Universitat de Girona
Facultat de Medicina

FINAL DEGREE PROJECT

PlGF (placental growth factor) as a predictor of macrosomia at 3rd trimester in pregnancies complicated with diabetes.

A multicenter prospective cohort study

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1. ABSTRACT

Background: Fetal macrosomia, defined as birth weight above 95th percentile, is one of the most frequent complications suffered by fetuses of diabetic mothers, happening in up to 45% of these pregnancies. For the infant, macrosomia increases the risk of shoulder dystocia, clavicle fractures and brachial plexus injury and increases the rate of admissions to the neonatal intensive care unit. For the mother, the risks associated with macrosomia are cesarean delivery, postpartum hemorrhage and perineal lacerations. Macrosomic newborns of women with diabetes are at an increased risk of becoming overweight or obese at a young age (during adolescence) and are more likely to develop type II diabetes later in life. Ultrasound follow-up and glucose monitoring during pregnancy are the two tools used in current clinical practice to predict fetal macrosomia. The problem is that, sometimes, despite good ultrasound and glycemic control, diabetic women appear to have macrosomic fetuses. Therefore, new elements are necessary to be included in the prenatal follow-up of diabetic mothers to get a better prediction of fetal macrosomia and achieve better perinatal outcomes. Few studies have evaluated the association of higher PlGF (placental growth factor) levels in maternal blood with higher birth weight, particularly among those with preexisting diabetes.

Objective: The aim of this study is to determine the predictive value of PlGF levels in pregnant women with pregestational diabetes (PGDM) and gestational diabetes (GDM) for macrosomia during the second and third trimesters of pregnancy.

Design and methods: This is a multicenter observational prospective cohort study. A sample of 410 pregnant women will enter into the study through a non-probabilistic consecutive recruitment. Participants will be pregnant women with diabetes who will be included in two cohorts (A: pregnant women with GDM; B: pregnant women with PGDM) from gestational age between 24-27 weeks to the end of the gestation. We will perform two determinations of PlGF in maternal blood, one at the end of the second trimester and another at the end of third trimester. We will collect the macrosomia rate in each cohort to perform a statistical analysis and confirm the association between PlGF levels and macrosomia adjusting for all the co-variables.

Keywords: macrosomia, pregestational diabetes, gestational diabetes, PlGF.

2. ABBREVIATIONS

DM: diabetes mellitus
WHO: world health organization
GDM: gestational diabetes mellitus
PGDM: pregestational diabetes mellitus
IUGR: intrauterine growth restriction
LGA: large for gestational age
EFW: estimated fetal weight
AHT: arterial hypertension
DKA: diabetic ketoacidosis
BMI: body mass index
ACE: angiotensin-converting enzyme
ARB: angiotensin receptor blocker
OGTT: oral glucose tolerance test
IADPSG: international association of the diabetes and pregnancy study groups
GA: gestational age
WG: weeks of gestation
PE: preeclampsia
BPD: biparietal diameter
AC: abdominal circumference
FL: femur length
HJT: hospital Josep Trueta
CEIC: comité ético de investigación clínica

3. INTRODUCTION

3.1. CONCEPTS

Diabetes mellitus (DM) is defined as a metabolic disorder of multiple causes characterized by chronic hyperglycemia associated to alterations in the metabolism of carbohydrates, proteins and fats, which occurs as a result of defects in the secretion of insulin or its action or both. Chronic hyperglycemia, typical of DM, is associated with long-term lesions, dysfunctions or failures of various organs, especially eyes, kidneys, nerves, heart and blood vessels. Symptoms (thirst, polyuria, polyphagia, weight loss) may be present, but they are not specific and are often completely lacking. Various pathogenic processes are involved in the development of diabetes, from the autoimmune destruction of β cells until peripheral resistance to insulin action, although the basis is always the deficiency in the action of insulin in its target tissues (1).

The World Health Organization (WHO) estimates that more than 422 million people worldwide have DM. Most cases belong to one of the two main categories, type 1 and type 2 (2)

Type 1 DM represents 5-10% of all cases. This form of diabetes is caused by an absolute deficiency of insulin secretion, due to a destruction of β cells in the pancreas.
This form includes cases attributable autoimmune pathogenesis and some of unknown etiology, in which there is no evidence of autoimmunity, and which are classified as idiopathic DM 1. It usually occurs in childhood and adolescence, however, it can appear in any age (3).

Type 2 DM represents 90-95% of diabetes cases. This is caused by a combination of insulin resistance and an inadequate compensatory response, with insufficient secretion for such resistance. The risk of developing it increases, among other factors, with age, obesity and sedentary lifestyle. It usually starts progressively after the fourth decade of life, although in recent years there has been a notable increase in young people and even children (1).

Gestational diabetes mellitus (GDM) is carbohydrate intolerance of varying severity, which begins or is first diagnosed during pregnancy. Diabetics who become pregnant should not be included in this category. Unlike the other types of diabetes, gestational is not caused by lack of insulin, but by the effects on its resistance, usually occurs from the second and third trimester. The normal response to this situation is an increase in insulin secretion, when this does not occur, GD occurs (3).
The prevalence of GDM in Spain at 2019 was 32.4% (4).
GD often reverts to normal after delivery and complicates approximately 7% of all pregnancies. The clinical recognition of this situation is important, because these patients have an increased risk of fetal morbidity and mortality if they don't receive adequate treatment and because more than one half of them will develop type 2 diabetes in the next 25 years after delivery (1).

3.2. DIABETES IN PREGNANCY

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Diabetes is one of the most common metabolic complications of pregnancy and is associated with an increased risk of maternal and fetal morbidity and mortality.
Approximately 1% of all pregnant women have pregestational DM (PGDM) and up to 12% of cases will have GDM during pregnancy.

We classify diabetes in relation to pregnancy. More than 90% of cases of diabetes encountered during pregnancy are GDM, the rest are in a group called "pregestational diabetes mellitus" (PGDM). PGDM is all diabetes diagnosed before the onset of pregnancy, so it can be type 1 DM, type 2 DM or other specific types of DM (genetic defects of β cell function, genetic defects of insulin action, diseases of the exocrine pancreas, endocrinopathies, drugs or other chemicals…)

Historically, any time diabetes was diagnosed during pregnancy, it was considered gestational diabetes. However, if diabetes is diagnosed in the first trimester or early second trimester with the standard diagnostic criteria, it is considered pregestational diabetes.

Both types of diabetes in pregnancy are a risk factor for the development of complications in the mother and in the offspring. In the following lines, we will mention the complications associated with diabetes around pregnancy, and later we will give special importance to macrosomia, which is of interest in this project.

3.2.1. Impact of diabetes mellitus on pregnancy

- Urinary infections
- Vaginal candidiasis
- Polyhydramnios
- Hypertensive states of pregnancy
- Prematurity

3.2.2. Impact of diabetes mellitus on the fetus and the newborn

Just in PGDM
- Malformations and/or abortions (organogenesis period)
- Intrauterine growth restriction (IUGR)

Both types PGDM and GDM
- Macrosomia (dystocia, obstetric trauma and increased caesarean section)
- Risk of loss of fetal wellbeing before or intrapartum
- Hypertrophic cardiomyopathy
- Fetal immaturity that can manifest as respiratory distress syndrome or metabolic disorders (hypoglycemia, hypocalcemia, hyperbilirubinemia, polycythemia)
- Perinatal mortality

3.2.3. Maternal prognosis

The onset of DMG is a marker of prediabetes, given the frequency of subsequent development of type 2 DM and metabolic syndrome (associated dyslipidaemia, obesity and arterial hypertension). Occasionally the GDM is manifesting a decrease in pancreatic reserve secondary to autoimmune destruction of the β cells (latent type 1 DM), subsequently resulting in a type 1 DM.

3.2.4. Offspring prognosis

Children who during the intrauterine period have been exposed to a hyperglycemic metabolic environment have a higher risk of developing obesity, alterations of carbohydrate metabolism and metabolic syndrome in adulthood.

3.3. MACROSOMIA

3.3.1. Definition

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Fetal macrosomia is defined as birth weight above 95th percentile and is associated with several maternal and fetal complications.

The definition of macrosomia is complex. Traditionally we talk about fetal macrosomia when the newborn's weight at birth is >4000g or when the weight of the newborn is above 95th percentile for a given gestational age. The first definition only looks at birth weight, while the second also takes into account the gestational age of the newborn, so the last one is stricter.

Prenatal diagnosis of a fetal macrosomia is not simple. Ultrasound is the most widespread method to estimate fetal weight, but it is not an exact technique and although its reliability increases as pregnancy progresses, it loses precision in extreme weight values.

The estimated fetal weight (EFW) can be calculated from the second trimester ultrasound. When the EFW is above 95th percentile for a given gestacional age, the fetus is classified as "large for gestational age" (LGA).

Therefore, during the ultrasound follow-up we will talk about LGA and the term "macrosomia" will be reserved at birth (8).

DM is an important risk factor to macrosomia. Fetal macrosomia affect 12% of newborns of normal women and 15-45% of newborns of women with GD(9). It occurs in approximately 50% of pregnancies complicated with PGDM(10).

3.3.2. Pathophysiology

The pathophysiology of macrosomia can be explained based on Pedersen's hypothesis **(Figure 1)** of maternal hyperglycemia leading to fetal hyperinsulinemia and increased utilization of glucose and, hence, increased fetal adipose tissue. When maternal glycemic control is impaired and the maternal serum glucose level is high, the glucose crosses the placenta. However, the maternal-derived or exogenously administered insulin does not cross the placenta. As a result, in the second trimester, the fetal pancreas, which is now capable of secreting insulin, starts to respond to hyperglycemia and secrete insulin in an autonomous fashion regardless of glucose stimulation. This combination of hyperinsulinemia (insulin being a major anabolic hormone) and hyperglycemia (glucose being a major anabolic fuel) leads to an increase in the fat and protein stores of the fetus, resulting in macrosomia (9).

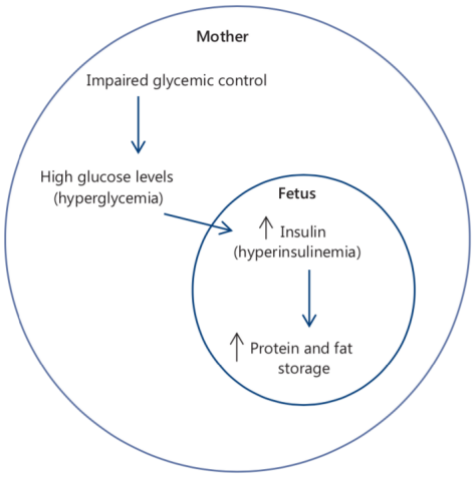

Figure 1. Results of maternal hyperglycemia modified according to Pedersen's hypothesis

While blood glucose during pregnancy is assuredly one of the pathways involved in fetal overgrowth, the inconsistency of studies associating blood glucose and fetal growth indicates that there are other factors at play. Despite good blood glucose control, women with diabetes appear to have large babies. As such, new factors need to be identified to better understand fetal overgrowth in the offspring of women with diabetes(10).

3.3.3. Risk factors

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A part from diabetes, there are other risk factors related to macrosomia (see **table 1**). We differentiate between constitutional (present before pregnancy) and gestational risk factors (they appear during pregnancy).

Constitutional	Gestational
▪ Previous child > 4000g ▪ Pregestational maternal BMI (obesity and overweight) ▪ Multiparity (>4) ▪ Ethnicity (African or Latin race) ▪ Maternal birth weight (>4000g) ▪ Maternal age <17y ▪ Previous diabetes ▪ Paternal obesity ▪ Hypertension ▪ Smoking habit	▪ Excessive weight gain during pregnancy (>16kg) ▪ Male sex ▪ Chronologically prolonged pregnancy ▪ Gestational diabetes

Table 1. Macrosomia risk factors

3.3.4. Macrosomia related complications

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3.3.4.1. Maternal Complications

If the baby is atypically large, vaginal birth will be more complicated. There is a risk of prolonged labor in which the fetus might be stuck in the birth canal, instrumental delivery (with forceps or vacuum) may be needed, and even unplanned or emergency caesarean section may be necessary. During birth, there is a greater risk of laceration and tear of the vaginal tissue than when the baby is of normal size, and the muscle between the vagina and the anus might tear (perineal tear).

There is also a high chance of uterine atony. The uterus muscle may not properly contract, resulting in heavy bleeding and postpartum haemorrhage. The risk of post-partum bleeding and genital tract injury was about 3–5 times higher in macrosomic deliveries. Besides, if the mother has had a previous caesarean section, there is a higher chance of uterus tear along the scar line of the previous surgery.

3.3.4.2. Fetal Complications

Immediate Complications

- Premature birth. Due to early induction of labor before 37 weeks of gestation and/or premature rupture of membranes, there is a risk of preterm delivery. Although all the necessary precautions are undertaken prior to induction of early labor, newborns are still under the risk of complications associated with prematurity, including difficulties in respiration and feeding, infection, jaundice, neonatal intensive care unit admission and perinatal death.
- Shoulder dystocia and Erb's palsy. One of the most serious complications of vaginal delivery in macrosomic babies is shoulder dystocia which is associated with birth trauma. Newborns with a birth weight of 4,500 g or more carry a 6 times higher risk of birth trauma, and the risk of brachial plexus injury is approximately 20 times higher when the birth weight is above 4,500 g.
- Hypoglycemia at birth. One of the most common metabolic disorders of the neonate of a diabetic mother is hypoglycemia. It occurs due to the hyperinsulinemia of the fetus in response to the maternal hyperglycemia in utero. Hypoglycemia can lead to more serious complications like severe central nervous system and cardiopulmonary disturbances. Major long-term sequelae include neurologic damage resulting in mental retardation, recurrent seizure activity, developmental delay and personality disorders.
- Neonatal jaundice. Factors which may account for jaundice are prematurity, impaired hepatic conjugation of bilirubin and increased enterohepatic circulation of bilirubin resulting from poor feeding. In macrosomia, neonates have a high oxygen demand causing increased erythropoiesis and, ultimately, polycythaemia. Therefore, when these cells break down, bilirubin (a byproduct of red blood cells) increases resulting in neonatal jaundice.

- Congenital anomalies. Heart defects and neural tube defects, such as spina bifida, are the most common types of birth defects. The high blood sugar level of women with diabetes can damage the developing organs of the fetus, leading to congenital anomalies.

Later Complications

- Childhood Obesity and Metabolic Syndrome. Many studies suggest that one of the reasons of childhood obesity is GDM. There has been evidence of fetal programming of later adiposity amongst offspring exposed to existing diabetes in utero. Offspring of diabetic mothers is also susceptible to the onset of metabolic syndromes such as increased blood pressure, hyperglycemia, obesity and abnormal cholesterol levels that occur together and increase the risk of heart disease, stroke and diabetes.

3.4. ASSISTANCE TO PREGNANT WOMEN WITH DIABETES

3.4.1. Pregestational diabetes

3.4.1.1. Preconception counseling

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Women with preexisting diabetes who are planning a pregnancy should ideally be managed beginning in preconception in a multidisciplinary clinic including an endocrinologist, maternal-fetal medicine specialist, registered dietitian nutritionist, and diabetes educator, when available.

The objective will be to determine, depending on the maternal complications secondary to diabetes, the risk involved in pregnancy and prevent and/or reduce the maternal and fetal complications with adequate control and medical care before conception.

There are very high risk situations, both for the mother and the fetus, which would make pregnancy unadvisable as long as they remain. These situations are:

- Levels of A1C> 10% (85.8mmol/mol).
- Severe kidney disease (plasma creatinine> 2mg/dl or proteinuria>3g/24h and/or AHT of difficult control).
- Ischemic heart disease.
- Severe proliferative retinopathy, with bad visual prognosis.
- Severe autonomic neuropathy.

The preconception care of women with diabetes should include the standard screenings and care recommended for all women planning pregnancy and also diabetes-specific counseling. Diabetes-specific counseling should include an explanation of the risks to mother and fetus related to pregnancy and the ways to reduce risk including glycemic goal setting, lifestyle management, and medical nutrition therapy. The most important

diabetes-specific component of preconception care is the attainment of glycemic goals prior to conception (see **table 2**). See **Annex 1. Preconception care for PGDM women** for all the details on elements of preconception care.

3.4.1.2. Control during pregnancy

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A. Metabolic control

Fasting and postprandial self-monitoring of blood glucose are recommended in both gestational diabetes mellitus and preexisting diabetes in pregnancy to achieve optimal glucose levels. Glucose targets are listed in **table 2**.

Fasting plasma glucose	70-95 mg/dL (3.9-5.3 mmol/L)
1h postprandial glucose	90-140 mg/dL (5-7.8 mmol/L)
2h postprandial glucose	<120 mg/dL (6.7 mmol/L)

Table 2. Glycemic targets in pregnancy

In patients with preexisting diabetes, glycemic targets are usually achieved through a combination of **medical nutrition therapy** and **insulin administration**. Patient follow-up should be carried out jointly by the obstetrician and the diabetologist. The frequency of visits will be adapted to the clinical situation of the pregnant woman, being advisable to take place every 2-4 weeks.

Medical nutrition therapy

We have to adapt the diet to the weight of the patient, the needs of pregnancy, the preferences of the woman and the insulin therapy scheme. The distribution of the macronutrients, in terms of global caloric intake, should keep the following percentages: carbohydrates (40-50%), proteins (20%) and fats (30-40%), with a predominance of monounsaturated.

Also recommend the practice of moderate daily physical exercise (more important in type 2 DM).

Glucose monitoring

Fasting and postprandial monitoring of blood glucose is recommended to achieve metabolic control in pregnant women with diabetes. Preprandial testing is also recommended when using insulin pumps or basal-bolus therapy so that premeal rapid-acting insulin dosage can be adjusted.

To perform home self-monitoring, capillary glycemia will be assessed by glucometer. It is advisable to perform 3 daily preprandial glycemias and 3 daily postprandial glycemias, with a nocturnal determination as needed.

Insulin

Insulin is the preferred agent for management of both type 1 and 2 diabetes in pregnancy.

Insulin physiology

Given that early pregnancy is a time of enhanced insulin sensitivity and lower glucose levels, many women with type 1 diabetes will have lower insulin requirements and increased risk for hypoglycemia. The situation rapidly reverses by approximately 16 weeks as insulin resistance increases exponentially during the second and early third trimesters to 2–3 times the preprandial requirement. The insulin requirement levels off toward the end of the third trimester with placental aging. A rapid reduction in insulin requirements can indicate the development of placental insufficiency. In women with normal pancreatic function, insulin production is sufficient to meet the challenge of this physiological insulin resistance and to maintain normal glucose levels. However, in women with diabetes, hyperglycemia occurs if treatment is not adjusted appropriately.

Insulin use

The physiology of pregnancy necessitates frequent titration of insulin to match changing requirements and underscores the importance of daily and frequent self-monitoring of blood glucose.
Due to the complexity of insulin management in pregnancy, referral to a specialized center offering team-based care is recommended if this resource is available.

A recent Cochrane systematic review was not able to recommend any specific insulin regimen over another for the treatment of diabetes in pregnancy. While many providers prefer insulin pumps in pregnancy, it is not clear that they are superior to multiple daily injections (12).

Regarding the type of insulin to use, human insulin is recommended; however, several studies support both the safety and efficacy of insulin analogs.

HbA1C in Pregnancy

The HbA1C test is a blood test that provides information about your average levels of blood glucose, over the past 3 months. The high limit of normality for HbA1C is between 5.7-5.9% (38.8-41mmol/mol).

In studies of pregnant women without preexisting diabetes, increasing HbA1C levels within the normal range are associated with adverse outcomes.
Observational studies in preexisting diabetes and pregnancy show the lowest rates of adverse fetal outcomes in association with HbA1C <6– 6.5% (42–48 mmol/mol) early in gestation.

In the second and third trimesters, A1C <6% (42 mmol/mol) has the lowest risk of LGA, preterm delivery, and preeclampsia.
Taking all of this into account, a target of <6% (42 mmol/mol) is optimal during pregnancy if it can be achieved without significant hypoglycemia.

Due to physiological increases in red blood cell turnover, HbA1C levels fall during normal pregnancy. Additionally, as HbA1C represents an integrated measure of glucose, it may not fully capture post-prandial hyperglycemia, which drives macrosomia. Thus, although HbA1C may be useful, it should be used as a secondary measure of glycemic control in pregnancy, after self-monitoring of blood glucose.

Given the alteration in red blood cell kinetics during pregnancy and physiological changes in glycemic parameters, HbA1C levels may need to be monitored more frequently than usual (e.g., monthly).

Type 1 Diabetes

Women with type 1 diabetes have an increased risk of hypoglycemia in the first trimester and, like all women, have altered counterregulatory response in pregnancy that may decrease hypoglycemia awareness. Education for patients and family members about the prevention, recognition, and treatment of hypoglycemia is important before, during, and after pregnancy to help to prevent and manage the risks of hypoglycemia.

Pregnancy is a ketogenic state, and women with type 1 diabetes, and to a lesser extent those with type 2 diabetes, are at risk for diabetic ketoacidosis (DKA) at lower blood glucose levels than in the nonpregnant state. Women with type 1 diabetes should be prescribed ketone strips and receive education on diabetic ketoacidosis prevention and detection. DKA carries a high risk of stillbirth.

Type 2 Diabetes

Type 2 diabetes is often associated with obesity. Recommended weight gain during pregnancy for overweight women is 15–25 lb and for obese women is 10–20 lb. There is no adequate data on optimal weight gain versus weight maintenance in women with a BMI >35 kg/m^2.

Glycemic control is often easier to achieve in women with type 2 diabetes than in those with type 1 diabetes but can require much higher doses of insulin, sometimes necessitating concentrated insulin formulations. As in type 1 diabetes, insulin requirements drop dramatically after delivery.

The risk for associated hypertension and other comorbidities may be as high or higher with type 2 diabetes as with type 1 diabetes, even if diabetes is better controlled and of shorter apparent duration.

B. Ophthalmic control

- Examination of the fundus: before the second half of pregnancy, around 28 weeks. In women with retinopathy, examinations are scheduled for an ophthalmologist's trial.
- Laser retinal photocoagulation if accuracy is considered.
- Avoid as much as possible the practice of retinal fluoresceingraphy.

C. Nephrological control

- Determination of urinary excretion of albumin in the first, second and third trimester of pregnancy.
- Stop treatment with ACE inhibitors, ARBs, statins and other potentially harmful drugs, if they had not been withdrawn in the preconception period. Blood pressure levels should try to stay below 130/80 mmHg.

D. Obstetric control

First visit

Goals:

- To confirm pregnancy and set gestational age.
- To assess embryo-fetal viability.
- To discard obstetric and/or associated gynecological pathology.

Method:

- Review of the medical history and obstetric-gynecological examination (uterine, cervical, adnexal and breast characteristics). Take for cytology if it has not been done in the preconception consultation.
- Ultrasound study in order to establish the chronology of pregnancy, to verify the vitality and normality of the embryo (or in the case of the fetus) and its location in the uterine cavity, as well as the characteristics of the annexes.

Follow-up visits

Goals:

- To monitor maternal wellness and discard associated complications.
- To assess the growth and fetal wellness, as well as the presence of possible complications.

Method:

- **Regular obstetric control**. Assessment of fetal heart rate, abdominal circumference, uterine height and fetal movements. Weight control and blood pressure.
- **Study of congenital anomalies**. They are subject to increased risk of malformations, and therefore should be subject to special attention.
- **Ultrasound monitoring:**

- Ultrasound between weeks 11 and 14, as in all pregnant women, to assess the viability, the number of fetuses, date gestation, calculate the risk of chromosomopathies and early morphological evaluation.
- It would be advisable to perform an early echocardiographic examination between 14-16 weeks, especially in pregnant women with diabetes who have an increased risk of malformations.
- Ultrasound at 20-22 weeks, for the screening of malformations, as in all pregnant women.
- Assess the performance of fetal echocardiography for the study of possible cardiomyopathy, usually between 28 and 32 weeks.

- Because pregnant women with diabetes do not have a higher risk of chromosomopathies, the same guidelines as in the general population will be followed in this regard.
- **Assessment of fetal growth and intrauterine environment.** Serial determination of fetal biometrics, volume of amniotic fluid and placental characteristics, by monthly ultrasound from 28-30 weeks.
- **Assessment of fetal wellbeing**: by cardiotocographic study, in each visit from 36-38 weeks, in patients with good control and in the absence of vasculopathy. In any case, the starting week and the cadence will depend on the maternal metabolic control and the state of the fetus.

Analytical determinations

Test	1st trimester	2nd trimester	3rd trimester
Blood group and Rh	+		
Indirect Coombs test	+	+ If Rh –	+ if Rh-
Syphilis and HIV serology	+		+ if not done or population at risk
Rubella serology	+	+ if antibodies-	+ if antibodies –
Blood count	+	+	+
Chemistry screen	+	+	+
HbA1c	monthly	monthly	monthly
Urine test	+	+	+
Urine culture	+	+	+
Hepatitis B serology	+		+ if not done or population at risk
Vaginal and rectal GBS culture			+
Thyroid hormones and antithyroid antibodies	advisable[a]	[a]	[a]

a If thyroid dysfunction and / or autoimmunity, the precise thyroid function controls for each entity will be performed throughout pregnancy.

Table 3. Recommended analytical determinations

End of gestation

With proper metabolic control and adequate monitoring of fetal wellness, pregnancy should be allowed to evolve until spontaneous onset of labor. From week 38, induction of labor in women with good obstetric conditions can be considered. If in the 40th week the delivery has not started, we will try to end the pregnancy.
In the remaining situations, when there is no guarantee of adequate follow-up and obstetric-diabetological control, we will try to end the pregnancy after week 37, or earlier if deemed necessary. If it is necessary to end gestation before the week 34 + 6,

corticosteroids should be administered to accelerate fetal lung maturation, considering the appropriate adaptation of insulin therapy.
In case of risk of loss of fetal well-being, the termination of pregnancy will be immediate.

Mode of delivery

The route of birth choice will be vaginal. Caesarean section indications are the same as for pregnant women without diabetes, except that the estimated fetal weight exceeds 4.500 g or there is a history of shoulder dystocia in a previous pregnancy.

3.4.2. Gestational diabetes

3.4.2.1. Diagnosis

(13)
The diagnosis and screening of GDM are current topics of debate. We have extracted the information to make this section from "the follow-up protocol for the pregnancy in Catalonia", as this is the guide that is currently used in clinical practice in Catalan hospitals. See **Figure 2** for a better understanding of this section.

As a definition, GDM is the diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation. However, there are some situations that will be descrived below, which require a screening before the 24 weeks of gestation.

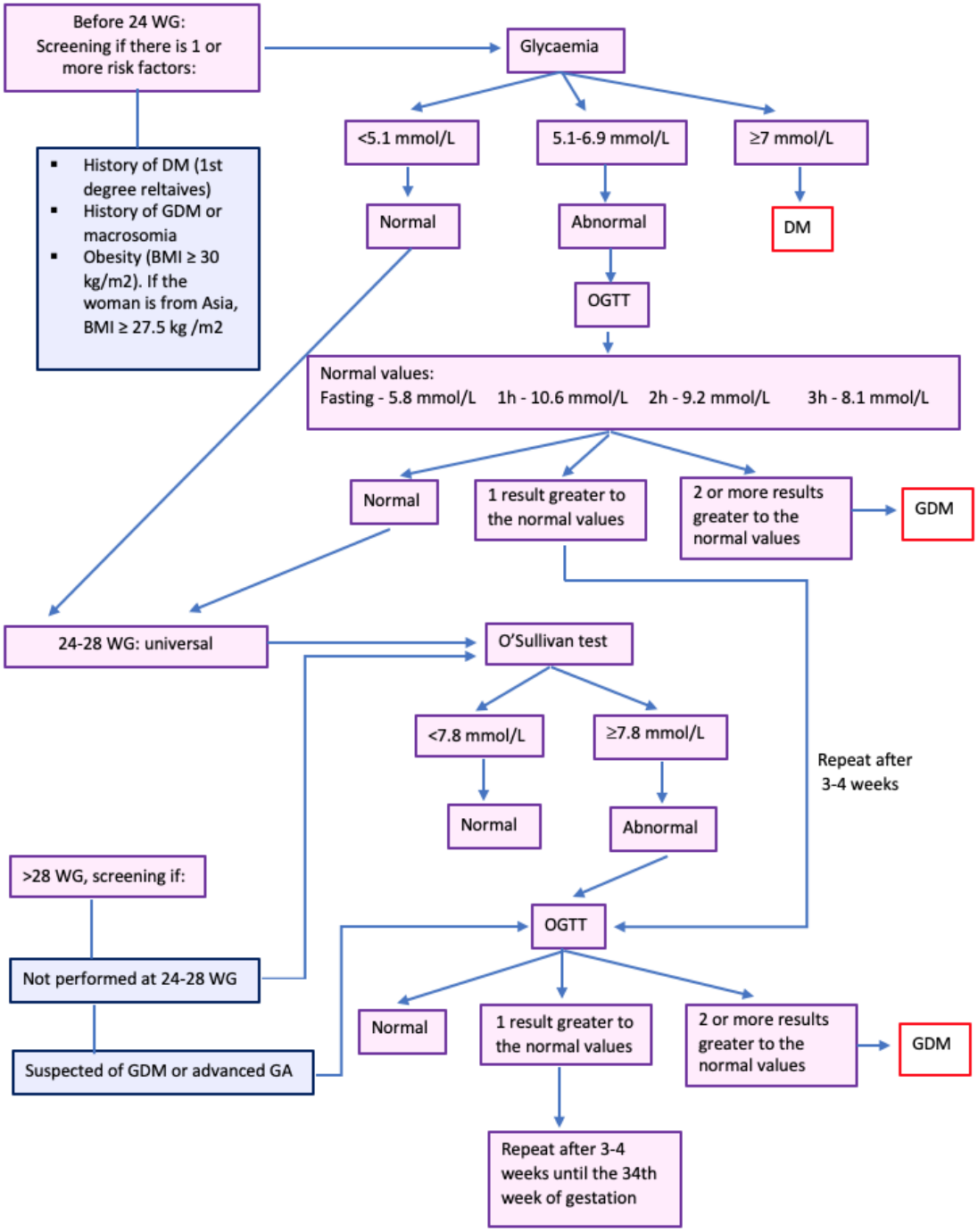

Figure 2. Screening of GDM

WG: weeks of gestation; GA: gestational age

Screening at the first visit (before 24 weeks of gestation)

Target population: Pregnant women at risk of DM/early GDM. We will use basal glucose determination only in those pregnant that complete one or more of these risk criteria:
- History of DM (first degree relatives)
- History of GDM or macrosomia
- Obesity (BMI ≥ 30 kg/m2). If the women is from Asia, BMI ≥ 27.5 kg /m2

Screening test: Basal plasma glycemia (see **table 4**)
Confirmation test: Oral glucose tolerance test (OGTT) (see **table 5**)

If the result is not diagnostic, it will be re-evaluated at 24-28 weeks with OGTT.

Screening at 24-28 weeks of gestation

Target population: universal; all pregnant women not previously diagnosed with diabetes.
Screening test: O'Sullivan test (see **table 6**)
Confirmation test: OGTT (see **table 5**)

Screening after 28 weeks of gestation

Target population: pregnant women not examined at 24-28 weeks or with clinical data compatible with gestational diabetes.
Screening test:

-For pregnant women not previously examined: O'Sullivan test (see **table 6**)
-Pregnant women with clinical data compatible with diabetes or not previously examined + advanced gestational age (provided that no impending birth is expected or reached): Required proceed directly to the confirmation test.

Confirmation test: OGTT (see **table 5**)

▪ It's fundamental that the pregnant is fasting during minimum 8 hours before blood collection. ▪ Assessment of laboratory results: -If it is <92 mg/dL (5.1 mmol/L) is considered normal and the next step will be the screening at 24-28 weeks with O'Sullivan test. -If it is 92-125 mg/dL (5.1-7 mmol/L) is considered abnormal and the next step is the confirmation test (OGTT). ▪ -If it is >125 mg/dL (7 mmol/L) is diagnosed with DM, no confirmation test is required, and the pregnant woman should be referred to specialist care immediately.

Table 4. Screening with basal plasma glycemia before 24 weeks of gestation

- Preparation for the OGTT
  - -Preparatory diet
  - -Fast for 8-10 hours
- Dose: 100g of glucose is administered in 25% solution (in 300cc of water) orally. The intake should be done in less than 5 minutes.
- The pregnant woman can't smoke or eat and have to rest until the extraction is performed.
- Venous blood extraction is performed before taking the glucose solution and after 1h, 2h and 3h of the intake.
- Assessment of laboratory results: we consider GDM confirmed in cases where we find 2 or more results equal to or greater than the following cut-off points:
  - -Fasting, before glucose solution: 105 mg/dL (5.8 mmol/L)
  - -After 1h: 190 mg/dL (10.6 mmol/L)
  - -After 2h: 165 mg/dL (9.2 mmol/L)
  - -After 3h: 145 mg/dL (8.1 mmol/L)

Table 5. Preparation and interpretation of oral glucose tolerance test

- Preparation for the O'Sullivan test
  - -Neither a fast nor a preparatory diet is required
  - -The test can be done in any moment of the day
- Dose: 50g of glucose is administered in 25% solution (in 200cc of water) orally. The intake should be done in less than 5 minutes.
- The pregnant woman can't smoke or eat and have to rest until the extraction is performed.
- Venous blood extraction is performed 1h after the start of glucose intake; it's not necessary a basal plasma glycemia determination.
- Assessment of the results of the O'Sullivan test:
  - -Negative test: less than 140 mg/dL (7.8 mmol/L)
  - -Positive test: equal to or greater than 140 mg/dL (7.8 mmol/L)
- The O'Sullivan test is not diagnostic of gestational diabetes, it only establishes suspicion. If the test is positive, a confirmatory test must be performed by the OGTT.

Table 6. Preparation and interpretation of screening with O'Sullivan test

GDM is often indicative of underlying β cells dysfunction, which confers marked increased risk for later development of diabetes, generally but not always type 2 diabetes, in the mother after delivery. As effective prevention interventions are available, women diagnosed with GDM should receive lifelong screening for prediabetes to allow interventions to reduce diabetes risk and for type 2 diabetes to allow treatment at the earliest possible time.

3.4.2.2. Control during pregnancy

(11)(6)

A. Metabolic control

The glucose targets in GDM are the same that the ones listed in PGDM (see **table 3**) and self-monitoring of blood glucose is recommended to achieve optimal glucose levels.

Lifestyle behaviour change is an essential component of management of GDM and may suffice for the treatment of many women. Insulin should be added if needed to achieve glycemic targets.

Lifestyle management

After diagnosis, treatment starts with medical **nutrition therapy**, **physical activity**, and **weight management** depending on pregestational weight and glucose monitoring aiming for the targets recommended.

The food plan should provide adequate calorie intake to promote fetal/neonatal and maternal health, achieve glycemic goals, and promote weight gain according to 2009 Institute of Medicine recommendations (14).

Pharmacologic therapy

Insulin is the first-line agent recommended for treatment of GDM. The insulin use aspects discussed in the PGDM section can be applied here.

Metformin and **sulfonylureas** (oral hypoglycemic drugs) should not be used as first-line drugs, as both cross the placenta to the fetus.

There are some women with GDM requiring medical therapy who, due to cost, language barriers, comprehension, or cultural influences, may not be able to use insulin safely or effectively in pregnancy. Oral agents may be an alternative in these women after a discussion of the known risks and the need for more long-term safety data in offspring. However, due to the potential for growth restriction or acidosis in the setting of placental insufficiency, metformin should not be used in women with hypertension, preeclampsia, or at risk for IUGR.

B. Obstetric control

Obstetric monitoring and control will be similar to that performed in normal pregnant women, with the following qualifications:

-Apart from the usual ultrasound studies of normal pregnancy, it is recommended to add one between the 28th and 32nd weeks to detect the presence of macrosomia.

-The end of gestation in these patients will depend on the severity of the condition. In patients with a mild DMG the ending criteria will not differ from those employed in the general obstetric population, while in those who we suspect an undiagnosed PGDM or who require insulin or have fetal macrosomia, the criteria of the PGDM will be applied.

3.5. PlGF

(15)(16)
Placental growth factor is a member of the vascular endothelial growth factor (VEGF) family and is predominantly expressed in the placenta, although it is also expressed at low levels in many other tissues, including the heart, lung, thyroid, liver, skeletal muscle and bone.

The human PlGF gene is located on chromosome 14q14 and encodes 4 isoforms of PlGF. The protein is secreted as a glycosylated homodimer and PlGF-1 and -3 are diffusible isoforms whereas PlGF-2 and PlGF-4 have heparin binding domains. Of these, PlGF-1 and -2 are the most abundant forms, and during pregnancy they are secreted in a strongly correlated manner, indicating a common regulation mechanism. The presence of a heparin binding domain suggests that PlGF-2 and -4 remain cell membrane-associated and act in an autocrine fashion, while the diffusible forms of PlGF probably affect targets in a paracrine manner.

PlGF is a **proangiogenic**, as it promotes angiogenesis by interacting with members of the VEGF receptor family found primarily on endothelial cells.
Angiogenesis is a vital process for embryonic development and growth and it is regulated by a complex interplay of a multitude of factors, including the VEGF family.
Circulating PlGF is prominently elevated in pregnancy with the source being the placenta. The function of PlGF in the placenta is likely to be in the promotion of development and maturation of the placental vascular system.

In a normal pregnancy, maternal PlGF levels have a nonlinear association with gestation. During the first trimester, the concentrations are low and they increase from week 11 to 12 onwards on a peak at week 30, after which it decreases (see Figure 1**Figure 3**).

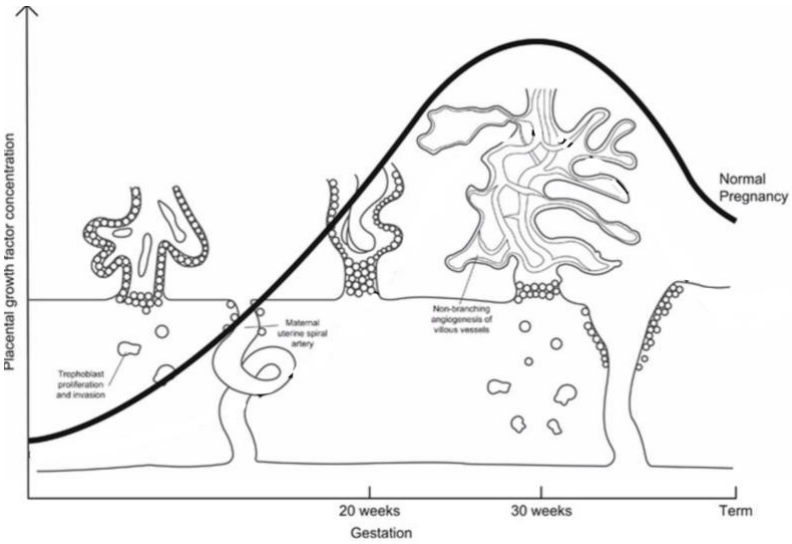

Figure 3. PlGF levels in a normal pregnancy

PlGF has been widely investigated as a biomarker for pregnancy complications associated with impaired placentation including **preeclampsia (PE)** and **intrauterine growth restriction (IUGR)** in the earlier duration of gestation (17)(18)(19).

In pregnancies complicated by preeclampsia, circulating levels of PlGF are decreased in response to the placental release of a soluble receptor (sFLT1) in response to oxidative and inflammatory stress (16). This soluble receptor has an "antiangiogenic" effect, because when it binds to PlGF reduces its bioavailability, therefore, the proangiogenic effect is also reduced.

Fetal growth depends on maternal, fetal and placental factors. Macrosomia, as a result of fetal overgrowth in pregnancies complicated by diabetes, is clearly associated with glycemic control in these patients (see Pathophysiology).
Specific follow-up during these patients' pregnancy in current clinical practice relies on glycemic control and ultrasound to evaluate fetal growth. Despite these controls, we still have a high incidence of macrosomia in pregnant women with diabetes, therefore, new elements are needed to predict this complication.

Determination of PlGF levels, as a biomarker in maternal blood, could have a predictive value for macrosomia. Higher PlGF levels may signal better vasculature, which could result in increased glucose transport in women with diabetes during hyperglycemic excursions, leading to excess glucose exposure to the offspring in utero (10).

Some studies have evaluated higher PlGF levels and their association with higher birth weight, particularly among those with preexisting diabetes ((20)(21)).

In this study, we want to see if high PlGF levels in maternal blood during the third trimester of pregnancy are a good predictor of macrosomia and this is independent of glycemic control in pregnant women with diabetes.

4. JUSTIFACTION

Prevention of fetal macrosomia in pregnancies complicated with diabetes mellitus (DM) would reduce serious perinatal morbidity and mortality.

Women with DM are at high risk for obstetric and perinatal complications; excessive fetal growth is a major driver of adverse pregnancy outcomes. Macrosomia, defined as birth weight above 95th percentile, is related to an increased risk for death in utero and shoulder systocia during labor, the latter related to asphyxia, clavicle fracture and/or Erbs palsy. During the neonatal period macrosomic infants are at increased risk for hypoglycemia, infant respiratory distress syndrome (IRDS), hyperbilirubinaemia and hypertrophic cardiomyopathy. A number of long-term population studies have shown that macrosomic newborns of women with diabetes have a higher risk to develop obesity and type 2 diabetes mellitus at young age.

Fetal macrosomia affect 12% of newborns of normal women and 15-45% of newborns of women with GD(9). It occurs in approximately 50% of pregnancies complicated with PGDM(10).

Hyperglycemia is considered a determinant of excessive fetal growth. Although the theory of maternal hyperglycemia and subsequent fetal hyperinsulinemia has long been thought to be the primary mechanism of fetal overgrowth in pregnancies affected by diabetes, as maternal glycemic control has generally improved in the past decades, a coinciding reduction in the rate of macrosomia has not been observed.

A number of studies (22)(23)(24)(25)(26)(27) have seen that the relationship between glycemic control during pregnancy and the occurrence of macrosomia is lower than might be expected. For example, in one study by Bethany M. Mulla, they saw that in in U.S the occurrence of LGA remains very high in women with Type 1 DM, despite the use of CGM and overall good glycemic control. Neither HbA1c nor glycemic variability predicted fetal overgrowth or birthweight (22). Similary, another study in The Netherlands by I.M.Evers, saw that despite apparent good glycemic control, the incidence of fetal macrosomia in a non-selected prospective nationwide cohort of 289 Type I diabetic women was very high (23).

Despite good blood glucose control, women with diabetes appear to have large babies. As such, new factors need to be identified to better understand fetal overgrowth in the offspring of women with diabetes.
Among other factors that may affect fetal growth is placental growth factor (PlGF), an angiogenic growth factor produced by the placenta(28). Higher PlGF levels may signal better vasculature, which could result in increased glucose transport in women with preexisting diabetes during hyperglycemic excursions, leading to excess glucose exposure to the offspring in utero. While several studies have shown that lower PlGF levels are associated with intrauterine growth restriction (29)(30)(17), few have evaluated higher PlGF levels and their association with higher birth weight, particularly among those with preexisting diabetes (20)(21).

Therefore, more elements are needed to improve the control of diabetic pregnant women, so that we can have better perinatal results. In this study we want to see if trimester-specific PlFG levels are a good predictor of fetal macrosomia and this is independent of glycemic control in pregnant women with diabetes. If this is the case, PlGF levels may be included as a useful marker during de follow-up of diabetic pregnant women.

5. HYPOTESIS

High levels of PlGF (placental growth factor) during the 3rd trimester of pregnancy are related to a higher incidence of macrosomia and this association is independent of glycemic control in the two groups of diabetic pregnant women (PGDM and GDM).

6. OBJECTIVES

In order to confirm our hypothesis, our **main objective** is:

1. To determine the independent association between glycemic control, PlGF levels and the incidence of macrosomia in pregnant women with PGDM and GDM.

Our **secondary objectives** are:

2. To estimate the incidence of macrosomia in general.

3. To estimate the incidence of macrosomia in women with GDM and in women with PGDM.

7. SUBJECTS AND METHODS

7.1. STUDY DESIGN

The study will be a multicenter observational prospective cohort. 3 hospitals will participate with the University Hospital Dr. Josep Trueta (HJT) as the coordinator center. These hospitals will be: Germans Trias i Pujol University Hospital, Vall d'Hebron University Hospital and Joan XXIII University Hospital.
We will follow two cohorts (A: pregnant women with GDM; B: pregnant women with PGDM) from GA between 24-27 weeks (at the time of performing the 2nd trimester blood test) to the end of the gestation.

7.2. STUDY POPULATION

The population of this study will be pregnant women with GDM (cohort A) and pregnant women with PGDM (cohort B) who undergo the pregnancy under the control of the selected hopitals in Catalonia and fulfill the inclusion and not the exclusion criteria (for each cohort).

7.3. SUBJECTS SELECTION

7.3.1. Inclusion criteria

Cohort A (GDM)

- Single pregnancy.
- Women older than 18 years old.
- Gestational age between 24-27 WG (at the time of performing the 2nd trimester blood test).
- Intact membranes.
- Normal umbilical morphology (two arteries and one vein).
- Diagnosis of gestational diabetes.

Cohort B (PGDM)

- Single pregnancy.
- Women older than 18 years old.
- Gestational age between 24-27 WG (at the time of performing the 2nd trimester blood test).
- Intact membrane.
- Normal umbilical morphology (two arteries and one vein).
- Diagnosis of pregestational diabetes.

7.3.2. Exclusion criteria

Cohort A (GDM)

- The presence of fetal congenital anomaly.
- Patient with a diagnosis such as oligohydramnios, preeclampsia and intrauterine growth retardation.
- Patient who used cigarette or alcohol during pregnancy.
- Women unable to give the informed consent.

Cohort B (PGDM)

- The presence of fetal congenital anomalies.
- Patients with a diagnosis such as oligohydramnios, pre-eclampsia and intrauterine growth retardation.
- Patients who used cigarettes or alcohol during pregnancy.
- Women unable to give the informed consent.

7.4. SAMPLING

7.4.1. Sample size

In a bilateral sample with a significance level (α) of 5% and a statistical power of 80% and foreseeing a moderate risk and a 5% of losses we will need 205 women in each subcohort.

We have assumed a moderate risk, that is, a not very high prevalence. The prevalence of macrosomia in PGDM women is of 50%; the prevalence of macrosomia in GDM women is between 15-45%. Using de 50% prevalence value, the sample size would have been too small, and, the value of GDM prevalence is too wide range for using it. Because of these last and because we don't know the prevalence in our study population, we decide to assume a moderate risk.

The computations were carried out with the Prof. Dr. Marc Sáez' software based on the "pwr" library of the free statistical environment R (version 3.6.2).

7.4.2. Sample collection

A consecutive non-probabilistic sampling will be followed in pregnant women who fulfill the inclusion criteria and not the exclusion criteria that undergo their pregnancy under the control of each selected center. Pregnant women will be recruited in the second trimester blood test as they appear for routine follow-up.

This study will be multicenter to recruit the whole sample and to have more external validity.

The centers that will be proposed to enrol this study are:

- Dr. Josep Trueta University Hospital.
- Germans Trias i Pujol University Hospital.
- Vall d'Hebron University Hospital.
- Joan XXIII University Hospital.

Dr. Josep Trueta University Hospital will be the coordinator center of this study, and one researcher will be assigned as the principal investigator in each collaborator hospital to set up good communication between all of them.

7.5. VARIABLES

7.5.1. Dependent variable

Macrosomia. It is a *dichotomic nominal qualitative variable*. It will be expressed by **yes** or **not**.

At the time of delivery, infants born to study participants will be weighed in grams and fetal macrosomia will be diagnosed if fetal weight is above 95th percentile.
We will use a calibrated pediatric balance beam scale, to measure the newborns' weight.

7.5.2. Independent variables

High levels of PlGF in maternal blood. It is a *dichotomic nominal qualitative variable*. It will be expressed by **yes** or **not**.

Blood samples will be collected at the 2nd and 3rd trimester blood tests and serum concentrations of PlGF will be measured using the Triage PlGF Test. The Quidel Triage PlGF Test is a single use fluorescence immunoassay device designed to determine the concentration of PlGF in EDTA anticoagulated plasma specimen. We will consider that the levels are high when they equal or exceed the 75th percentile (see **table 7**), based on the values of this marker obtained in other study (10) carried out in a population similar to ours and that we estimate that provide solid evidence to establish this cut-off point.

GA interval (weeks)	*N*	Mean GA	Percentiles of PlGF (pg/mL)						
			5th	10th	25th	50th	75th	90th	95th
20 + 0–23 + 6	242	21.7	76	98	147	218	324	500	604
24 + 0–28 + 6	238	26.1	141	173	291	417	662	937	1181
29 + 0–31 + 6	226	30.1	139	189	341	536	923	1380	1815
32 + 0–34 + 6	223	33.2	65	105	240	464	798	1330	1621
35 + 0 to 36 + 6	222	35.9	32	47	101	274	558	1010	1465
37 + 0–40 + 6	215	37.7	23	34	79	176	335	557	727

Table 7. Normal reference range percentiles of PlGF by GA interval (16)

Glycemic control:

HbA1C values, a *categorical qualitative variable*. We will measure this variable in three different points during pregnancy (1st trimester; 2nd trimester; 3rd trimester). The values in each determination (%) will be classified in these three groups:

- HbA1C ≤6.0% → excellent.
- HbA1C 6.1-7.0% → good.
- HbA1C>7.0% → not optimal.

7.5.3. Co-variables

7.5.3.1. Maternal co-variables

Maternal age at delivery, we will treat it like a *categorical qualitative variable*, divided in 3 groups:

- <25 years.
- 25-35 years.
- 35-40 years.
- >40 years.

Maternal weight (kg)
Maternal height (m)
} **BMI before pregnancy (kg/m^2)** = weight (kg) / height^2 (m^2)

We will treat the maternal BMI before pregnancy like a *categorical qualitative variable*, divided in 4 groups:

- <18,5→low weight.
- 18,5 – 24,9→normal weight.
- 25 – 29,9→overweight.
- ≥30→obesity.

Origin, treated like a *nominal qualitative variable*:

- Arabic/ North African.
- Caucasian.
- Gypsy.
- Indian/ Pakistani.
- Latin American.
- Sub-Saharan/ Caribbean.
- South East Asia/ Chinese

Socioeconomic status, an ordered polychotomous variable (social class **1** to social class **5**), built from the educational level and job, according to Domingo et al. (31).

Parity, a *dichotomic qualitative variable*:

- Nulliparous
- Multiparous

Maternal birth weight >4000g, a *dichotomic nominal qualitative variable*, expressed by **yes** or **not**.

Previous macrosomic child, a *dichotomic nominal qualitative variable*, expressed by **yes** or **not**.

7.5.3.2. Gestation co-variables

Gestational weight gain, we will treat it like a *categorical qualitative variable*, divided in 3 groups:

- Low.
- Normal.
- Excessive.

The weight of the pregnant woman is measured at each control visit (monthly) using a calibrated platform scale with the woman barefoot and in light clothing. To know the value of weight gain, we will look in the medical history for the weight at the first pregnancy visit and subtract it from the current weight.

To classify the weight gain during all pregnancy among the three categories mentioned above, we will rely on the recommendations of the Institute of Medicine (IOM) revised guidelines published in 2009 (32), summarized in **table 8.**

Pre-pregnancy BMI (kg/m²)	Total weight gain (kg)
Underweight (<18.5)	12.5-18
Normal weight (18.5-24.9)	11.5-16
Overweight (25-29.9)	7-11.5
Obese (≥30)	5-9

Table 8. Institute of Medicine 2009 recommendations for total amount of weight gain during pregnancy based on pre-pregnancy BMI. Adapted from (32).

Treatment with insulin, a *nominal qualitative variable*:

- Not use of insulin.
- Continuous subcutaneous insulin infusion (CSII).
- Treatment with multiple daily insulin injections (MIT).

Type of insulin, a *nominal qualitative variable*:

- Not use of insulin.
- Human regular insulin.
- Insulin-analogue.

Presence of polyhydramnios, a *dichotomic nominal qualitative variable*, expressed by **yes** or **not**.

Estimated fetal weight (EFW), a *continuous quantitative variable*. It will be expressed in grams (g). We will measure this variable in three different points during pregnancy (28 WG; 32 WG; 36 WG). We will determine EFW from the fetal biometric parameters (BPD, AC, FL) calculated during abdominal ultrasound examinations, using customized birth weight tables for Spanish population and Hadlock C formula.

Gestational age at delivery, a *continuous quantitative variable*. It will be expressed in weeks of gestation (WG).

Type of delivery, treated like a *nominal qualitative variable*:

- Eutoctic.
- Assisted vaginal delivery.
- Caesarean section.

Type of labour, treated like a *nominal qualitative variable*:

- Spontaneous labour.
- Induced labour.
- Scheduled caesarean section.

Another variable will be the **presence of complications during pregnancy**, listed below, as *qualitative nominal variables*, expressed as yes (presence) and not (absence).

- Preterm labour.
- Premature rupture of membranes.
- Chorioamnionitis.
- Umbilical cord prolapse.
- Placental abruption.
- Uterine rupture.
- Stillbirth

7.5.3.3. Newborn co-variables

Newborn sex, a *dichotomic nominal qualitative variable*, expressed by **male** or **female**.

Postnatal hypoglycemia, a *dichotomic nominal qualitative variable*, expressed by **yes** or **not**.

Cord blood metabolic acidosis, a *dichotomic nominal qualitative variable*, expressed by **yes** or **not**.

7.6. DATA COLLECTION

Before starting the protocol

In each of the involved centers we will assign a principal investigator (an obstetrician), and a co-investigator (a midwife) in order to control all the variables evaluated in the pregnant women involved in the study.

Enrolement procedures

The enrolement procedures will be different in each cohort:

Cohort A (GDM): As we explained in the "Gestational diabetes" section, all pregnant women not previously diagnosed with diabetes undergo the screening of GDM test (O'Sullivan and OGTT if indicated) at the same moment of 2nd trimester analytical determination (24-27 WG). The post-test visit, where the results of this and the analytical results are collected, will be the point at which the enrolement is carried out. If the pregnant woman is diagnosed with GDM and recognized as a possible candidate for the study, because she seems to accomplish the study criteria, the obstetrician or midwife (part of the work team) will ask her to take part of it giving the necessary information. If the patient wants to participate, the obstetrician or midwife will give an information sheet (see Annex 2. Protocol information sheet) explaining in an easy and understandable way how the study will be conducted. The informed consent (see Annex 3: Informed consent document) will be signed the same day of the first visit. The midwife will perform a blood extraction (10 mL) on the same visit, in order to determine de PlGF levels.

Cohort B (PGDM): Pregnant women with PGDM do the 2nd trimester analytical test, same as the rest pregnant women, between 24-27 WG. Just before performing the blood extraction, woman recognized as possible candidates for the study, will be asked to take part of it, following the same procedures explained above. If they want to participate and sign the informed consent, we will use part of the blood drawn in the 2nd analytical test to determine the PlGF levels. Therefore, in this cohort, we won't have to perform an "extra" blood extraction (unlike the cohort A).

Variables information

Except the determination of PlGF levels in maternal blood, the rest of variables we will include in the study are systematically collected in any pregnancy. Therefore, we will register them directly from the medical history to the database anonymously.

In both cohorts, once a woman is enroled in the study, a member of the research team (a midwife) will record the following information: HbA1C values at 1st trimester, BMI before pregnancy, origin, socioeconomic status, parity, maternal birth weight >4000g and previous macrosomic child.

In both cohorts, part of the blood drawn in the 3rd trimester analytical determination will be used to quantify PlGF levels at 3rd trimester.

The rest of variables will be registered after delivery by a member of the work team.

8. STATISTICAL ANALYSIS

8.1. DESCRIPTIVE ANALYSIS

8.1.1. Main objective

In each one of the subcohorts (A: pregnant women with GDM; B: pregnant women with PGDM), we will summarize the "presence or absence of macrosomia" in women with "high levels of PlGF/ not high levels of PlGF" and in women with "good glycemic control/ bad glycemic control" through proportions in a contingency table.

8.1.2. Secondary objective

In each one of the subcohorts, we will also summarize the presence or absence of macrosomia in general.

We will stratify these analyzes by the co-variables.

8.2. BIVARIATE INFERENCE

We will evaluate the association between macrosomia (yes/not), high levels of PlGF (yes/not) and glycemic control (good/bad) through chi-square contrast test. In case that in any of the cells of the table of contingency the expected counts were lower than 5, we will use the exact Fisher's test.
We will stratify these analyzes by the co-variables.

8.3. MULTIVARIATE ANALYSIS

8.3.1. Main objective

We will evaluate the association between macrosomia (yes/not), high levels of PlGF (yes/not) and glycemic control (good/bad) through logistic regressions, controlling for the co-variables.

We will also introduce in the logistic regression the interaction between the high levels of PlGF (yes/not) and glycemic control (good/bad). In this way, apart from checking our hypothesis that they are independent predictors, we will analyze whether one or the other are effect modifiers.

8.3.2. Secondary objectives

We will use the above logistic regressions, in this case without interaction, to estimate the incidence adjusted by the independent variables and the co-variables.

9. ETHICAL AND LEGAL ASPECTS

All the women included in the study will sign a "**Annex 3: Informed consent document**"

Each person of the research team and each hospital direction must sign a statement attesting to having read and approved the final protocol and agree with the national and international ethical aspects of research.

This protocol will be presented to the CEIC of the HJT. The objections performed by the CEIC will be considered and introduced.

This study will be conducted according to the requirements expressed in the *Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects* signed by the World Health Association in October 2013, and to *ministerial order SAS/3470/2009* defined in the current legislation in Spain related with the conduct of observational studies.

The processing of personal data required in this study, the personal data cession of all the patients and their confidentiality and communication will obey the *Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)* and the *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los derechos digitales.*
Patients data including names, surnames, telephones, addresses and clinical history information will remain anonymous after their introduction and processing into a database which will also be handled according to the mentioned Law and exclusively used for the development of the study. The data access will only be available for the research team. The access to this information for a third person will not be allowed.

10. STUDY LIMITATIONS

Study design: as an observational study, without a randomization of the subjects in the different categories of the independent variable, it is possible that there are some confounding factors that we have not included as co-variables.

Glycemic control: an important limitation of the study is how to assess glycemic control in the pregnant woman. We decided to use the HbA1C values in each trimester of pregnancy for determine if the control is excellent/good/not optimal, since this was the only way available. During pregnancy, as we explained in "HbA1C in Pregnancy" section, self-monitoring of blood glucose is performed in every diabetic pregnant woman and it's the main tool for metabolic control, while HbA1C is used a secondary measure of glycemic control.

HbA1C may not be the best measure of glycemic control. Multiple studies shown that people with diabetes can have considerable episodic hyperglycemia and maintain normal A1C values (33)(23)(34). Therefore, glucose variability and post-prandial hyper-glycemic episodes, which are not reflected in the HbA1C value, could be involved in macrosomia and not detected in our study. Finding a more accurate way to assess glycemic control would help in further studies.

PlGF: Although there are many studies that quantify the values of PlGF in maternal blood to relate it to various pregnancy complications, in clinical practice the values of this marker in pregnant women are not determined. This implies that there are no values established as altered, whether they have been studied what are the values in normal pregnancy. In our study we want to treat this variable as a qualitative variable (high levels yes or not). We have set the cut-off point at the 75th percentile, based on the values of this marker obtained in other study (10) carried out in a population similar to ours and that we estimate that provide solid evidence to establish this cut-off point.

PGDM: another limitation of our study is the lack of distinction between type-1 and type-2 PGDM pregnancies. In the cohort B (PGDM) we include women with type 1 or type 2 diabetes. Mechanisms involving birth weight may differ by type of diabetes, particularly due to other lifestyle and comorbid chronic disease factors that are involved that may differ between these two groups.

Sample size: Although we will probably have an enough sample size to represent our whole population, the lack of information on data on the prevalence of macrosomia in diabetic pregnant women in our population was a limitation when determining the sample size.

11. WORK PLAN

The research team involved in the study will be composed by:

- 1 general coordinator (GC)
- 4 obstetricians (O), one from each center
- 4 midwives (M), one from each center
- 1 statistical specialist (SS)

The study will be completed in 34 months. It's organized in the following stages:

Stage 1: Coordination (3 months)

Activity 1. Presentation and approval by the CEIC. The GC will be the responsible of this. The current protocol will be presented to the CEIC of HJT.

Activity 2. Determination of the collaborating professionals in the study. The GC will contact each participating center. All the hospitals who agree with this collaboration will receive a copy of the current protocol. One investigator/reference person per hospital will be determined, who will be an obstetrician.

Activity 3. Research team meeting. The investigators of each hospital will have a meeting in HJT to deal with any doubt or problem about the protocol or the organization before starting the recruitment.

Stage 2: Recruitment and data collection (21 months)

Activity 4. Consecutive and non-probabilistic recruitment.

Activity 5. Data collection. The SS will create a common database. All the women recruited will be listed encoded with a number, in order to avoid confidentiality problems, and all the data collected will be computerized.

Stage 3: Statistical analysis (3 months)

Activity 6. Statistical analysis will be done by the SS.

Stage 4: Interpretation of results (1 months)

Activity 7. The research team will redact conclusions from the obtained results.

Stage 5: Redaction, publication and dissemination of the research findings (6 months)

Activity 8. The corresponding articles will be written and research findings will be published.

12. CHRONOGRAM

WORK PLAN STAGES	ACTIVITIES	STAFF	2020												2021												2022									
			J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
STAGE 1	**Protocol approbation**	GC																																		
	Determination of the collaborators	GC																																		
	Research team meeting	ALL																																		
STAGE 2	**Recruitment**	O, M																																		
	Data collection	O, M																																		
STAGE 3	**Statistical analysis**	SS																																		
STAGE 4	**Interpretation of results**	O, M, SS																																		
STAGE 5	**Publications and dissemination of results**	GC																																		

13. BUDGET

TYPE OF COST	DESCRIPTION	UNIT COST	HOURS/UNITS	SUBTOTAL
Material	Triage PlGF test	10€	820 tests	8.200€
Staff	Statistical specialist	30€	100 hours	3.000€
Laboratory	Sample preservation and analysis			2.500€
Coordination	Meeting of the investigators of the different hospitals (travels, accommodation, diets)	250€	4 investigators	1.000€
Article publication	Revision, edition, formatting layout, graphic design and preparation of the digital metadata			2.000€
TOTAL				**16.700€**

14. IMPACT

Macrosomia is the most characteristic complication of fetuses of diabetic pregnant women. Macrosomic fetuses have a great impact in obstetric outcomes due to the risk of both maternal and fetal complications.

Nowadays, the prenatal diagnosis of macrosomia is mainly performed by ultrasound, estimating fetal weight. However, the scan assessment is not a precise technique, with errors in fetal weight estimation up to 10 and 20%.

In diabetic pregnant women with suspected macrosomia, the only way to avoid this fetal overgrowth is to optimize the metabolic control in order to prevent an excess of glucose transfer from the mother to the fetus. However, despite good glycemic control, many diabetic women appear to have large babies.

If the results obtained in this study are relevant and our hypothesis is validated, we could state that the determination of PlGF levels in maternal blood during pregnancy is useful for predicting fetal macrosomia. This could be a good tool that allows to advance in the decision making about adjusting insulin doses or schedule the end of gestation. Therefore, with the introduction of the PlGF in the clinical practice, we could improve perinatal outcomes and late complications of these children.

One way to apply the determination of this marker in clinical practice, assuming our hypothesis was confirmed, could be by constructing a risk equation that will help us to predict the incidence of macrosomia.

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16. ANNEXES

Annex 1. Preconception care for PGDM women

Preconception education should include:

☐ Comprehensive nutrition assessment and recommendations for:
- Overweight/obesity or underweight
- Meal planning
- Correction of dietary nutritional deficiencies
- Caffeine intake
- Safe food preparation technique

☐ Lifestyle recommendations for:
- Regular moderate exercise
- Avoidance of hyperthermia (hot tubs)
- Adequate sleep

☐ Comprehensive diabetes self-management education

☐ Counseling on diabetes in pregnancy per current standards, including: natural history of insulin resistance in pregnancy and postpartum; preconception glycemic targets; avoidance of DKA/severe hyperglycemia; avoidance of severe hypoglycemia; progression of retinopathy; PCOS (if applicable); fertility in patients with diabetes; genetics of diabetes; risks to pregnancy including miscarriage, still birth, congenital malformations, macrosomia, preterm labor and delivery, hypertensive disorders in pregnancy, etc.

☐ Supplementation
- Folic acid supplement (400 mg routine)
- Appropriate use of over-the-counter medications and supplements

Medical assessment and plan should include:

☐ General evaluation of overall health

☐ Evaluation of diabetes and its comorbidities and complications, including: DKA/severe hyperglycemia; severe hypoglycemia/hypoglycemic unawareness; barriers to care; comorbidities such as hyperlipidemia, hypertension, NAFLD, PCOS, and thyroid dysfunction; complications such as macrovascular disease, nephropathy, neuropathy (including autonomic bowel and bladder dysfunction), and retinopathy

☐ Evaluation of obstetric/gynecologic history, including history of: cesarean section, congenital malformations or fetal loss, current methods of contraception, hypertensive disorders of pregnancy, postpartum hemorrhage, preterm delivery, previous macrosomia, Rh incompatibility, and thrombotic events (DVT/PE)

☐ Review of current medications and appropriateness during pregnancy

Screening should include:

☐ Diabetes complications and comorbidities, including: comprehensive foot exam; comprehensive ophthalmologic exam; ECG in women starting at age 35 years who have cardiac signs/symptoms or risk factors, and if abnormal, further evaluation; lipid panel; serum creatinine; TSH; and urine protein-to-creatinine ratio

☐ Anemia

☐ Genetic carrier status (based on history):
- Cystic fibrosis
- Sickle cell anemia
- Tay-Sachs disease
- Thalassemia
- Others if indicated

☐ Infectious disease
Neisseria gonorrhea/Chlamydia trachomatis
- Hepatitis C
- HIV
- Pap smear
- Syphilis

Immunizations should include:
☐ Rubella
☐ Varicella
☐ Hepatitis B
☐ Influenza
☐ Others if indicated

Preconception plan should include:
☐ Nutrition and medication plan to achieve glycemic targets prior to conception, including appropriate implementation of monitoring, continuous glucose monitoring, and pump technology
☐ Contraceptive plan to prevent pregnancy until glycemic targets are achieved
☐ Management plan for general health, gynecologic concerns, comorbid conditions, or complications, if present, including: hypertension, nephropathy, retinopathy; Rh incompatibility; and thyroid dysfunction

Annex 2. Protocol information sheet

HOJA DE INFORMACIÓN PARA LA PACIENTE

Estimada paciente,

Nos dirigimos a usted para informarla de que se está realizando un estudio y nos gustaría solicitar su participación. Este estudio ha sido aprobado por el Comité Ético de Investigación Clínica del Hospital Josep Trueta.

Título del estudio: PlGF (placental growth factor) como predictor de macrosomia en el 3r trimestre en embarazos complicados con diabetes.

¿Por que se realiza este estudio y cual es su objetivo?

Las mujeres embarazadas que padecen diabetes mellitus tienen más probabilidades de que aparezcan complicaciones durante el periodo de gestación o durante el parto. La complicación más característica de las embarazadas diabéticas es la macrosomia. Tener un bebé macrosómico ("bebe grande") implica riesgos durante el parto para la madre (mayor posibilidad de parto instrumentado, cesárea, lesiones perineales, hemorragia postparto, rotura uterina) y para el feto (distocia de hombros, lesiones nerviosas y fracturas), y también riesgos después del parto para el bebé (hipoglicemia, asfixia, mayor frecuencia de ingresos, mayor mortalidad…). Para prevenir que el feto crezca demasiado, debemos controlar los niveles de azúcar para intentar tener siempre unos niveles óptimos y no transferir más de la cuenta a nuestro bebé. La única forma que tenemos de prever la macrosomia es el control ecográfico, con el que valoramos el crecimiento y el bienestar del feto durante la gestación. A pesar de un buen control glucémico y ecográfico, hay mujeres que siguen teniendo "bebes grandes", por lo que necesitamos encontrar otras herramientas para saber, durante el embarazo, que mujeres tienen más riesgo de macrosomia y así adecuar su seguimiento a ese riesgo. Una posible herramienta es la determinación de un marcador en sangre de la madre llamado "PlGF" (placental growth factor) durante la analítica del segundo y del tercer trimestre. Por todo esto, en el presente estudio tenemos como objetivo evaluar la utilidad de este marcador para prever la macrosomia en las mujeres embarazadas con diabetes.

¿Por que la invitamos a usted?

La invitamos a usted porque es una mujer que actualmente está embarazada y padece diabetes (pregestacional o gestacional), por lo que podría estar en riesgo de tener un bebé macrosómico.

¿Qué tengo que hacer si decido participar?

Recuerde que su participación es voluntaria y si decide no participar esto no afectará a su asistencia o a su relación con el investigador y su equipo. Los investigadores

recogerán datos personales, obstétricos, del seguimiento del embarazo actual y de su hijo/hija en el periodo neonatal, mediante la revisión de su historia clínica. Siempre de forma que estos datos sean anónimos.

Tratándose de un estudio observacional, no se realizará ninguna intervención adicional a las que se realizan de forma habitual en su centro. Tampoco se va a realizar ninguna prueba extraordinaria (exceptuando las diabéticas gestacionales, explicado en el siguiente párrafo), y por supuesto, no se dejará de hacer ninguna prevista. Tampoco requiere que usted tenga que realizar más visitas al hospital.

En el caso que usted haya sido diagnosticada de diabetes gestacional, le realizaremos una extracción adicional de 10 ml de sangre venosa mediante las técnicas habituales en el momento en que decida participar en el estudio y firme el consentimiento informado, con tal de determinar los valores del marcador en estudio.

¿Obtendré algún beneficio por participar?

Al tratarse de un estudio de investigación orientado a generar conocimiento no se espera que usted obtenga beneficio directo por participar ni va a recibir ninguna compensación económica por ello, si bien usted contribuirá al avance del conocimiento y al beneficio social.

¿Qué riesgos o inconvenientes tiene el participar?

Este es un estudio observacional, por lo tanto, el seguimiento de su embarazo no va a cambiar por participar en este estudio.

Muchas gracias por molestarse en leer esta hoja de información.
Si tiene alguna pregunta no dude en realizarla.
Si está de acuerdo en participar en este estudio, se le entregará una copia de esta hoja y del formulario de consentimiento informado.

Annex 3: Informed consent document

HOJA DE CONSENTIMIENTO INFORMADO

Yo, _____, con DNI/NIE _____ acepto voluntariamente participar en el estudio *"PlGF (placental growth factor) as a predictor of macrosomia at 3rd trimester in pregnancies complicated with diabetes"* y confirmo que:

- He sido informada adecuadamente por el Dr./Dra. _____
- He leído toda la información de la hoja de información para la paciente y he entendido el contenido.
- He preguntado cualquier duda relacionada con el estudio a los responsables y ellos me la han resuelto.
- He recibido una copia de la hoja de información para la paciente.
- Entiendo y acepto que mis datos se recogerán a partir de mis registros médicos, por los investigadores del estudio o por cualquier otra parte designada que esté involucrada en el estudio (médicos, autoridades reguladoras, comités de ética). Se mantendrá la confidencialidad de los datos facilitados y no se utilizarán en las publicaciones mi nombre ni otras características identificativas.
- Comprendo que mi participación es voluntaria y que podré tomar la decisión de abandonar mi participación comunicándoselo a mi médico cuando yo quiera, sin tener que dar ninguna explicación y sin que afecte a mi cuidado médico.

Firma de la paciente **Firma del investigador/a**

Lugar y fecha: _____, ____ de _____ del _____