Universitat de Girona

Facultat de Medicina

Influence of Assisted Reproductive Techniques in the occurrence of weight discordance in twin gestations

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ABSTRACT

Background: There is no actual evidence that the *ART* are directly related to the occurrence of weight discordance. In some studies, *ART*-conceived twin pregnancies are at greater risk than non-*ART*-conceived ones for pregnancy complications and adverse perinatal outcome: the incidences of pregnancy-induced hypertension, uterine bleeding, premature contractions, *IUGR*, fetal death, discordance, and cesarean section were significantly higher. Discordance rate was elevated (25.3% vs. 17.0%) among *ART* twins, which can increase perinatal risk (increased incidence of *SGA* and *NICU* admission). Other studies say that perinatal and neonatal morbidity, gestational age at delivery, and birth weight are not affected by *ART*.

Regarding the first trimester ultrasound, some studies didn't notice significant differences in *CRL* disparity or birth weight discordance between spontaneous and *ART*-conceived dichorionic twin pregnancies. In *ART*-conceived dichorionic twin pregnancies, *CRL* disparity may be associated with birth weight discordance. In some studies, *CRL* discordance in twin pregnancies in the first trimester was a frequent finding.

Objectives: To analyze the association of the *ART* in the occurrence of weight discordance in the pregnancies between 2010 and 2013 in the Hospital Universitari de Girona Doctor Josep Trueta, and to describe the proportion of diagnosis of growth discordance in the first trimester by the ultrasonography technology.

Methods: A retrospective cohort study will be performed in those patients with twin pregnancies between 2010 and 2013, within the Hospital Universitari de Girona Doctor Josep Trueta (*HUJT*). A retrospective and descriptive study will be done in those cases with discordance weight in the moment of the birth, in which the *CRL* will be studied in the first trimester ultrasound, describing the percentage of discordance detected in that moment.

The general characteristics of the sample are going to be analyzed by Logistic Regression.

INTRODUCTION

Epidemiology Of The Twin Gestation

Multiple gestations account for 1-2% of all births and represent 10-14% of the overall perinatal mortality, a rate of five to ten times higher than that of singletons.[1] Because of the increased use of Assisted Reproductive Technologies (*ART*), the number of multiple gestations has steeply increased over the past 20 years.

Embryology Of The Twin Gestation [2–4]
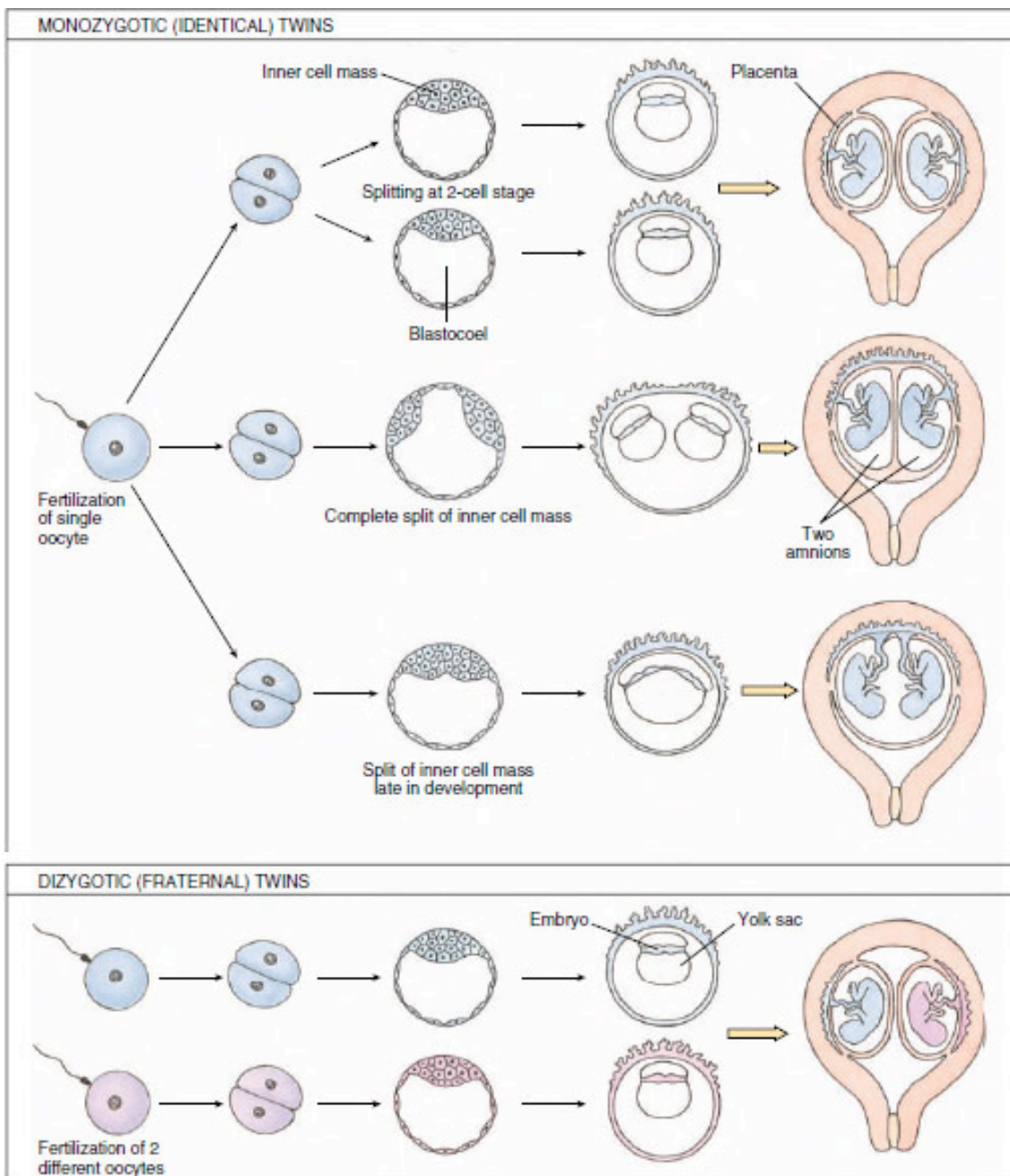
Langman J, Sadler TW. Langman's Medical embryology.

Dizygotic twins:

More or less 2/3 of the twin gestations are dizygotic or fraternal ones. They come from the simultaneous expulsion of two oocytes and their fertilization by two different sperms. Both zygotes have totally different genetic information: they have the same resemblance as two siblings of different age. They can be of the same or different sex.

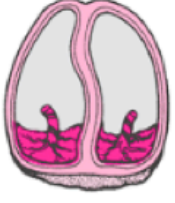The placentation of the dizygotic twins will always be **Diamniotic Dichorionic**. Two complete placental units are produced, each composed of an amnion and a chorion. As a result, the membrane separating dizygotic twins will consist of four layers – an amnion and a chorion from each fetus. The placentas will be separated or fused, but there will always be four layers of dividing membrane.

Monozygotic twins:

This second type of twins develops from one unique oocyte fertilized by one unique sperm, resulting two genetically identic twins.

The placentation depends on the time at which the twin division occurs:

- If it occurs in the **first three days**, two complete placental units will be formed and the dividing membrane will contain two amnion and two chorion layers, just as the dizygotic twins.

- If embryonic division occurs **between days 3 and 8**, the placentation will be a single chorion that was by now already developed and two amnions that have not yet began to form. As a result, the dividing membrane will be thin and wispy: it consists of only two opposed amniotic membranes without the intervening chorionic layers. This placentation is referred to as **Diamniotic Monochorionic**.

- If the division occurs **between days 8 and 13**, the twins will share a single amnion and chorion (the amnion begins to differentiate by day 8). There will be no dividing membranes separating the fetuses: a **Monoamniotic Monochorionic placentation**.

- If the embryonic division occurs **after day 13**, it also results in Monochorionic Monoamniotic placentation, but with physical attachment of the fetuses producing **conjoined twins**.

Zygote	Dizygotic	Monozygotic		
Day of division		0–3	3–8	8–13
Placenta				
Central membrane	2 Amnion 2 Chorion	2 Amnion 2 Chorion	2 Amnion	None

Gibbs RS. Danforth's Obstetrics and Gynecology,2008.

Ultrasound In Twin Gestation

Ultrasound plays numerous critical roles in the antepartum care of multiples. This includes: diagnosis, determination of amnionicity and chorionicity, identification of fetal growth and amniotic fluid volume, evaluation of fetal biophysical parameters and determination of presentation.[2]

Diagnosis:[5]

In the **first trimester**, the ultrasound scan can identify the embryo (clearly visible from the eighth week), limbs and head. The Crown Rump Length (*CRL*) will help dating the gestation. It can see movement. The heart movement can be identified and demonstrable with Doppler ultrasonography. It can detect multiple pregnancy, the placental site, and possible fetal abnormalities.
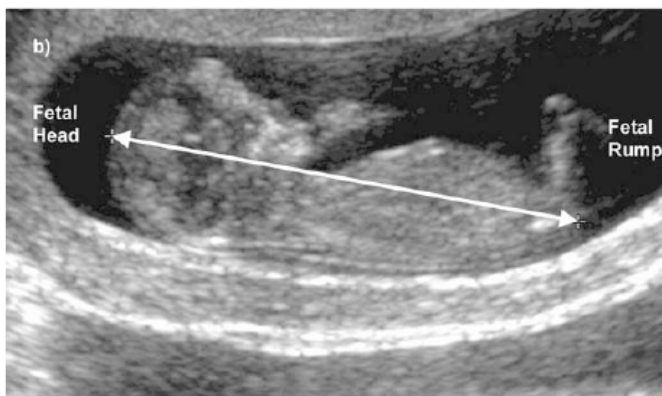
Measurement of *CRL* at 13 weeks gestation. *Loughna P. Fetal Size and Dating: Charts Recommended for Clinical Obstetric Practice. Ultrasound. 2009.*

Determination of amnionicity and chorionicity:

Dichorionic twins are easier to recognize from monochorionic twins in the first trimester. The criterion is simply that dichorionic twins have a thick membrane (actually with some interposing tissue) while monochorionic twins have either a very thin or barely visible membrane:[6]
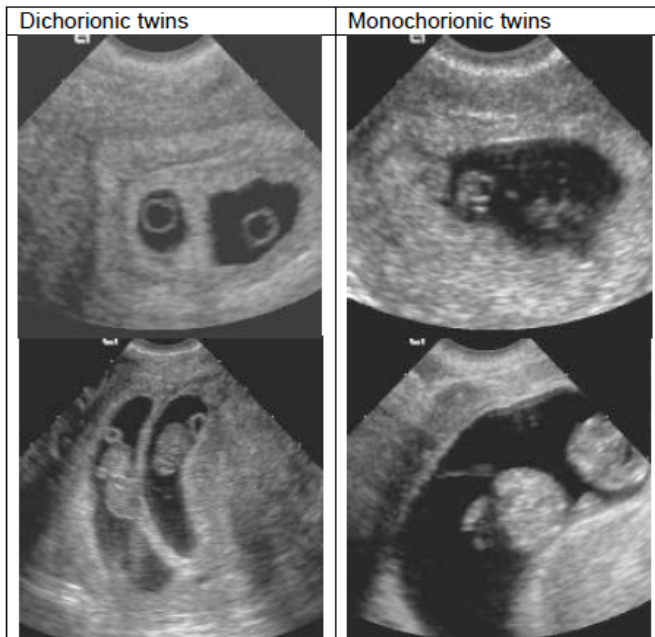
Sonography of Multiple Gestations. Reyes J, Silva SR, Domingo S, Republic D. Sonography of multiple gestations.

Transvaginal ultrasonography allows a reliable, simple and rapid determination: the dichorionic twin pregnancy in 4 weeks, monochorionic in 5 weeks, and differentiation of mono- or diamniotic in 7 weeks of gestation.[7] In monochorionic twins, there is a single placental mass, with or without a dividing membrane. When there is a dividing membrane, it is composed of two layers representing the two layers of amnion. In contrast, the inter-twin membrane of dichorionic twins is composed of a layer of chorion between two layers of amnion: it is thicker, especially between 6 to 9 weeks, when a septum can be observed between the chorionic sacs. After 9 weeks, the septum becomes progressively thinner, but it remains thick and easy to identify at the insertion point into the placental mass as a triangular projection called the *lambda* or *twin-peak sign*.[8]

Monoamniotic twins can be suspected in the absence of a dividing membrane demonstrated on two studies at least 12-15 hours apart.[9] Other features observed could be:[10]

- Single placenta and same sex twins

- Close approximation of the cord insertions

- Entanglement of the cords

- Normal and identical amniotic fluid volume around both fetuses

- Unrestricted fetal movement

Fetal growth and amniotic fluid volume:

At the **18-20 week** routine ultrasound visit, the following are assessed:[5]

- The Biparietal Diameter (*BPD*)

- The Head Circumference (*HC*)

- The Abdominal Circumference (*AC*)

- Femur Length (*FL*)

These measurements are used to confirm the gestational age of the fetus. It can also detect fetal abnormalities: spina bifida, double bubble of dilated stomach and duodenum in duodenal atresia, some cardiac abnormalities, hydrocephaly, renal pelvic dilatation, sacral agenesis or major limb defects.
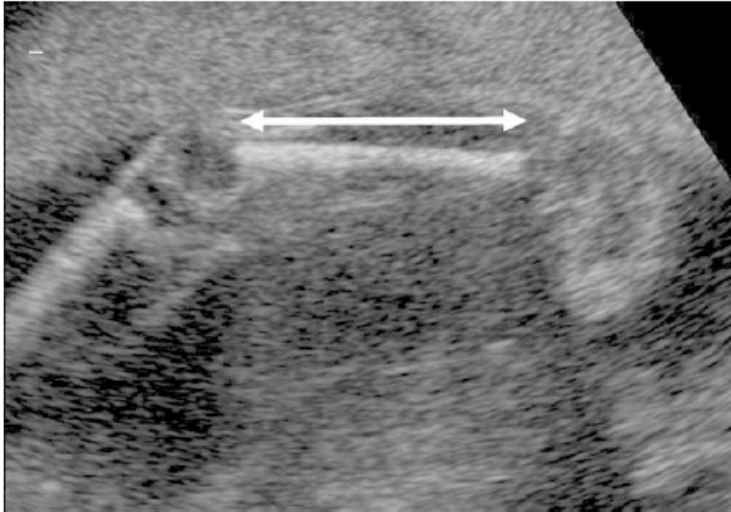
Measurement of *FL*.

Loughna P. Fetal Size and Dating: Charts Recommended for Clinical Obstetric Practice. Ultrasound. 2009.
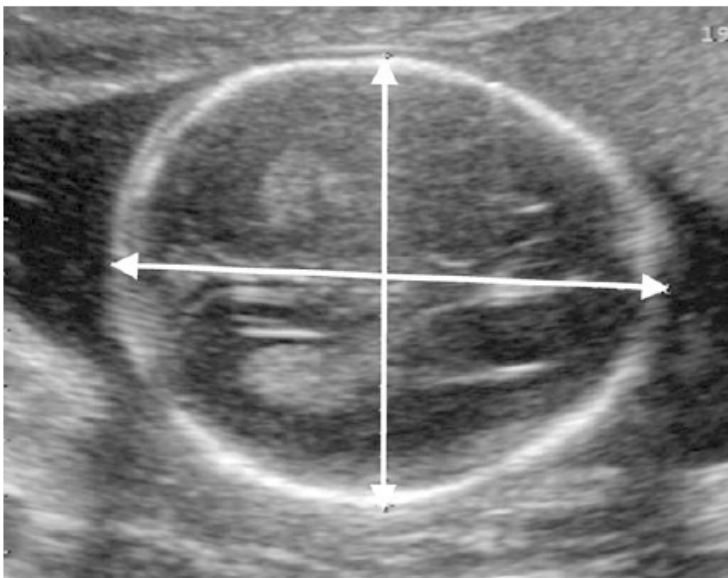
Estimation of *HC*, obtained from the measurements of *BPD* and the Occipital-Frontal Diameter (*OFD*). *Loughna P. Fetal Size and Dating: Charts Recommended for Clinical Obstetric Practice. Ultrasound. 2009.*
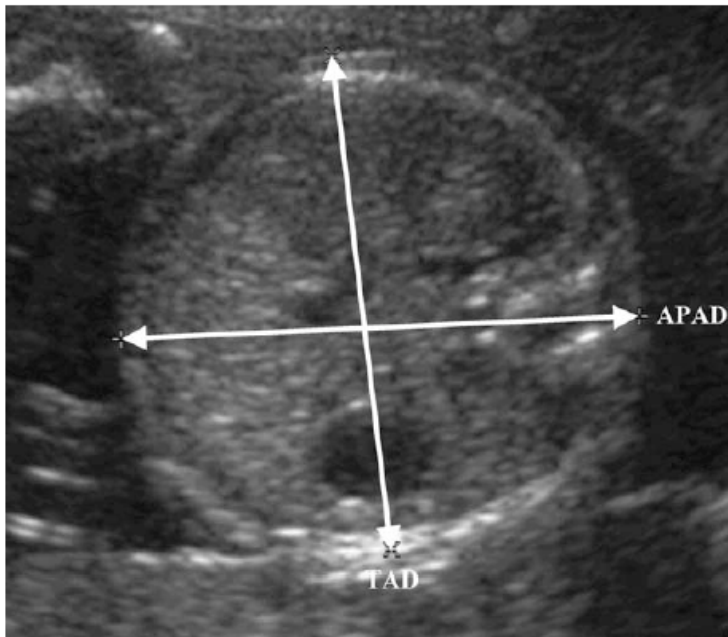
Estimation of *AC*.

Loughna P. Fetal Size and Dating: Charts Recommended for Clinical Obstetric Practice. Ultrasound. 2009.

At the **third trimester**:

Measurements of fetal growth are best achieved by measurement of the *HC* and the *AC*. Doppler waveforms from the uteroplacental circulation: Maternal Uterine Arteries, Umbilical Artery and Middle Cerebral Artery.

Appendix 1-5

Six tables had been attached:

      Appendix 1: Crown Rump Length dating table

      Appendix 2: Crown Rump Length size chart

      Appendix 3: Biparietal Diameter dating table

      Appendix 4: Abdominal Circumference size table

      Appendix 5: Femur Length size table

Data obtained from the information of the ultrasounds in Hospital Universitari de Girona Doctor Josep Trueta (HUJT).

Assisted Reproductive Technology [12–14]

ART is by definition any treatment or procedure that includes the handing of oocytes and sperm or embryos outside the body with the purpose of establishing a pregnancy.

- **Artificial Insemination:** instrumental introduction of the semen in the female genital tract, preferably in the uterine cavity. The semen has been processed in the

laboratory, using techniques designed to improve its quality. It can be from the spouse or a donor.

- **In Vitro Fertilization (*IVF*):** ovulation induction, oocyte retrieval, and fertilization of the oocytes in the laboratory; embryos are then cultured for 3-5 days with subsequent transfer transcervically under abdominal ultrasound guidance into the uterine cavity.

In general, *ART* increases the risk of multiple pregnancies by 10-fold above baseline (35% vs. 3% in the general population). Another risk factor for multiple pregnancies is maternal age: younger woman tend to be at higher risk of multiple pregnancy when more than one embryo is replaced.[15]

The problem with multiple gestations lies in the risk during pregnancy to both the fetuses and the mother. Risks of multiple pregnancies include higher rates of perinatal mortality, preterm birth, low birth weight, gestational hypertension, placental abruption, and placenta previa. Perinatal mortality in assisted conception twin pregnancies appears to be lower than in spontaneously conceived twin pregnancies.[16]

Discordant Twin Growth:

Discordance is the difference in the weights of the fetuses, and it is a common phenomenon reported to complicate over **15% of twin pregnancies**.[17]

According to the *American College of Obstetricians and Gynecologists (ACOG)*, Discordant Growth is associated with increased likelihood of anomalies, Intrauterine Growth Restriction (*IUGR*), preterm birth, infection of one fetus, stillbirth, Umbilical Artery pH <7.10, admission to neonatal intensive care unit, respiratory distress, and death within one week of birth.

Discordance is defined with the larger twin as a standard of growth and is calculated by the following equation:

<u>(Larger estimated or actual weight – Smaller estimated or actual weight)</u>
Larger estimate or actual weight

The *ACOG* considers a **15-25% difference in actual weight** among twins to be discordant.[18]

The diagnosis of divergence by the *Society of Obstetricians of Canada* is based on the difference in *AC* of 20 mm (Sensitivity 80%, Specificity 85% and Predictive Value Positive of

62%) or an **Estimated Fetal Weight (*EFW*) difference of 20%**. It is recommended to obtain the *EFW* from *DBP* and *AC* or *AC* and *FL* (Sensitivity of 25 to 55%).[18,19]

Twin Discordance is believed to result from known factors that can be categorized as maternal, fetal or placental, as shown in the table below:

TABLE 3
Factors that influence discordant growth of twins

Variable	Increase	Decrease	No Influence
Maternal	Maternal age of ≥30 years[20]	Nulliparity[28]	Maternal age[26]
	Assisted reproduction vs spontaneous twins[40,46]	Constant maternal weight gain throughout gestation[50]	Pregnancy with assisted reproduction[26]
	Nulliparity[15,47]		Parity[26]
	Tobacco use[48]		
	No prenatal care[49]		
	Fluctuating change in body mass index[50]		
Fetal	Monochorionic[51]	Dichorionic[51]	Chorionicity[26]
	Sex-discordant twins[28,52]		Reduced and nonreduced twins[24]
	Small for gestational age in either fetus[28]		
	Viral infection[53]		
Placenta	Velamentous cord insertion[54-57]	—	—
	Placental sharing[57-59]		
	Placental weight[51]		
	Pattern and size of vascular anastomoses[60]		

Miller. Discordant twins. Am J Obstet Gynecol 2012.

There is a disagreement whether the use of an *ART* is a risk factor for discordant growth.[18]

Traditionally, variation in fetal growth has been thought to emerge during the latter one half of pregnancy: antenatal ultrasound predictions of twin discordance are usually made in the second and third trimesters of pregnancy. Recent studies have challenged this with evidence of prediction of *IUGR* and adverse perinatal outcome in the first trimester. Disparate fetal growth has been noted as early as 10-14 weeks gestation and it would appear that differences at this early stage in fetal development are as accurate as biometric measurements within 1-month delivery.[17,20] Discordant fetal size is identified in the first trimester by the difference in *CRL* between twin pairs divided by the *CRL* of the larger twin. This discordance can be used as screening tests to predict second and third trimester complications:[18] in 38% of fetuses with growth discordance at birth, first trimester biometric disparity for both *CRL* and *BPD* were detected.[20]
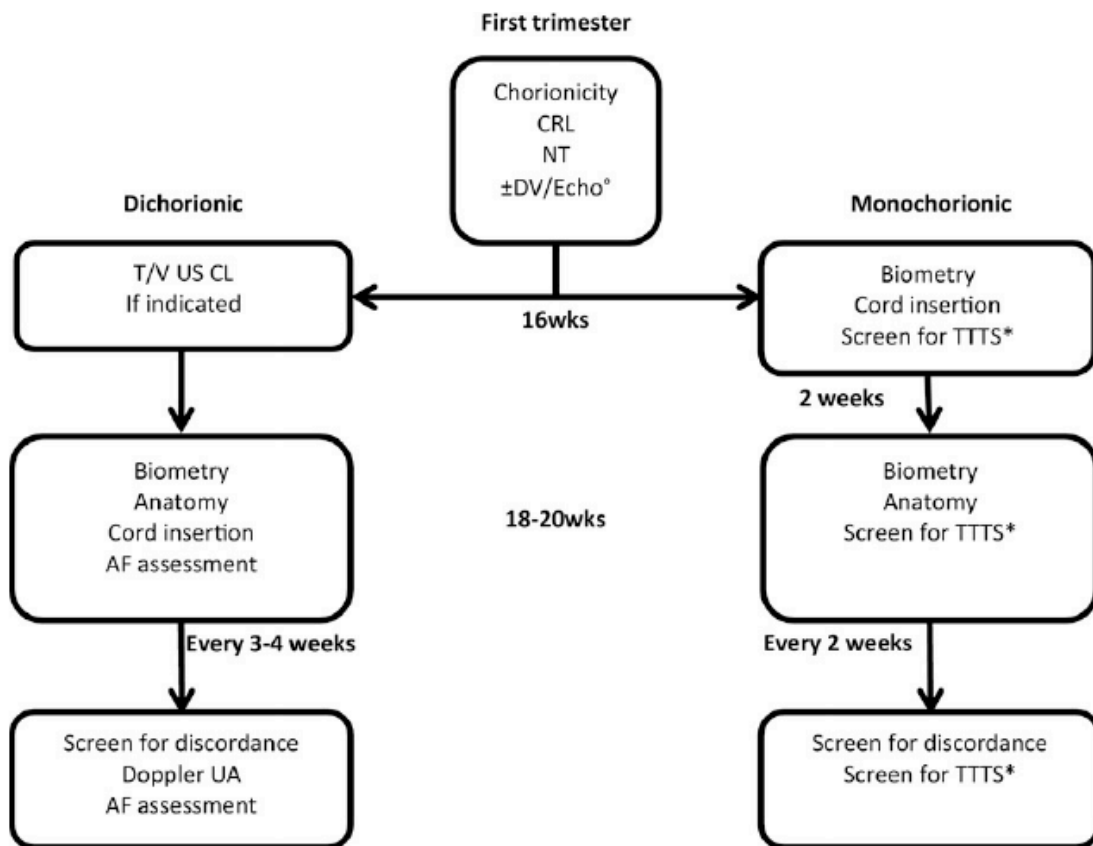
The *degree sign* indicates that the data may be limited to specialized centers for specific indications; the *asterisk* indicates bladder filling, amniotic fluid assessment, Doppler finding (umbilical artery, ductus venosus, umbilical venous).

AF, amniotic fluid; *CRL*, crown-rump length; *DV*, ductus venosus; *ECHO*, echocardiogram; *NT*, nuchal translucency; *T/V US CL*, transvaginal ultrasound cervical length; *TTTS*, twin-twin transfusion syndrome; *UA*, umbilical artery.

Miller. Discordant twins. Am J Obstet Gynecol 2012.

As we can see in the figure, first and third trimester can identify twins who are at increased risk of morbidity and death, classifying into high and low risk pregnancies, and identifying those who are likely to experience discordance. After 24 weeks gestation, the aim of the ultrasound study is to identify discordant or insufficient fetal growth, with the objective of preventing the morbidity of the smaller twin.[18]

From the 18th week, some studies had shown that real-time ultrasonography (measuring *EFW*, *BPD*, *AC* and *FL*) combined with Umbilical Artery Doppler was useful in predicting discordant twins.[20,21]

Management of Discordant Twins[22]

They should have a growth evaluation every 2-3 weeks, which include amniotic fluid volume assessment and arterial and venous fetal Doppler. The timing of delivery must be after 32 weeks gestation, which maximizes the prospect of survival of the growth-restricted twin, and decreases the handicaps in the appropriately grown co-twin.

As it has been exposed, the selective growth restriction confers substantial risks on the normally grown twin even if both fetuses are born alive, mainly neurologic complications. There can be an expectant approach or it can be done a fetal therapy, either cord occlusion in the deteriorating smaller twin or selective laser photocoagulation of communicating placental vessels; but these approaches are still on international trial.

Outcome of Discordant Twins

These infants are much more likely to suffer from various neonatal complications, especially respiratory and central nervous system diseases; and congenital malformations[23], but it may not be an independent risk factor for adverse perinatal outcomes in twin pregnancies.[24] Their mortality is increased in discordant twins, and they are more related to preterm birth and low birth weight.

Weight discordance and ART nowadays

In some studies, In Vitro Fertilization twins are known to be more likely to suffer higher incidences of preterm birth and prematurity-related respiratory complications with a longer nursery stay.[25] *ART*-conceived twin pregnancies are at greater risk than non-*ART*-conceived ones for pregnancy complications and adverse perinatal outcome: the incidences of pregnancy-induced hypertension, uterine bleeding, premature contractions, *IUGR*, fetal death, discordance, and cesarean section were significantly higher.[26,27] Discordance rate was elevated (25.3% vs. 17.0%) among *ART* twins. Small for Gestational Age (*SGA*) newborns were increased and *NICU* admission was more frequent in discordant group. *ART* can increase discordance rate, which can elevate perinatal risk.[28]

Other studies say that perinatal and neonatal morbidity, gestational age at delivery, and birth weight are not affected by *ART*.[29–31]

Ultrasound and early discordance detection:

Some studies didn't notice significant differences in *CRL* disparity or birth weight discordance between spontaneous and *ART*-conceived dichorionic twin pregnancies. In *ART*-conceived dichorionic twin pregnancies, *CRL* disparity may be associated with birth weight discordance.[32]

In some studies, *CRL* discordance in twin pregnancies in the first trimester was a frequent finding. Discordance>95th centile indicates major growth delay of one twin.[33]

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JUSTIFICATION

Advances in *ART* have contributed to the increase in the incidence of twin pregnancies since the 1980s. It is known that maternal and perinatal complications are higher in twins than in singleton pregnancies, increasing the use of health care resources and the associated costs. As it has been already exposed, there are different well-known factors (maternal, fetal and placental) that contribute to the appearance of these complications, specially the discordant growth, but there is disagreement whether the use of *ART* is a risk factor to these complications.

Studies done till nowadays show different results, and those showing a conclusion of difference between the outcomes in *ART* and non-*ART* pregnancies don't eliminate the confusion variables such as the age of the mother and the type of placentation, as it is in our objective of study.

Ultrasonography is a valuable tool in the diagnosis and management of twin pregnancy. Early diagnosis of twins and their chorionicity, close fetal surveillance, particularly of monochorionic twins, and prompt therapeutic intervention in diverse complications are necessary to reduce perinatal mortality.

We will center our attention on the effect of *ART* in the incidence of the discordant growth, mainly because knowing the relationship between them, we will be able to make a more conservative use of ovarian stimulation and to give all the necessary information about the risks of a multiple gestation to the patients[34]. They will receive a more cautious monitoring, mainly ultrasound every four weeks and evaluation of the cervix. Strict maternal control of diet, weight, blood pressure and plasma glucose will be done; maternal rest will be recommended, especially in lateral decubitus[34,35]. The patient control will also include pre and postnatal multidisciplinary control in multiple pregnancies, in charge of physicians with experience with ultrasound and multiple pregnancies[34].

In case of advanced discrepancies or when any of the fetuses runs severe danger, there's the option of a conservative treatment (mentioned above), feticide or abortion[36].

HYPOTHESIS

Our **main hypothesis** is that there are more growth discordances in the gestations accomplished by In Vitro Fertilization (*IVF*).

Our **secondary hypothesis** is that the ultrasound in the first trimester is capable of diagnosing difference in the *CRL* of 30% of the cases analyzed.

OBJECTIVES

Our **main objective** is to analyze the association of the *ART* in the occurrence of weight discordance in the pregnancies between 2010 and 2013 in the Hospital Universitari de Girona Doctor Josep Trueta.

Our **secondary objective** is to describe the proportion of diagnosis of growth discordance in the first trimester by the ultrasonography technology, within those cases in which there was weight discordance in the third trimester.

METHODOLOGY

Design Of The Study:

This is a retrospective, observational and analytical cohort study: a cohort of twin pregnancies between 2010 and 2013, within the Hospital Universitari de Girona Doctor Josep Trueta (*HUJT*).

Within those cases with discordance weight in the moment of the birth, the *CRL* will also be studied in the first trimester ultrasound, describing the percentage of discordance detected in this first trimester: it will be a retrospective and descriptive study.

Population In Study:

The population in study will be those twin pregnancies between January 1st 2010 and December 31st 2013 within the HUJT.

Inclusion criteria:

- Twin pregnancies with date of birth between January 1st 2010 and December 31st 2013.

- To belong to the field of the HUJT.

- Monitoring of the pregnancy in the HUJT, mainly the first trimester ultrasound and the labor.

Exclusion criteria:

- Pregnancies that are not twin pregnancies.

- Patients which first ultrasound or labor weren't controlled in the HUJT.

Selection Of The Sample:

This is a consecutive non-probabilistic sampling from the data we have in HUJT, from the first ultrasound of the pregnancy and the childbirth.

Sample Size:

It is determined by the cases of the twin pregnancies in the hospital. The power of the sample (*n*) will be calculated from this sample: this study is designed to detect a minimum difference of 10% between the spontaneous gestations and the ones obtained with ART. Accepting an alpha risk of 0,05 and a beta risk of 0,2 in a two-sided test, **522** subjects are necessary in the group of spontaneous gestations, and **174** subjects in either group of the *ART*, to find a statistically significant proportion difference of 0,10 (0,15 in the spontaneous group, and 0,25 in either *ART* group).

It has been anticipated a dropout rate of 5%.

The GRANMO Calculator has obtained the size of the sample needed. The ARCSINUS approximation was used.

Study Variables:

In our main objective, our independent variable is the use of *ART* (spontaneous gestation, *IVF*, Insemination), and our effect will be the weight discordance in the birth in twins: it will be the dicotomic dependent variable YES/NO.

Discordance is defined with the larger twin as a standard of growth and is calculated by the following equation:

(Larger estimated or actual weight – Smaller estimated or actual weight)

Larger estimate or actual weight

The *ACOG* considers a 15-25% difference in actual weight among twins to be discordant.[18] In our study we will consider a 20% or more as a discordance between twins' weight.

The covariables we will adjust to eliminate confusion will be: mother's age and the type of placentation. We will put them in categories and number them: the placentation of the fetuses (240: Bicorial Biamniotic; 241: Monocorial Biamniotic; and 242: Monocorial Monoamniotic); and the age of the mother (categories: <30 years; 30-38 years; >38 years).

In our secondary objective, we will take into account all those cases of established discordance between newborn twins, and take a look to the ultrasound of the first trimester, to see in which cases a difference between the *CRL* was detected. This difference is defined as a variation in the *CRL* of 5 or more days of gestational age, which is equivalent to two standard deviations, between the 6th and the 13th week.[18]

Measuring instruments:

Different scales were used in the delivery room and the operating room for weighing newborns; the data was obtained in grams. The users of these scales were mainly trained and experienced staff in HUJT for this function mainly midwives of the center.

For the ultrasound data, ultrasound queries from the HUJT were used; measures were taken in mm and compared to the reference tables of the hospital (in appendixes). Qualified personnel used these machines: doctors and residents from the center.

Data Collection Method:

For the recollection of the data, there were two computerized programs:

- *SIAO:* for the recollection of the weight of the newborns and all the data of the mother and the baby in the moment of the delivery. The data that this program recollects is mainly: the mother's age, the week of gestation, the type of placentation and the due date. It also has the information about the type of gestation.

- *ECOBS:* for the recollection of the mm of *CRL*, the type of gestation and all the growth parameters during the different trimesters of the pregnancy. It collects the same information as the SIAO program, about the mother and the gestation.

STATISTICAL ANALYSIS

The management and analysis of data was performed using the following tools.

Database:

To manage computer data, the *MS Access database* and *Microsoft Excel* tool will be used. Through them, a relational database will be compiled containing many fields as variables had been elected.

Statistical Handling. Statistical Test. Significance Level:

The general characteristics of the sample are going to be analyzed by *Logistic Regression*. The general characteristics of a sample will be analyzed with the bivariant analysis. The logistic helps to evaluate the main association (*ART*-discordance) adjusted by the effect of the rest of the covariables.

The dependent variable (weight discordance: YES/NO) is categorical. The independent variable or predictor is the use of the *TRA* (spontaneous gestation, *IVF*, Insemination). Covariables are the age of the mother and placental type: these variables have to be adjusted.

The relationship between qualitative variables will be analyzed using the *Odds Ratio (OR)*: the ratio of the odds of exposed and unexposed.

Logistic Regression Objectives:
- Predict a particular response from the predictor or independent variables, obtaining a mathematical formula that serves to calculate the probability of the event studied in a new individual by reason of the present values of the different variables included in the model.
- Calculate the (unbiased) adjusted or controlled risks for each independent variable. In this case it is important to determine the set of control variables that will fit in the analysis, including those with adequate theoretical justification. The steps are:
    1. Assess whether there is interaction (modifying effect) between any of the control variables and the independent variable, with tests of statistical significance, leaving the model interaction terms that are statistically significant.
    2. Assess whether there is confusion between any of the control variables and the main relationship evaluated without applying statistical significance test. In this situation to be analyzed is whether the introduction of a control variable in

the Logistic Regresion model produces a clinically significant change in the association measure that estimates the effect of exposure (X) on the response (Y). If not control that variable must be removed from the model, leaving it as it may decrease the precision of the study without providing adjustment (overfitting).

3. If at the end of the process more than a subset of control variables that offer a similar degree of fit, you must choose the one that considers more accurately evaluated main effect (X → Y) in the investigation.

Transversal study:

In our secondary objective, we will use a transversal study, to describe a process in a determined moment: the detection of *CRL* differences between twins in the first trimester, in those twins that were born with weight discordance.

ETHICS

This is an investigation without risk, since the information will be collected retrospectively. There will be no changes on the biological, psychological, physiological or social individuals participating in the study variables performed.

The study is designed following the principles of medical ethics defined on *the WMA Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects, last revision in the 64th WMA General Assembly, Fortaleza, Brazil, October 2013*.

According to the *Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal* in Spain, patients who take part in this study should sign an informed consent where there is an explanation of the procedure. All patients should sign voluntarily the informed consent for conducting various studies with these data consent, so it is not an ethical problem using the data anonymously.

As a retrospective study, all the data analyzed had been already recorded in a database.

Appendix 6: *Example of Informed Consent*

STUDY LIMITATIONS

Selection Bias:

This bias decreases by being a short follow-up study (approximately 6 months) of minimizing the loss of patients for this reason. Since they are routine pregnancy checks, the interest of the patient in the fetus makes the loses of patients decrease. In the medical records is the patient data to facilitate its location in case of conducting satisfaction surveys.

Information bias (measured):

This bias is reduced because we will be obtaining information for both groups from the same source, allowing comparability. Trained professionals collected all the information, using standardized instruments and compared it to the reference charts used in the hospital (attached in appendixes) so that this procedure can reduce this information bias.

Confusion of baseline characteristics.

Sample size:

In the database of 2010-2013 from HUJT there's a small population of twin gestations, obtaining a sample with small power, a limitation that can be solved by increasing the number of years of the study, or by creating a multicenter study, in a Catalonian level, for example.

EXECUTION PLAN

The study will be developed following the next steps:

January 2010 – December 2013:

The physicians of the HUJT will be aware that this study is being carried on and will offer to their patients the possibility to entry on the study, and will make them sign the informed consent. They are already trained on the collection of the data in the different informatics programs of the service (*ECOBS* and *SIAO*).

The professional personnel will collect all the data of the ultrasound of the first trimester in the *ECOBS*, and this data will be a part of the *Data Collection Notebook*. During all the pregnancy, the mother will have her routine medical controls with the obstetrician and the midwife.

When the labor arrives, the midwives will collect the weight of the twins in the *SIAO*, and it will also take part of the *Data Collection Notebook*.

First month:

Request permission to the HUJT research service to access to the statistical data.

Request approval of the Ethics Committee (CEIC).

Second month:

Request the list of agenda ultrasound (*ECOBS*) of twin pregnancies in HUJT and the list of newborns' weights (*SIAO*) with the objective of creating a database with the variables of interest: in the same chart we will have altogether the data of the pregnancy (gestation, age of the mother, placentation, *CRL*, weight of the newborns…).

Third and fourth month:

Organize the data anonymously and introduce it in the new database. Verify that the data is correctly computerized.

While the data is collected, we should design coding rules of the variables, in elements compatible with the computer analysis program.

Transfer the data to this statistical program.

Fifth and sixth month:

Data analysis:

- Obtaining statistical data

- Rating statistics

- Interpretation of data

Drawing conclusions.

Seventh month:

Translate the study in a document.

Presentation of the study to the HUJT and the journal for publication.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7
1. Request permissions							
2. Request lists of ECOBS and SIAO							
3. Organize data in new database							
4. Obtain statistical data							
5. Rating statistics							
6. Interpretation of data							
7. Draw conclusions							
8. Translate to a document							
9. Presentation							

IMPACT IN THE NATIONAL HEALTH SYSTEM

As prevention, by knowing the relationship between them, we will make a more conservative use of ovarian stimulation and to give all the necessary information about the risks of a multiple gestation to these patients.

In case a relationship between the use of *ART* and the occurrence of discordance is displayed, those patients with twin gestations obtained by an *ART* will immediately become part of the high-risk obstetrics patients (*OBAR*). They will receive a more cautious monitoring, mainly ultrasound every four weeks and evaluation of the cervix. Strict maternal control of diet, weight, blood pressure and plasma glucose will be done; maternal rest will be recommended, especially in lateral decubitus. The patient control will also include pre and postnatal multidisciplinary control in multiple pregnancies, in charge of physicians with experience with ultrasound and multiple pregnancies.

There will also need to rule out chromosomal abnormalities in early pregnancy, either by chorionic villus sampling or amniocentesis.

In case of advanced discrepancies or when any of the fetuses runs severe danger, there's the option of a conservative treatment (mentioned above), feticide or abortion.

BUDGET

The study has a low budget, as in the health center they will provide all the material and equipment needed. Our budget will mainly count of the *technical study costs*:

- Administrative authorizations: 50€
- Data managers: 8.400€ per year/per person
- Publication costs and congress presentations: 3.000€
- Travels and subsistence: 0€
- Other expenses (professional training, e.g.): 1.000€

|	
ADMINISTRATIVE AUTHORIZATIONS	50€
DATA MANAGERS (ONE PERSON/SIX MONTHS)	4.200€
PUBLICATION COSTS AND CONGRESS PRESENTATIONS	3.000€
TRAVELS AND SUBSISTENCE	0€
OTHER EXPENSES	1.000€
TOTAL	**8.250€**

APPENDIX

Appendix 1: *Crown Rump Length dating table*

weeks	pc5	pc10	pc25	pc50	pc75	pc90	pc95
6	2,45	3,14	3,76	5,21	6,02	7,3	8,32
6,1	2,78	3,5	4,24	5,68	6,63	8,02	9,05
6,2	3,13	3,88	4,73	6,17	7,26	8,75	9,79
6,3	3,49	4,27	5,23	6,68	7,91	9,49	10,54
6,4	3,87	4,68	5,75	7,2	8,56	10,25	11,3
6,5	4,27	5,1	6,28	7,73	9,23	11,01	12,07
6,6	4,68	5,55	6,82	8,29	9,9	11,78	12,85
7	6,48	7,46	9,13	10,63	12,73	14,97	16,07
7,1	6,97	7,98	9,75	11,26	13,47	15,79	16,9
7,2	7,48	8,52	10,37	11,9	14,22	16,63	17,74
7,3	8	9,07	11,01	12,55	14,98	17,47	18,59
7,4	8,54	9,63	11,66	13,22	15,76	18,32	19,46
7,5	9,1	10,22	12,33	13,91	16,54	19,19	20,33
7,6	9,67	10,81	13,01	14,61	17,34	20,06	21,21
8	12,11	13,36	15,86	17,55	20,65	23,66	24,85
8,1	12,76	14,04	16,61	18,32	21,51	24,58	25,78
8,2	13,49	14,8	17,45	19,19	22,46	25,61	26,82
8,3	14,11	15,44	18,14	19,92	23,26	26,46	27,68
8,4	14,8	16,16	18,93	20,73	24,15	27,42	28,65
8,5	15,52	16,9	19,73	21,57	25,05	28,38	29,63
8,6	16,25	17,65	20,55	22,41	25,97	29,36	30,61
9	19,33	20,82	23,94	25,95	29,76	33,37	34,66
9,1	20,14	21,66	24,83	26,88	30,73	34,39	35,7
9,2	20,96	22,51	25,72	27,81	31,72	35,43	36,75
9,3	21,8	23,37	26,63	28,77	32,72	36,48	37,81
9,4	22,66	24,25	27,56	29,73	33,74	37,54	38,88
9,5	23,54	25,14	28,49	30,71	34,76	38,6	39,96
9,6	24,43	26,06	29,45	31,71	35,8	39,68	41,05
10	28,14	29,86	33,38	35,85	40,06	44,09	45,52
10,1	29,11	30,85	34,4	36,92	41,16	45,22	46,66
10,2	30,1	31,85	35,43	38	42,27	46,36	47,81
10,3	31,1	32,87	36,48	39,1	43,39	47,51	48,97
10,5	32,63	34,43	38,07	40,78	45,09	49,25	50,74
10,5	33,15	34,96	38,61	41,35	45,66	49,84	51,33
10,6	34,2	36,03	39,7	42,5	46,82	51,02	52,52
11	38,55	40,46	44,18	47,23	51,56	55,84	57,4
11,1	39,68	41,6	45,33	48,45	52,78	57,07	58,65
11,2	40,83	42,76	46,5	49,68	54,01	58,31	59,91
11,3	41,99	43,94	47,68	50,93	55,24	59,56	61,17
11,4	43,16	45,13	48,88	52,2	56,5	60,83	62,45
11,5	44,36	46,34	50,09	53,47	57,76	62,1	63,74
11,6	45,56	47,57	51,31	54,77	59,04	63,38	65,03
12	50,56	52,62	56,33	60,09	64,26	68,61	70,33
12,1	51,85	53,93	57,62	61,46	65,59	69,94	71,68
12,2	53,15	55,24	58,93	62,85	66,94	71,28	73,04
12,3	54,47	56,58	60,24	64,25	68,3	72,64	74,41
12,4	55,81	57,93	61,57	65,66	69,67	74	75,79
12,5	57,16	59,29	62,92	67,09	71,05	75,37	77,18
12,6	58,53	60,68	64,27	68,53	72,45	76,76	78,58
13	64,16	66,36	69,84	74,45	78,15	82,39	84,29
13,1	65,61	67,82	71,27	75,97	79,6	83,83	85,74
13,2	67,07	69,29	72,71	77,5	81,07	85,27	87,2
13,3	68,55	70,78	74,16	79,05	82,55	86,73	88,68
13,4	70,05	72,29	75,62	80,61	84,04	88,19	90,16
13,5	71,56	73,81	77,1	82,19	85,54	89,67	91,65
13,6	73,09	75,35	78,6	83,78	87,05	91,16	93,16

Appendix 2: Crown Rump Length size chart
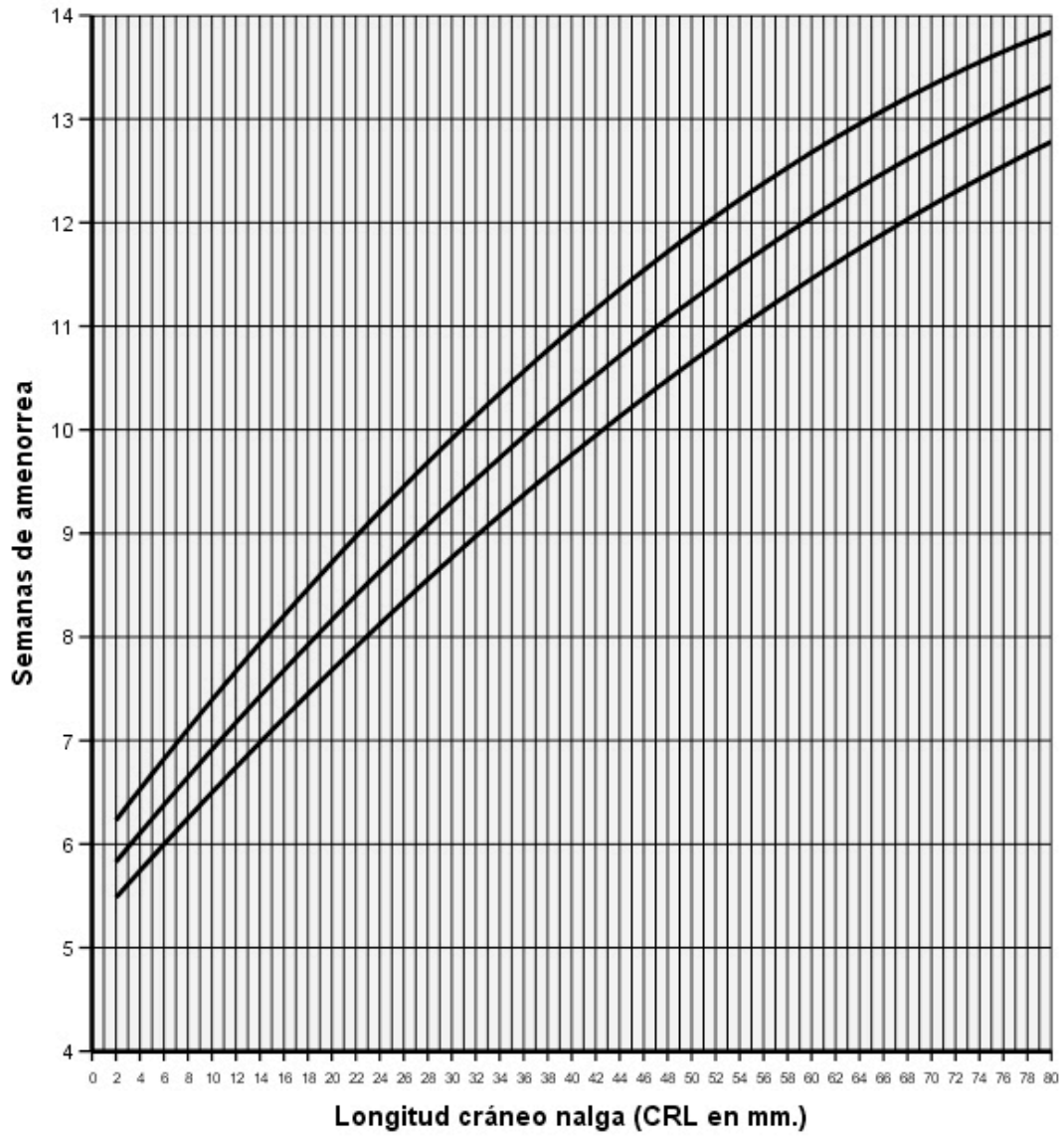
Appendix 3: *Biparietal Diameter dating table*

weeks	pc5	pc10	pc25	pc50	pc75	pc90	pc95
13	19	20	21	23	24	24	25
14	22	23	25	26	27	28	29
15	26	27	28	30	31	32	33
16	29	30	32	34	35	36	37
17	33	34	35	37	38	40	41
18	36	37	39	40	42	43	44
19	39	40	42	44	45	47	48
20	42	43	45	47	49	50	51
21	45	46	48	50	52	53	54
22	48	49	51	53	55	56	57
23	51	52	54	56	58	59	61
24	54	55	57	59	61	62	63
25	56	57	59	62	64	65	66
26	59	60	62	64	66	68	69
27	62	63	65	67	69	71	72
28	64	65	67	69	72	73	74
29	66	67	70	72	74	76	77
30	69	70	72	74	76	78	79
31	71	72	74	76	79	80	81
32	73	74	76	79	81	83	83
33	75	76	78	81	83	85	85
34	77	78	80	83	85	87	87
35	79	80	82	85	87	89	89
36	80	82	84	87	89	90	91
37	82	84	86	88	90	92	93
38	84	85	87	90	92	94	94
39	85	87	89	92	93	95	96
40	87	88	91	93	95	96	97
41	88	90	92	95	96	98	98

Appendix 4: *Abdominal Circumference size table*

weeks	pc5	pc10	pc25	pc50	pc75	pc90	pc95
17	92,39	95,87	100,99	105,87	113,18	118,8	126,7
18	102,98	106,58	112,11	117,72	125,24	131,13	139,03
19	113,4	117,14	123,06	129,37	137,11	143,29	151,18
20	123,66	127,54	133,85	140,81	148,78	155,26	163,15
21	133,76	137,78	144,47	152,04	160,25	167,06	174,96
22	143,7	147,87	154,93	163,06	171,52	178,67	186,59
23	153,48	157,81	165,23	173,87	182,59	190,11	198,05
24	163,1	167,59	175,36	184,48	193,46	201,37	209,34
25	172,55	177,21	185,32	194,88	204,13	212,44	220,46
26	181,85	186,68	195,12	205,07	214,61	223,34	231,4
27	190,99	196	204,75	215,05	224,88	234,06	242,17
28	199,96	205,16	214,22	224,82	234,95	244,6	252,77
29	208,77	214,17	223,53	234,39	244,83	254,96	263,19
30	217,42	223,02	232,67	243,75	254,51	265,14	273,45
31	225,91	231,72	241,64	252,9	263,98	275,14	283,53
32	234,24	240,26	250,45	261,84	273,26	284,96	293,44
33	242,41	248,65	259,09	270,58	282,34	294,6	303,17
34	250,42	256,88	267,57	279,1	291,22	304,07	312,74
35	258,27	264,96	275,89	287,42	299,9	313,35	322,13
36	265,95	272,88	284,03	295,53	308,38	322,45	331,35
37	273,48	280,65	292,02	303,43	316,66	331,38	340,39
38	280,84	288,26	299,84	311,13	324,74	340,12	349,27
39	288,04	295,72	307,49	318,61	332,63	348,69	357,97
40	295,08	303,02	314,98	325,89	340,31	357,07	366,5
41	301,96	310,17	322,3	332,96	347,79	365,28	374,86

Appendix 5: *Femur Length size table*

weeks	pc5	pc10	pc25	pc50	pc75	pc90	pc95
17	17,8	18,57	19,96	21,56	22,9	24,33	25,74
18	20,69	21,48	22,88	24,56	25,96	27,46	28,83
19	23,51	24,33	25,74	27,49	28,95	30,51	31,85
20	26,28	27,12	28,53	30,35	31,87	33,49	34,8
21	28,98	29,84	31,26	33,13	34,71	36,38	37,67
22	31,61	32,5	33,93	35,85	37,48	39,2	40,46
23	34,19	35,09	36,54	38,5	40,18	41,93	43,19
24	36,7	37,63	39,08	41,07	42,81	44,59	45,84
25	39,16	40,1	41,55	43,58	45,36	47,17	48,41
26	41,55	42,5	43,97	46,01	47,84	49,67	50,92
27	43,87	44,85	46,32	48,37	50,25	52,09	53,34
28	46,14	47,13	48,6	50,66	52,59	54,44	55,7
29	48,34	49,34	50,82	52,89	54,86	56,7	57,98
30	50,48	51,5	52,98	55,04	57,05	58,88	60,19
31	52,56	53,59	55,08	57,12	59,17	60,99	62,32
32	54,58	55,62	57,11	59,13	61,22	63,01	64,38
33	56,53	57,58	59,08	61,07	63,19	64,96	66,37
34	58,42	59,48	60,98	62,94	65,09	66,83	68,28
35	60,25	61,32	62,82	64,73	66,93	68,62	70,12
36	62,02	63,09	64,6	66,46	68,68	70,33	71,88
37	63,73	64,81	66,31	68,12	70,37	71,96	73,57
38	65,37	66,45	67,96	69,71	71,98	73,51	75,19
39	66,95	68,04	69,55	71,22	73,52	74,99	76,73
40	68,47	69,56	71,07	72,67	74,99	76,38	78,2
41	69,92	71,02	72,53	74,04	76,39	77,7	79,59

Appendix 6: *Example of Informed Consent*

FORMULARIO DE CONSENTIMIENTO INFORMADO

Yo,(nombre apellidos)…………………………………………………………………….

- ✓ He leído la hoja de información que se me ha entregado
- ✓ He podido hacer preguntas sobre el estudio
- ✓ He recibido respuestas satisfactorias a mis preguntas
- ✓ He recibido suficiente información sobre el estudio y la he comprendido
- ✓ He hablado con (nombre y apellido del investigador)

………………………………………….....................…………………..

- ✓ Comprendo que mi participación es voluntaria
- ✓ Cedo mis datos para posibles estudios futuros
- ✓ Comprendo que puedo retirarme del estudio

ƒ Cuando quiera

ƒ Sin tener que dar explicaciones

ƒ Sin que esto repercuta de ninguna manera en mis cuidados médicos

o Presto libremente mi conformidad a participar en el estudio y recibiré una

copia de éste documento

....

(fecha) (firma del paciente)

....

(fecha) (firma del investigador)