\hat{\beta}$ depends on ψ the solution to (3.19) must be found by iterations.

The modified profile log-likelihood in (3.19) is called the residual maximum likelihood (REML)-method. The REML-method sets the fixed effects estimates equal to the weighted least squares (WLS) solution from (3.17) in the likelihood function and then maximizes it to find the variance component terms only. Because of this approach the ordinary likelihood function should be used in this study, instead of the restricted likelihood function, as models with different fixed effects will be formulated, why the results will not be comparable when using REML [13].

3.4.2 Estimation of Random Effects

The random effects are not parameters in the model, why the likelihood approach described in section 3.4.1 cannot be used for estimating random effects. The random effects are seen as latent variables and is estimated using a so-called hierarchical likelihood which includes the joint density for observed and unobserved random quantities. To formulate the hierarchical likelihood the probability functions from Equation (3.12) and (3.13) are used. The hierarchical likelihood is

$$f(y, u; \beta, \psi) = f_{Y|u}(y; \beta) f_U(u; \psi)$$

Then (appart from an additive constant)

$$\ell(\beta, \psi, u) = -\frac{1}{2}\log|\Sigma| - \frac{1}{2}(y - X\beta - Zu)^T\Sigma^{-1}(y - X\beta - Zu) - \frac{1}{2}\log|\psi| - \frac{1}{2}(u^T\psi^{-1}u)$$

hence the score function is

$$\frac{\partial}{\partial u}\ell(\beta, \psi, u) = Z^T\Sigma^{-1}(y - X\beta - Zu) - \psi^{-1}u$$

As before the maximum likelihood estimate can be determined by setting the score function equal to zero and solving with respect to u

$$(Z\Sigma^{-1}Z - \psi^{-1})u = Z^T\Sigma^{-1}(y - X\beta) \tag{3.20}$$

The solution to (3.20) is called the best linear unbiased predictor. The estimate $\hat{\beta}$ is used instead of β.
The observed Fisher information with respect to u is used to assess the accuracy obtained when determining the parameters for $\hat{u}$

$$\begin{aligned} I(\hat{u}) &= \frac{\partial}{\partial u}(Z\Sigma^{-1}Z - \psi^{-1})u \\ &= Z^T\Sigma^{-1}Z - \psi^{-1} \end{aligned}$$

3.5 Test of Significance

To test for significance among the included variables different tests was performed. The fixed effects were tested using likelihood ratio test and the random effects were tested using rANOVA, which will be described in the following.

3.5.1 Test of Fixed Effects

To test the fixed effects parameters in the mixed model for significance, a likelihood ratio test can be performed. Since the likelihood ratio test of mixed models only is approximately χ^2 distributed the p-value will be smaller. Therefore, for p-values which are only slightly under the cut-off value, chosen to be 0.05, there is a need for additional testing to insure, that the variable is in fact significant. For p-values higher than the p-value one can be confident in the result. [18]

Likelihood Ratio Test

The likelihood ratio test can be used when comparing two nested models. It is a statistical test which can be used when comparing the goodness of fit of two models where one (the null model) is a special case of the other model (the alternative model). This is done in order to determine whether a model can be reduced to a simpler complexity or not. The test is based on the likelihood ratio, which is a expression of how many times more likely the data are under one model compared to the other.
The likelihood ratio principle consist of three main steps

- Find the maximum likelihood estimate for any $\theta \in \Theta_0$. Substituting the value of θ back into the likelihood function provides a value of the likelihood function denoted $L(\Theta_0)$
- Find the maximum likelihood estimate for any $\theta \in \Theta_1$. Call this likelihood function $L(\Theta_1)$
- Form the ratio by calculating the likelihood ratio statistic λ as

$$\lambda = \frac{L(\Theta_0)}{L(\Theta_1)} \tag{3.21}$$

If λ is small, it indicates that the data are more plausible under the alternative hypothesis than under the null hypothesis. Hence, the hypothesis ($\mathcal{H}_0$) is rejected for small values of λ.

Theorem 3.4 (Wilk's likelihood ratio test)
For $\lambda(y)$ defined in (3.21) then under the null hypothesis $\mathcal{H}_0$ the random variable $-2\log(\lambda(Y))$ converges in law to a χ^2 random variable with (k-m) degrees of freedom, i.e.

$$-2\log(\lambda(Y)) \to \chi^2(k-m) \tag{3.22}$$

under $\mathcal{H}_0$

Note that $-2\log(\lambda(Y))$ can be written as the statistic $D = -2(\log L(\Theta_0) - \log L(\Theta_1))$ which is called the deviance.

3.5.2 Test for Random Effects

To test for significance among the random effects the function `ranova` in `R` will be used. The function computes an ANOVA-like table where the random effects terms in the model are being tested for significance. This is done by removing one random effect at the time, and computing the likelihood ratio test of models reductions. [14]

3.5.3 Model selection

Doing this study a number of different models were fitted. In order to chose the best model the Akaike information criterion (AIC) og Bayesian information criterion (BIC) will be assessed. Both are defined as

$$AIC = -2\log(\hat{\theta}) + 2p$$
$$BIC = -2\log(\hat{\theta}) + \log(n)p$$

where n is the number of observations and p is the number of parameters.
Using AIC og BIC allow us to deal with the trade off between model accuracy and model complexity. Both AIC and BIC includes a penalty term which is an increasing function of the number of parameters included.

4 | Models

Statistical modeling and the way of doing so, depends highly on the purpose of the model. Galit Shmueli distinguish between three types of statistical modeling in his study "To Explain or to predict?" [16]. The three modeling types are listed in the following

- Explanatory modeling: models which are used to test a causal theory. In such models a set of underlying factors that are measured by some variables, X, are assumed to cause an underlying effect which are measured by variable Y.
- Predictive modeling: models which purposes are to predict new or future observations. Involves the process of applying statistical models to data in order to obtain a model which can be used for prediction of any kind.
- Descriptive modeling: models which purposes are to summarize or represent the data structure in a compact manner. Involves capturing the association between the dependent and independent variables. [16]

In this study the aim was to create a statistical model which captures the association between the estimated fetus weight and the explanatory variables. Hence a descriptive model, why all data was included in the modeling process. In order to select the most accurate model Akaike information criterion (AIC) and Baysian information criterion (BIC) were used. Additionally, all models were tested to check if the model assumptions were met.

4.1 Mixed model

Maximum likelihood estimates of the parameters in the linear mixed effects models were obtained using the function `lmer` in `R`. In order to incorporate the correlation between fetuses from the same mother, and fetuses who had longitudinal measurements, random effects were included through the ID number of both the mother and the fetus. Random effects are written in `R` as (`1|ID_fetus`) indicating a random intercept and fixed mean. In order to assed the interaction of `T2star` and `singleton` an interaction term was included in the model as `T2star` $*$ `singleton`

The first model fitted included all explanatory variables. To test for significance the command `drop1` in `R` was used. A log of the output is seen below.

```
Model:
EFW ~ GA_MRI + as.factor(para) + as.factor(Ex_EFW) + T2star *
    as.factor(singleton) + GA_Birth + Birth_weight + BW_zscore +
    as.factor(proteinuria) + BMi + as.factor(gender) + as.factor(smoking)+
    age + (1 | ID_mother) + (1 | ID_Fetus)
                           Sum Sq  Mean Sq NumDF DenDF F value Pr(>F)
GA_MRI                     13201888 13201888 1 187.20 178.9761 <2.2e-16***
as.factor(para)            86798    21700    4 144.62 0.2942  0.8813767
as.factor(Ex_EFW)          2103261  2103261  1 104.01 28.5136 5.499e-07***
GA_Birth                   79911    79911    1 167.49 1.0833  0.2994516
Birth_weight               472      472      1 168.34 0.0064  0.9363522
BW_zscore                  344615   344615   1 175.27 4.6719  0.0320166*
as.factor(proteinuria)     26325    26325    1 134.07 0.3569  0.5512472
BMi                        916669   916669   1 134.92 12.4271 0.0005785***
as.factor(gender)          58511    58511    1 189.42 0.7932  0.3742564
as.factor(smoking)         596845   298422   2 121.66 4.0457  0.0199042*
age                        9794     9794     1 122.36 0.1328  0.7161971
T2star:as.factor(singleton)1020644  1020644  1 189.46 13.8367 0.0002626***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

The log indicates that not all variables were significant. Before trying to reduce the model, different tests was made to check if the model assumptions were met, to clarify whether or not mixed models could be used to model the estimated fetal weight. First the observed values for each explanatory variable were plotted against the residuals to look for patterns in order to determine if the assumption of linearity in the residuals were met. The residual plots are seen in Figure 4.1.

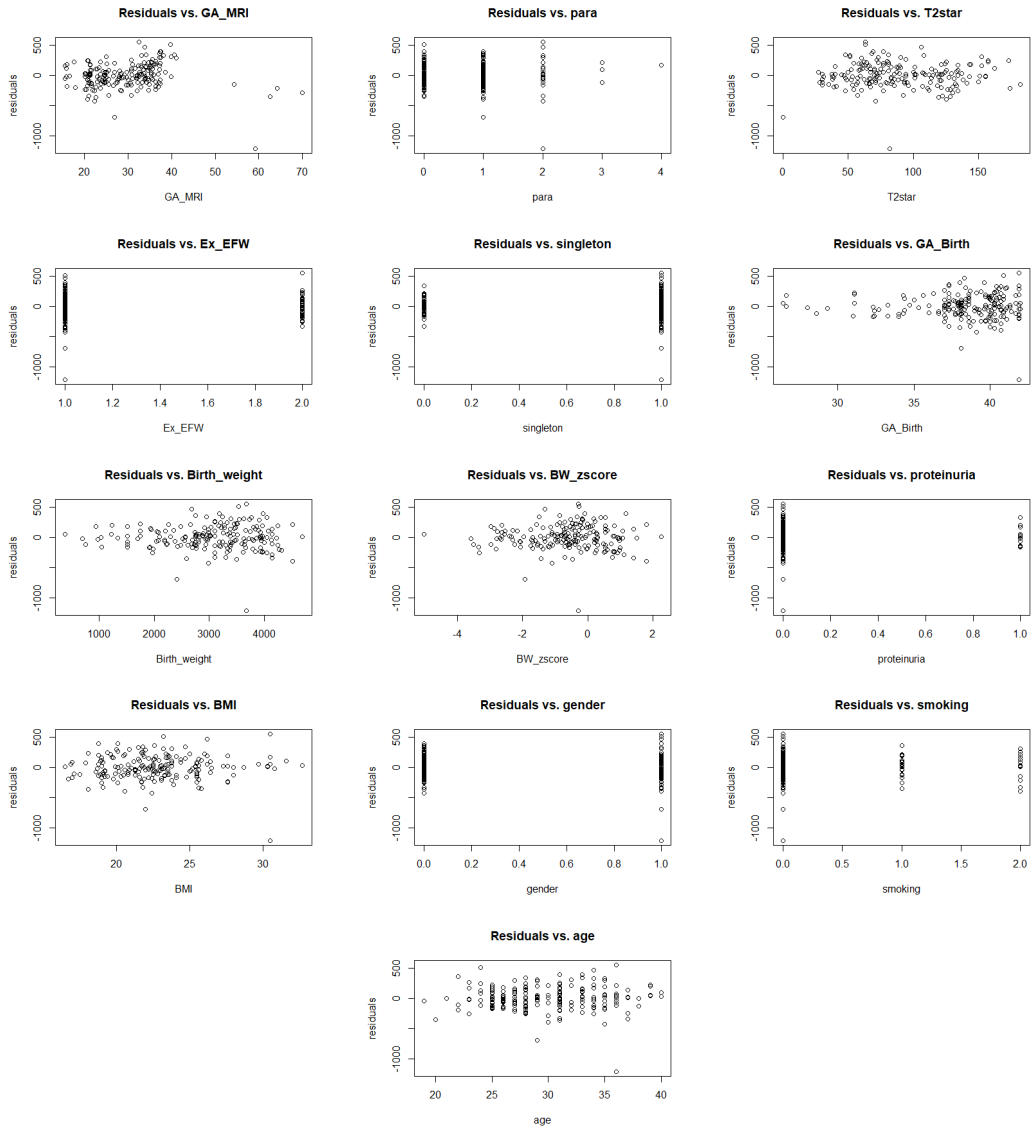

Figure 4.1: The plots of residuals against observed values for each of the explanatory variables did not indicate any violation of the assumption of linearity, as no obvious patterns are seen

The plot of residuals versus observed values for each explanatory variable did not indicate any obvious pattern in the residuals.Therefore, the assumption of linearity was not violated. Note however, that the plot for residuals versus `GA_MRI` had observations with gestational age as high as 70 weeks, which is not possible. Looking away from these, no patterns are seen in the residuals.
In order to check if the variance was constant a plot of the fitted values against residuals was made. (See Figure 4.2).

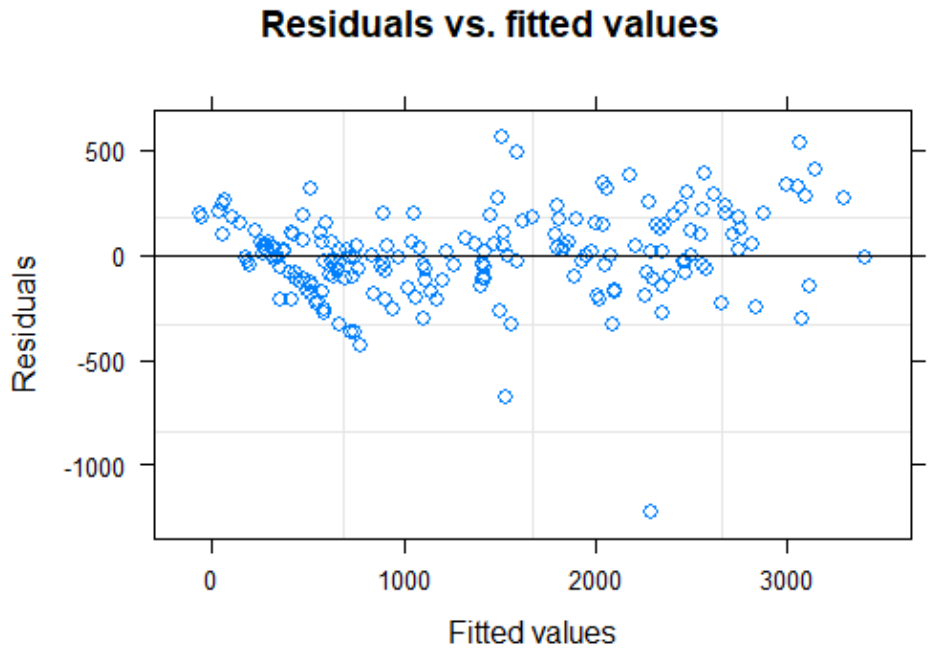

Figure 4.2: Residuals plotted against fitted values. Fairly symmetric around zero. The variance looks relatively constant across the fitted range which implies that the assumption of constant variance are met

The plot of residuals versus fitted values (See Figure 4.2) did not indicate any obvious patterns. Hence the assumption of constant variance was not violated.
To check if the residuals were normally distributed a histogram was made. Additionally, a quantile-quantile plot of the model against a theoretical distribution was made. Both plots are presented in Figure 4.3.

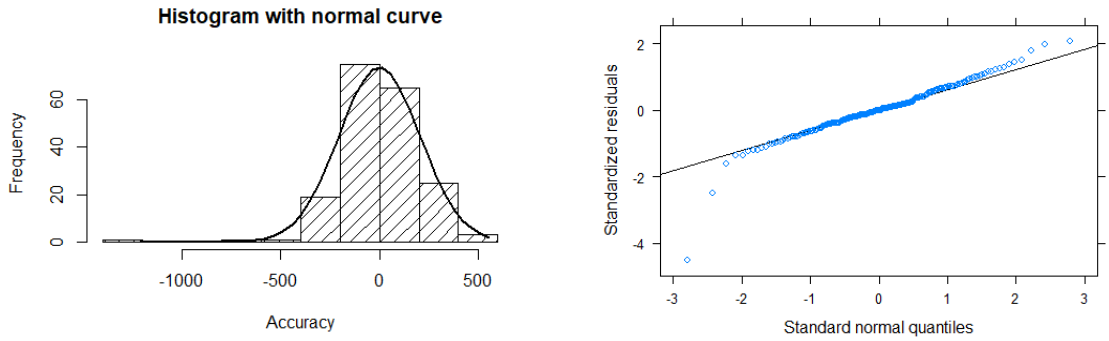

Figure 4.3: The histogram of the residuals and the quantile-quantile plot confirms the assumption of normallity of residuals.

The histogram and quantile-quantile plot confirmed the assumption of normal distributed residuals. All the above indicates that a mixed effects model could be used to model the estimated fetal weight.

4.1.1 Model Reduction

In section 2.1 the scatterplot of T2* for the same fetus at different scans did not indicate any obvious correlation (See Figure 2.2). Therefore, a `ranova` in `R` was performed to look for significance among the random effects to see if both should be included in the model. The output can be seen in the following log.

```
Model:
EFW ~ GA_MRI + as.factor(para) + as.factor(Ex_EFW) + T2star +
    as.factor(singleton) + GA_Birth + Birth_weight + BW_zscore +
    as.factor(proteinuria) + BMi + as.factor(gender) +
    as.factor(smoking) + age + (1 | ID_mother) + (1 | ID_Fetus)+
    T2star:as.factor(singleton)
                npar  logLik    AIC    LRT Df Pr(>Chisq)
<none>            22 -1397.0 2838.0
(1 | ID_mother)   21 -1402.2 2846.5 10.459  1   0.001221 **
(1 | ID_Fetus)    21 -1397.0 2836.0  0.000  1   1.000000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

According to the `ranova` the only significant random effect was `ID_mother`, why `ID_fetus` was removed from the model.
The fixed effects were tested for significance using the command `drop1` in `R`. This was tested based on the likelihood ratio test described in Section 3.5.1. Insignificant variables were removed based on backwards selection. In each step the least significant variable was removed until only significant variables remained. Whenever a variable was removed an ANOVA test was performed to make sure that the variable could be removed without causing a significant change in the model.
The first variable to be tested was `Birth_weight`, as it was found to be the least significant one. An ANOVA test confirmed that the variable could be removed.

```
Data: PlacentaData
Models:
model2: EFW ~ GA_MRI + as.factor(para) + as.factor(Ex_EFW) + T2star *
model2: as.factor(singleton) + GA_Birth + BW_zscore +
model2: as.factor(proteinuria) + BMi + as.factor(gender) +
model2: as.factor(smoking) + age + (1 | ID_mother)
model1: EFW ~ GA_MRI + as.factor(para) + as.factor(Ex_EFW) + T2star *
model1: as.factor(singleton) + GA_Birth + Birth_weight + BW_zscore +
model1: as.factor(proteinuria) + BMi + as.factor(gender) +
model1: as.factor(smoking) + age + (1 | ID_mother)
       Df  AIC    BIC logLik deviance  Chisq Chi Df Pr(>Chisq)
model2 20 2834 2898.9  -1397     2794
model1 21 2836 2904.2  -1397     2794 0.0064      1     0.9363
```

4.1.2 Model Selection

After reducing the models according to the backward selection described in Section 4.1.1 seven models were fitted. The models were:

Table 4.1: Model deskriptions of all seven models

Models	Model description
Model	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{para} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{proteinuria} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + \texttt{age} + \texttt{BW\_weight} + (1\|\texttt{ID\_mother}) + (1\|\texttt{ID\_fetus})$
Model1	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{para} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{proteinuria} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + \texttt{age} + \texttt{BW\_weight} + (1\|\texttt{ID\_mother})$
Model2	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{proteinuria} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + \texttt{age} + \texttt{para} + (1\|\texttt{ID\_mother})$
Model3	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{proteinuria} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + \texttt{age} + (1\|\texttt{ID\_mother})$
Model4	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + \texttt{age} + (1\|\texttt{ID\_mother})$
Model5	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{BMI} + \texttt{gender} + \texttt{smoking} + (1\|\texttt{ID\_mother})$
Model6	$\texttt{EFW} \sim +\texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{BMI} + \texttt{smoking} + (1\|\texttt{ID\_mother})$

To choose the right model for further work, a comparison of AIC and BIC values was performed. The AIC and BIC values are listed in Figure 4.2

Table 4.2: Akaike information criterion (AIC) and Bayesian information criterion (BIC) for each model

	AIC	BIC
Model	2838.00	2909.44
Model1	2836.00	2904.19
Model2	2834.01	2898.95
Model3	2827.18	2879.13
Model4	2825.47	2874.17
Model5	2823.74	2869.20
Model6	2822.71	2864.92

According to the results presented in Figure 4.2 Model6 had the best score with regard to both AIC and BIC, why this model was chosen for further work.
The summary is seen in the following:

```
Formula: EFW ~ GA_MRI + as.factor(Ex_EFW) + T2star * as.factor(singleton) +
GA_Birth + BW_zscore + BMi + as.factor(smoking) + (1 | ID_mother)
Data: PlacentaData

AIC      BIC   logLik deviance df.resid
2822.7   2864.9  -1398.4   2796.7      177

Scaled residuals:
Min      1Q  Median      3Q     Max
-4.4646 -0.4337 -0.0054  0.4537  2.0416

Random effects:
Groups    Name        Variance Std.Dev.
ID_mother (Intercept) 84143    290.1
Residual              74182    272.4
Number of obs: 190, groups:  ID_mother, 140

Fixed effects:
                            Estimate Std. Error       df t value Pr(>|t|)
(Intercept)                 -413.057 637.627 139.310  -0.648 0.51818
GA_MRI                       53.300  4.021   186.732  13.256 < 2e-16  ***
as.factor(Ex_EFW)2           365.324 69.607  106.237   5.248 7.91e-07 ***
T2star                      -6.689   2.185   183.386  -3.062 0.00253  **
as.factor(singleton)1        952.363 218.433 182.827   4.360 2.17e-05 ***
GA_Birth                     43.829  13.461  142.059   3.256 0.00141  **
BW_zscore                    237.378 35.231  178.677   6.738 2.13e-10 ***
```

```
BMi                           -38.320  10.367  131.850  -3.696 0.00032  ***
as.factor(smoking)1           -265.405 109.903 135.853  -2.415 0.01707  *
as.factor(smoking)2           -174.519 127.721 116.326  -1.366 0.17444
T2star:as.factor(singleton)1 -8.701   2.238   189.007  -3.888 0.00014  ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
       (Intr)  GA_MRI Ex_EFW  T2star sn1   GA_Brt BW_zsc BMi    sm1 as.()2
GA_MRI -0.093
Ex_EFW  2     -0.058 -0.129
T2star -0.456  0.158  0.159
sn(1)  -0.309 -0.104  0.016  0.794
GA_Bir -0.844 -0.161  0.025  0.160  0.055
BW_zsc  0.486 -0.092 -0.213 -0.334 -0.084 -0.465
BMi    -0.420  0.016 -0.030 -0.060 -0.021  0.082 0.104
sm1    -0.151 -0.064  0.002 -0.031 -0.066  0.102 0.165  0.247
sm2     0.093 -0.174 -0.078 -0.132 -0.025  0.071 0.112  0.075  0.108
T2:sn   0.410  0.049 -0.006 -0.866 -0.915  0.189 0.111  0.012  0.008 0.021
```

Before concluding anything based on the summary, tests were performed to make sure that all model assumptions were met. To test if the assumption of linearity in the independent variables was met, plots of the residuals against each independent variable were made. These are seen in Figure 4.4.

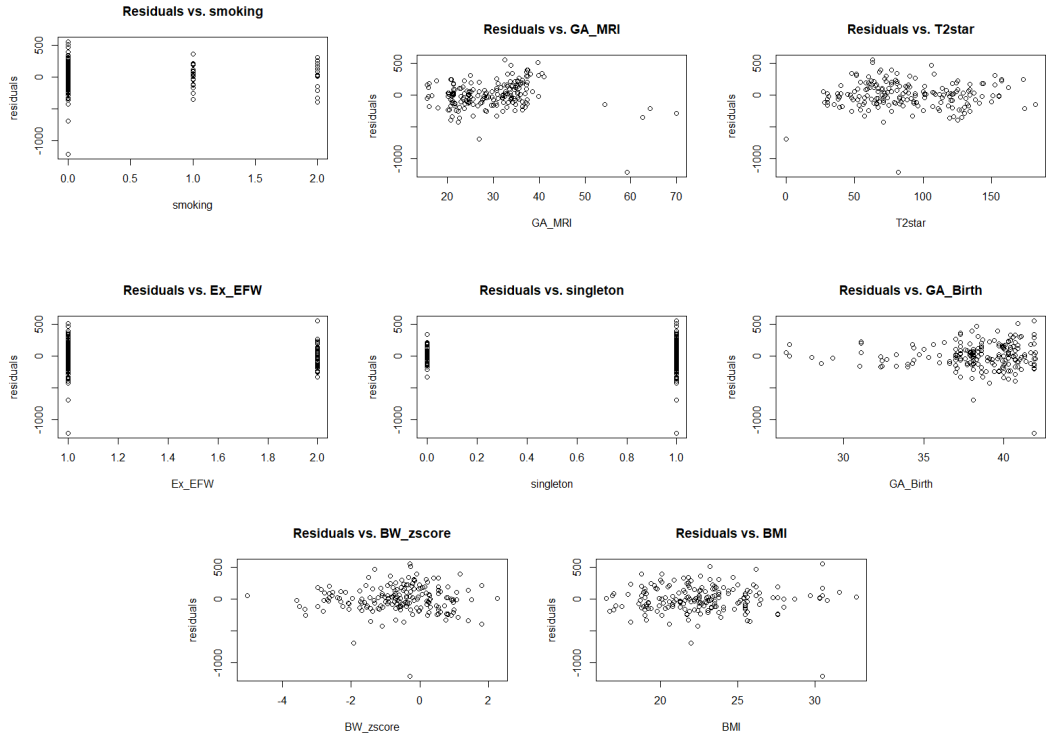

Figure 4.4: Plots of the residuals plotted against the observed values for the explanatory variables in the model. The plot does not indicate any violation of the assumption of linearity in the variables

The plot of the residuals against the observed values for the explanatory variables indicates that the assumption of linearity in the independent variables was met. The plot for residuals versus `GA_Birth` revealed some observations at gestational age as high as 70 weeks, which is not possible. When looking at the plot for gestational age up to 40 weeks, no obvious pattern in the residuals was found.
Next, to test if the assumption of constant variance was met, a plot of residuals against fitted values was made. The plot is seen i Figure 4.5.

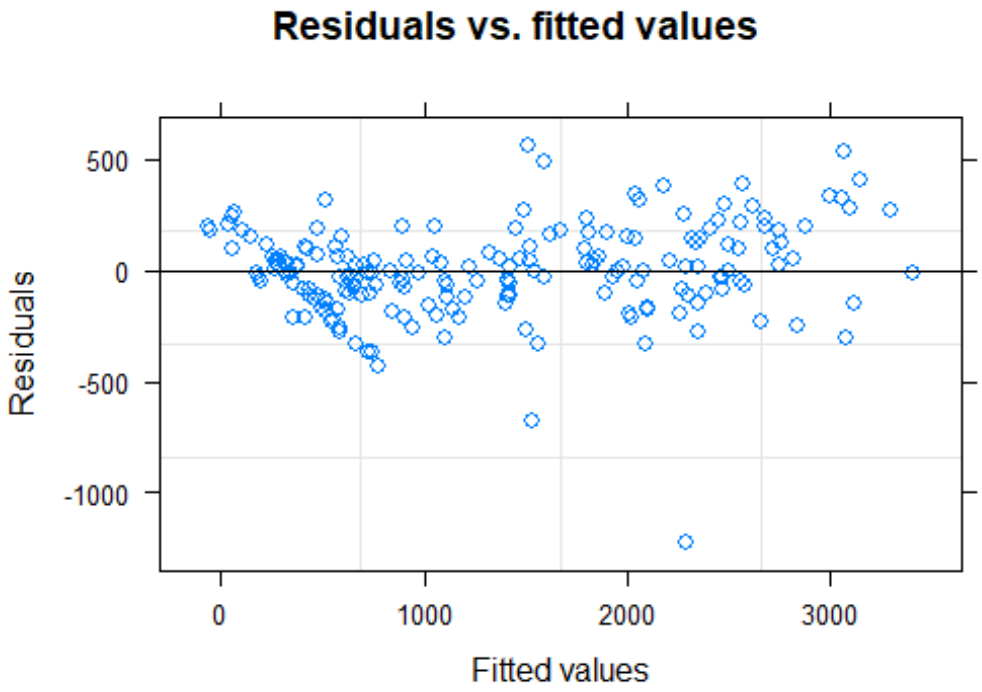

Figure 4.5: Residuals plotted against fitted values for model6. The plot indicates that the assumption of constant variance is met, as no obvious patterns are seen

The plot seen in Figure 4.5 shows no indication of violation of the assumption of constant variance, as no obvious patterns are seen.
In order to test if the residuals were normally distributed a histogram of the residuals was plotted along with a quantile-quantile plots of the model against a theoretical distribution. Both plots are seen in Figure 4.6.

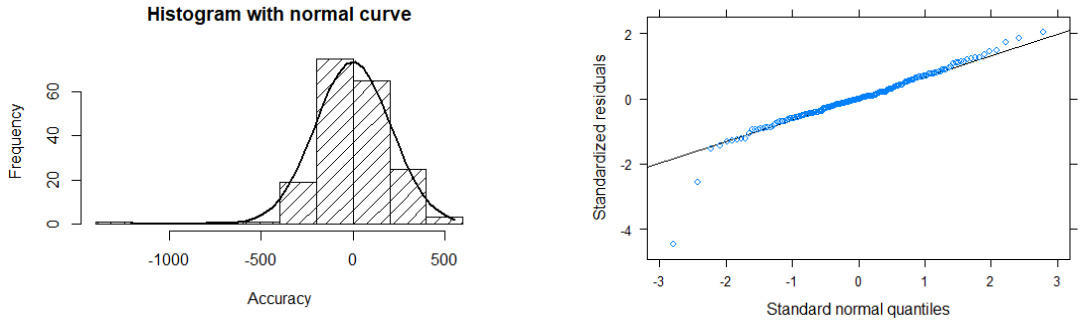

Figure 4.6: The two plots indicate that the assumption of normally distributed residuals are met

The plots in Figure 4.6 does not indicate that the assumption of normally distribution residuals was violated. This lead to the conclusion that this mixed effect model can be used to model estimated fetal weight for twins and singletons. It was found, that the estimated fetal weight depended significantly on interaction between placental T2* values and singleton. This implies that the estimated fetal weight at a given placenta T2* value is significantly different for singletons and twins.
To assess the fixed effects in the model a plot showing the confidence interval was made in Figure 4.7.

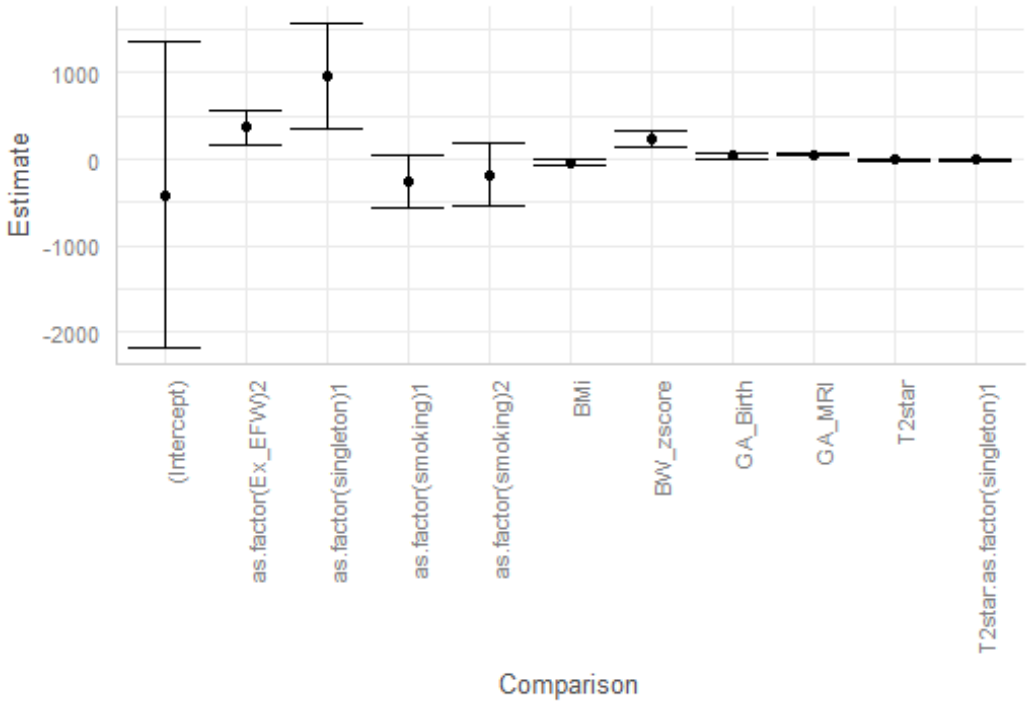

Figure 4.7: A plot showing the confidence intervals of the fixed effects in model6

5 | Conclusion

The placenta plays a key role when it comes to fetal development, as the placenta is responsible for suppling nutrient to the fetus during pregnancy. However, in some cases the placenta fails to meet the requirements from the fetus due to a dysfunctional placenta. A consequence of having a dysfunctional placenta could be the fetus' inability to reach its genetic growth potential. This is known as fetal growth restriction (FGR) and is caused by an insufficient oxygen supply. FGR is associated with approximately 50% of all stillbirths. Diagnosing FGR is complicated by the difficulty in separating the normal small fetuses from the growth restricted ones. [7, 8, 17] Today when assessing whether a fetus is able to reach its genetic growth potential the same reference curves are used for both twins and singletons. This is done despite the fact that birth weight is significantly smaller for twins than singletons. Multiple studies have pointed on the difference in estimated fetus- and birth weights for singleton and twins, but one question is yet to be answered: Is the difference in weights for singleton and twins caused by a higher tendency to dysfunctional placentas for twins than singletons or is it caused by some genetic factors leading to a smaller normal weight for twins? [22, 19] The aim of this study was to answer this particular question. Using data from Aalborg University Hospital mixed effects models were fitted in order to model the estimated fetal weight. A study by Sinding et. al (2016) found that placental MRI transverse relaxation time, T2*, could be used as a marker of dysfunction placenta [7]. Therefore, placental T2* values for each fetus was included as a indicator of the placental function. In order to answer the question raised the size of the fetus was compared to placental T2* values for twins and singletons. It was hypothesized that if the estimated fetal weights were the same for both twins and singletons at a given placental T2* value then the same reference curves can be used for both twins and singletons. In contrast, if the weight for twins are significantly different from singletons at a given placental T2* value then there is a need for new reference curves for twins.

Initially, data underwent some cleaning where variables were removed due to high percentage of missingness or because they were considered irrelevant for the aim of this study. The composition of data was changed in order to provide ID numbers to all fetuses. A plot in `R` revealed that 11% of data was missing, but as data was found to be missing based on the missing completely at random mechanism, all missing values could be removed without causing bias. This resulted in at dataset with 190 observations of 16 variables.

Data had a hierarchal nature composed of two levels; one level for each mother and a second level for each fetus with the latter being caused by longitudinal measurements. Because of the nature of data, correlation patterns were examined as one would expect to see some correlation between fetuses from the same mother and between longitudinal measurements for the same fetus. Different plots were made, as an exploratory data analysis, from which the suspicion of correlated data was confirmed. In order to incorporate this behavior a linear mixed effect model was used to model the estimated fetal weight, as mixed effects models allows a wide variety of correlation patterns to be modeled. To make sure a mixed effects model was the right choice for the data different plots was made to examine if all model assumptions were met. No plots indicated a violation of any of the model assumptions, why mixed effects models were used to model the data.

The models were fitted with random effects associated with the individual mothers and random effects from each individual fetus. However, an `ranova` test showed that the random effect for each fetus was not significant, why this was excluded from the model.
A total of seven models were fitted using the 16 variables from the dataset. The first model included all variables whereas the additional models only included some. Variables which was not significant based on the likelihood ratio test, was removed using backward selection. This way one variable was removed at the time, after which an `ANOVA` test was performed to determine whether or not the variable could be removed without changing the model significantly.

The seven models were compared according to Akaike information criterion (AIC) and Bayesian informaton criterion (BIC). Model6 was found to be the best fit according to these measures. This corresponded to the model which only included significant variables. The model was

$$\texttt{EFW} \sim \texttt{GA\_MRI} + \texttt{T2star} * \texttt{singleton} + \texttt{Ex\_EFW} + \texttt{GA\_Birth} + \texttt{BW\_zscore} + \texttt{BMI} + \texttt{smoking} + (1|\texttt{ID\_mother})$$

This indicates that the gestational age at the MRI scan along with gestational age at birth, birth weight zscores, information about the mothers smoking habits and the interaction between placental T2* value and whether the fetus was singleton or twin all influenced the estimated fetal weight. Based on these results it was found that the estimated fetal weight was significantly different for singletons and twins at a given placental T2* value. This implies that there could be a need for a new reference curve when assessing the normal size for twin fetuses, and hence evaluating the placenta function. However, when making such conclusions one should keep in mind, that there was some mistakes in the data, that might have lead to wrong conclusions. Therefore, there is a need for refitting the models using data where mistakes are corrected.

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Appendix

A | R-scripts

```
PlacentaData <- read.table('C: 10. semester filer.txt')

library(Amelia)
missmap(PlacentaData, main="Missing values vs. observed", x.cex=0.9)

# Test for MCAR
library(BaylorEdPsych)
LittleMCAR(PlacentaData)$p.value
# weak evidence against the null hypothesis.
# Hence missing values can be removed.

PlacentaData <- na.omit(PlacentaData)
library(lmerTest)
library(lme4)

model<-lmer(EFW ~ GA_MRI +as.factor(para) +as.factor(Ex_EFW)+
T2star*as.factor(singleton)
+ GA_Birth +Birth_weight+ BW_zscore+ as.factor(proteinuria)+ BMi +
as.factor(gender)+as.factor(smoking)+ age+(1|ID_mother) + (1|ID_Fetus),
data=PlacentaData, REML=FALSE)
ranova(model)
res <- resid(model)
# Test model assumption of linearity
plot(PlacentaData$GA_MRI, res, xlab="GA_MRI", ylab="residuals",
main="Residuals vs. GA_MRI")
plot(PlacentaData$para, res, xlab="para", ylab="residuals",
main="Residuals vs. para")
plot(PlacentaData$T2star, res, xlab="T2star", ylab="residuals",
main="Residuals vs. T2star")
plot(PlacentaData$Ex_EFW, res, xlab="Ex_EFW", ylab="residuals",
 main="Residuals vs. Ex_EFW")
```

```
plot(PlacentaData$singleton, res, xlab="singleton", ylab="residuals",
main="Residuals vs. singleton")
plot(PlacentaData$GA_Birth, res, xlab="GA_Birth", ylab="residuals",
main="Residuals vs. GA_Birth")
plot(PlacentaData$Birth_weight, res, xlab="Birth_weight", ylab="residuals",
main="Residuals vs. Birth_weight")
plot(PlacentaData$BW_zscore, res, xlab="BW_zscore", ylab="residuals",
main="Residuals vs. BW_zscore")
plot(PlacentaData$proteinuria, res, xlab="proteinuria", ylab="residuals",
 main="Residuals vs. proteinuria")
plot(PlacentaData$BMi, res, xlab="BMI", ylab="residuals",
main="Residuals vs. BMI")
plot(PlacentaData$gender, res, xlab="gender", ylab="residuals",
 main="Residuals vs. gender")
plot(PlacentaData$smoking, res, xlab="smoking", ylab="residuals",
 main="Residuals vs. smoking")
plot(PlacentaData$age, res, xlab="age", ylab="residuals",
main="Residuals vs. age")
# Test for constant variance
plot(model, main="Residuals vs. fitted values", ylab="Residuals",
xlab="Fitted values")
# test for normallity
library(lattice)
qqmath(model)

h <- hist(res, breaks = 10, density = 10, xlab = "Accuracy",
 main = "Histogram with normal curve")
xfit <- seq(min(res), max(res), length = 40)
yfit <- dnorm(xfit, mean = mean(res), sd = sd(res))
yfit <- yfit * diff(h$mids[1:2]) * length(res)

lines(xfit, yfit, col = "black", lwd = 2)



# fjerner fetus
model1<-lmer(EFW ~ GA_MRI + as.factor(para) +as.factor(Ex_EFW)+
T2star*as.factor(singleton)  + GA_Birth +Birth_weight+ BW_zscore+
as.factor(proteinuria)+ BMi +as.factor(gender)+as.factor(smoking)+
age+(1|ID_mother), data=PlacentaData, REML=FALSE)
drop1(model1, test="LRT")
anova(model, model1)
```

```
#Birth_weight
model2<-lmer(EFW ~ GA_MRI + as.factor(para) +as.factor(Ex_EFW) +
T2star*as.factor(singleton)  + GA_Birth+ BW_zscore+
as.factor(proteinuria)+ BMi +as.factor(gender)+as.factor(smoking)+
age+(1|ID_mother),
data=PlacentaData, REML=FALSE)
anova(model2, model1)
drop1(model2)
# para
model3<-lmer(EFW ~ GA_MRI  +as.factor(Ex_EFW) + T2star*as.factor(singleton)
+GA_Birth+ BW_zscore+ as.factor(proteinuria)+ BMi +as.factor(gender)+
as.factor(smoking)+ age+(1|ID_mother), data=PlacentaData, REML=FALSE)
anova(model3, model2)
drop1(model3)

#proteinuria
model4<-lmer(EFW ~ GA_MRI  +as.factor(Ex_EFW) + T2star*as.factor(singleton)
+GA_Birth+ BW_zscore+ BMi +as.factor(gender)+as.factor(smoking)+
age+(1|ID_mother), data=PlacentaData, REML=FALSE)
anova(model3, model4)
drop1(model4)
#age
model5<-lmer(EFW ~ GA_MRI  +as.factor(Ex_EFW) + T2star*as.factor(singleton)
+GA_Birth+ BW_zscore+ BMi +as.factor(gender)+as.factor(smoking)+
(1|ID_mother),
data=PlacentaData, REML=FALSE)
anova(model5, model4)
drop1(model5)

#gender
model6<-lmer(EFW ~ GA_MRI  +as.factor(Ex_EFW) +
T2star*as.factor(singleton)+ GA_Birth+ BW_zscore+ BMi+as.factor(smoking)+
(1|ID_mother), data=PlacentaData,
REML=FALSE)
anova(model5, model6)
drop1(model6)
summary(model6)
```