Title: BEhavior of biomarkers during pregnanCy and lactatiOn through a biological Multi-paradigm modEl**.** BECOME study protocol.

Autores: PhD**.** Aguilar-Cordero, María José[1]; BSc. Baena-García, Laura[2]; BSc. Rodríguez-Blanque, Raquel[3]; BSc. Sánchez-García, Juan Carlos[3]; PhD. Capel-Tuñón, Manuel[4]; BSc. Sánchez-López, Antonio Manuel[3]; BECOME study group.

Correspondencia:

Dra. María José Aguilar Cordero

Departamento de Enfermería

Facultad de Ciencias de la Salud

Universidad de Granada

Av/ de la Ilustración s/n - CP: (18071)

Granada (España)

Teléfono: +34 657841751.

E-mail: **mariajaguilar@telefonica.net**

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1. PhD. Departamento de Enfermería. Facultad de Ciencias de la Salud. Universidad de Granada. Hospital Clínico San Cecilio de Granada (España) Grupo de Investigación CTS 367. Plan Andaluz de Investigación. Junta de Andalucía (España).
2. BSc. Grupo de Investigación CTS 367. Plan Andaluz de Investigación. Junta de Andalucía (España). Programa de Doctorado Medicina y salud Pública. Universidad de Granada (España)
3. BSc. Grupo de Investigación CTS 367. Plan Andaluz de Investigación. Junta de Andalucía (España). Departamento de Enfermería. Universidad de Granada (España)
4. PhD. Departamento de Lenguajes y Sistemas Informáticos. ETS Ingenierías Informática y Telecomunicación (ETSIIT). Universidad de Granada (España)

ABSTRACT

Background

Despite of advances in research, at the moment, various points related to the physiology of gestation and the etiology of severe diseases that can be developed in the course of it remain unknown. One of those aspects is the behavior of biomarkers (triglycerides, prolactin, glucose and cholesterol) during pregnancy, which experience a gradual increase in their levels until they reach the peak of hypertriglyceridemia, a few days before delivery. Several studies have reported that biomarkers experience a higher elevation in diabetic and obese pregnant women and in those women who suffer preeclampsia. The description of their behavior in different population of pregnant women (healthy women and women at risk) would identify the relation of these with some of the alterations that occurs more frequently during pregnancy.

Objective

The aim of this study is to develop a multi-paradigm biological model of systems to determine triglyceride, prolactin, glucose and cholesterol levels during pregnancy and its relation with lactogenesis in healthy and risk pregnant women.

Methods

A prospective cohort study will take place with women during pregnancy and lactation. Participating women will be divided into two groups. One group will be integrated by healthy women and the other group by pregnant women with a risk medical history. The personal, family and a detailed medical history will be collected in each group. A study of all the variables which influence the level of the mentioned biomarkers (triglycerides, cholesterol, glucose and prolactin) will be done.

The universe consists in 4,300 women, who constitute the historical average deliveries during the semester in the city of Granada (Spain). The sample collection will be made in medical office's pregnancy control in Granada's hospitals, in their respective health centers and during the second half of 2015. The sample will be stratified and probabilistic. Peculiarities of pregnant women will be taken into account when calculating the size of the study sample. This sample will be made up of 224 women who comply with the inclusion criteria and that have signed the informed consent.

To achieve the project objectives an organization comprising six theoretical and practical phases enabling the scientific development of the project.

During the first phase, the technical and administrative preparation of the project is constructed. Thereafter, the work is divided into two action areas which encompass the collection and data modeling.

The creation of a biological multi-paradigm computer simulation model of the levels of biomarkers in different months of pregnancy and in the various pathologies of pregnant women can be very effective to know the risks that involve high levels of lipids for the mother and for the baby.

KEYWORDS: Pregnancy; Gestational diabetes; Hypertension; Obesity; Biological multi-paradigm model; Biomarkers; Triglycerides; Cholesterol; Prolactin; Glucose.

INTRODUCTION

Pregnancy is considered as a physiological state that is part of the reproductive life of women. However, it causes several modifications in pregnant woman metabolism that in some cases can become in a risk factor to the development of pathologies.

Increase of motherly mortality is one of the aims of Development of Millennium of United Nations (United Nations General Assembly, 2000). In the tenth edition of Illness International Classification it is define motherly death when it takes place while the woman is pregnant or after 42 days of delivery, with independency of duration and location by causes related to pregnancy or getting worse by this, excluding those of accidental origin (WHO, 1992). Despite advances in progress of reduction of childhood and motherly mortality, it has been more slowly than the objective proposed by 2015 (Lozano et al., 2011). In 2013 almost 300 000 women of the entire world died by causes related to pregnancy and delivery (WHO, 2010). Hemorrhages and hypertensive disorders related to pregnancy are the main causes of motherly death in underdeveloped countries. However in developed countries anesthesia and cesarean complications are the most frequently causes (Khan et al, 2006).

In Spain were 425 390 deliveries in 2013, which means a reduction of 6,4 % with regard to the last year; it is the fifth consecutive year with decrease of birth rate. Besides, the middle age of motherly continues increasing, at this moment it is 32,2 years (INE, 2014).

A higher motherly age is related with an increase of the risk factors during pregnancy, even in women between 30 and 34 years old (Kenny et al., 2013).

That is why complications during pregnancy are still frequents also in developed countries and it is necessary a higher understanding of the physiological mechanism in this stage.

Otherwise, some of the pathologies that can be presented during pregnancy (gestational diabetes, hypertension and preeclampsia) has been related with an increased risk of experienced cardiovascular diseases in older ages, something that should be assess in futures considerations of women health (Bhattacharya et al., 2012; Fraser et al., 2012).

One of the most important biomarkers of cardiovascular diseases is the triglycerides levels which experience an important change during pregnancy. (Malhotra, Sethi & Arora, 2014) Triglycerides are lipids presents in blood flow. They are synthetized into the endoplasmic reticulum of almost the totality of human cells, especially in hepatocytes. High levels produce a narrowness of the arterial light, increasing the risk of hypertension, stroke and acute myocardial infarction (Miller et al, 2011).

During a normal pregnancy occurs a decrease of the lipid levels in the first weeks, followed by a progressive increase, especially after 12 weeks of pregnancy (Ghio et al., 2012).

This rise of the plasmatic concentrations of lipids flow follow an straight line until reach the top peak a few days before delivery (Wiznitzer et al., 2009). This rising is caused by the increase of estrogens levels and insulin resistance (Butte, 2000; Herrera & Ortega-Senovilla, 2010).

By the other hand, those changes in motherly metabolism are considered necessaries to the fetus development, considering that a rate of rising of triglycerides levels below 0,01 mg/dl per day is associated with an increased risk of abortion and premature delivery (Herrera et al., 2006; Laughon et al., 2014). However, this rise occurs in an accentuate way in women with obesity, diabetes and preeclampsia whose etiology remains unknown (Aragon-Charris et al., 2014; Charlton et al., 2014; Ephraim et al., 2014; Olmos et al., 2014; Scifres, Catov & Simhan, 2014).

The main part of studies analyzed triglycerides during the last weeks of pregnancy. In some studies it has been proved that a higher level of triglycerides during the first weeks of pregnancy is strongly related to weight in birth, an early development of insulin resistance and metabolic syndrome (Agius, Savona-Ventura & Vassallo, 2013; Gonzalez-Jimenez et al., 2014).

Between 24 and 48 hours after delivery, a decrease of the triglycerides levels occurs and it depends on the magnitude of the levels in blood during the final days of pregnancy (Hubel et al., 1996).

Lactation brings important benefits to the mother because it promotes more beneficial metabolic parameters (Gunderson, 2014) which are very important in those women with risk factors added. In spite of it, women with a risk pregnancy have lower rates of successful lactation even if they have more hospital support (Kozhimannil et al., 2014).

Determination of lipid profile in pregnancy is obtained through the trimestral blood test. However, its value continue considered unspecific because of the absence of standardized percentile tables for the evaluation of different kinds of population according gestational age. If so, they will allow determining those levels in which elevation is not physiological anymore, and it becomes in a risk factor to develop pathologies in pregnant woman (Ywaskewycz et al., 2010).

The purpose of this study is to carry out an informatics biological model that allow knowing the levels of triglycerides, prolactin, glucose and cholesterol during pregnancy and the relation with lactogenesis.

Lactogenesis includes three stages. Stage 1: It is initiated by pregnancy. Stage 2: begins with secretion of milk, two or three days before delivery coinciding with the decrease of progesterone levels and a high level of prolactin. Stage 3: Lactopoyesis, that includes the establishment and maintaining of the secretion of milk.

This last stage is defined by the suction reflex during lactation; mechanism begins with the stimulation of nipple sensitive receptors that provoke a response of the hypophysis to prolactin segregation. This hormone stimulates the synthesis of the proteins of milk and lactose, main components of the human milk. It will be discussed the synthesis of triglycerides and the levels of fatty acids specially Acetate and Butyrate also.

This model will be very helpful in obstetrics and gynecology's services because in a quickly and simple way provides information about the risk of a high level of triglycerides in woman during pregnancy and its relation with lactogenesis in the last days.

MULTIPARADIGM MODEL OF AN INTEGRATED ORGANIC SYSTEM

Biology of systems is a branch of knowledge and investigation relatively new whose main interest is the study of the interactions between the different components of biological systems, for that, they are described in an strict way, based on mathematics,

physics, biochemical, and biological models the interactions that provoke the operation and behavior of those systems.

The objective of the biology of Systems is to get a vision of organism as a net of genes, proteins and biochemical reactions that response in an accurate and realistic way to the conditions and stimulation of their natural environment (Benghazi Akhlaki et al., 2007).

This approach has been denominated a multiparadigm model of an integrate organic system to get the expected results, an expert in informatics must work in a cooperating way with biologist, medical people, physicians and mathematicians (Di Ventura et al., 2006; Hall, 2010).

It is a goal obtaining data that involve several organs in the human body that allow to make endocrines, metabolic, biochemical, genomic, and physiological changes who take places when a pregnant woman experienced the transition of pregnancy to lactation. These changes are caused and coordinated by several tissues and take place in multiples levels of organism understanding as an integrate organic system (Flatt, 2004).

Physiological and metabolic data of pregnant woman and the lactating mother that are analyzed allow to develop a complete database of the metabolic changes that occurs in the tissues during the transition of pregnancy to lactation and how this fact affects the synthesis of mother's milk.

To realize this project, it is created a multiparadigm model that describe in an accurate way interactions between physiological, biochemical and genetic process during transition of pregnancy to lactation in real time (Hall & Jordan, 2008).

HYPOTHESIS

The study of biomarkers (triglycerides, cholesterol, prolactin and glucose) in pregnant woman healthy and with risk associated; allow obtaining an analytic database to regulate the physiological, biochemical, metabolic and endocrine changes that are produced in pregnancy, lactation and after delivery. That makes possible to create a biological multiparadigm model that determine differences existent in the evolution of

biomarkers and their restore after delivery. It is related with lactogenesis, the kind of milk and its characteristics. Evaluation of diet and physical activity allow knowing healthy habits and its relation with lipid profile in pregnant woman.

SPECIFIC AND GENERAL OBJECTIVES

General objective 1: To analyze recording the behavior of biomarkers during pregnancy and after delivery in each women group (healthy and with risk). Specific objectives:

O1.1 To determine the nutritional state, the stress and physical activity levels in participating women.

O1.2 To assess overweight and obesity and their influence over biomarkers.

O1.3 To assess the relation between familial and sociodemographic variables with triglycerides, prolactin, glucose and cholesterol levels during pregnancy.

General objective 2: To relate biomarkers with lactogenesis in different groups of pregnancy (healthy and with risk).Specific objectives:

O2.1 To determine the influence of biomarkers in the moment of appearance of lactogenesis and composition of mother's milk.

O2.2 To analyze the relation between the pathologies of pregnant woman in risk and lactogenesis, comparing with healthy woman.

O2.3 To know the process of regulation of biomarkers after delivery.

General objective 3: To analyze the characteristics of the human milk in different groups of pregnant women (healthy and with risk) in relation to the biomarkers of mothers. Specific objectives:

O3.1 To evaluate specifically the biomarkers presents in the synthesis of fatty components in milk: triglycerides, total and free fatty acids, proteins and free aminoacids.

O3.2 To compare the rates of beginning and maintenance of lactation in risk women regarding healthy women, determining the causes of leavening it or the choice of artificial lactation.

O3.3 To describe the effects of lactation into the metabolic parameters and lipid profile of each group.

General objective 4: To develop computational models that describe with a different level of detail, the dynamic changes that occurs in human metabolism during transition of pregnancy to lactation. Specific objectives:

O4.1 To design continuous and non-continuous models that allow representing in a dynamics way the most relevant factors of metabolism of fats in blood and tissues of human body.

O4.2 To develop design patterns of software that can be useful to adjust different biological systems that allowed their use by researchers of Biology and Medicine.

General objective 5: To confirm *in silici* (through a computational simulation) the causes of changes in biomarkers and the delates in the synthesis process of fats in mammary glands that take place at the beginning of lactation. Specific objectives:

O5.1 To confirm and validating the utility of the developed models in project to predict changes in fats synthesis that occurs in the beginning of lactation.

O5.2 To schedule software components for different implementation platforms that can be combined and used to confirm the hypothesis about the behavior of others biological systems.

MATERIALS & METHODS

It is to carry out a prospective cohort study with women during pregnancy and lactation. Participating women were divided into two groups. The first one is composed by healthy women and the second one by women with a risk history (obesity, hypertension and diabetes). It is taken in each group the familiar and personal history and a detail medical history. It is analyzed the possible variables that influence the level of biomarkers.

Sample

The universe is composed by 4300 women that constitute the historical average of deliveries during a semester in the city of Granada (Spain). The research is projected over women that attend to the pregnancy control consultation into Granada's hospitals during the second semester of 2015. The sample is probabilistic and stratified. To calculate the size of the sample they were considered the characteristics of pregnant women that to be formed by 224 women that comply with the inclusion criteria and that sign the informed consent.

Exclusion criteria

- Impossibility to participate in the research.
- Restrictive pulmonary disease.
- Cardiac pathology with hemodynamic repercussion.
- Familiar hyperlipidemia.
- Multiple pregnancies.
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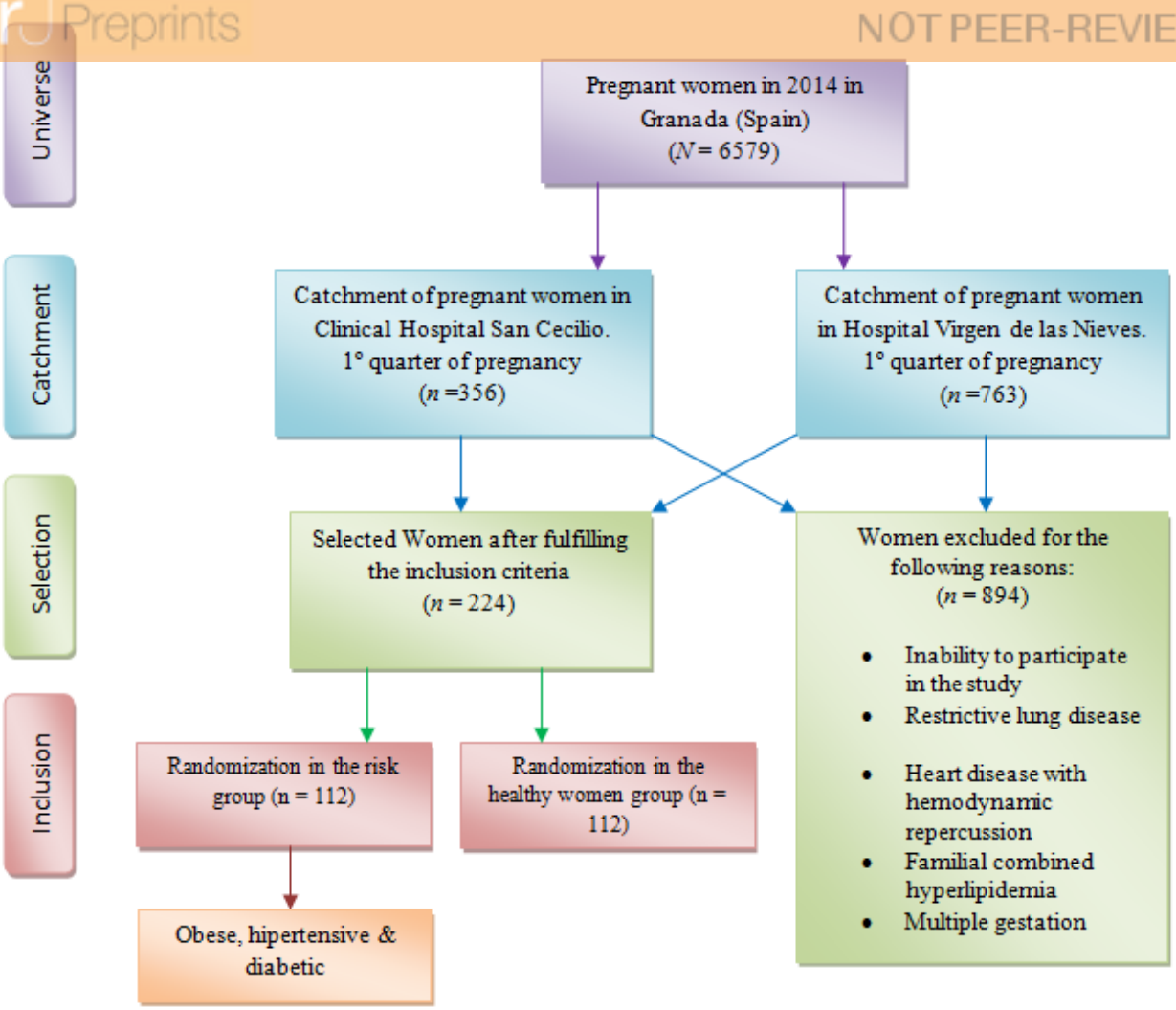
Inclusion criteria

- Pregnant women healthy and with risk that want participate in a voluntary way in the research and sign the informed consent.

Figure 1: *Diagram of collection of sample*:



Variables

Dependent variables:

Biomarkers (triglycerides levels and other parameters related to lipid and glucose metabolism: total cholesterol, HDL, lipoproteins, glucose tolerance, creatine, apolipoproteins) during pregnancy and lactation.

Independent variables:

- Nutritional assessment
- Use of contraception methods.
- Risk factors (stress, toxic habits, personal and familiar history).
- Socio-demographics (profession, age, number of pregnancies, educational level).

-Obesity, Diabetes and Motherly Hypertension.

METHODOLOGY

To carry out the objective of the research it is proposed and organization that includes 6 theoretical/practical stages that allowed the scientific development. During the first stage a technical and administrative preparation is carry out and after this the work is divided into two lines of performance:

1) Collection data stage: in this stage it is contemplated a prospective cohort study where the samples of pregnant women are collected.
2) Adjusted data stage: this stage includes three aspects that ended with generation of a biological multiparadigm model.

In those stages a continuous collaboration is kept because the interchange of information and collection of data during a cohort prospective study is essential to development of biological models.

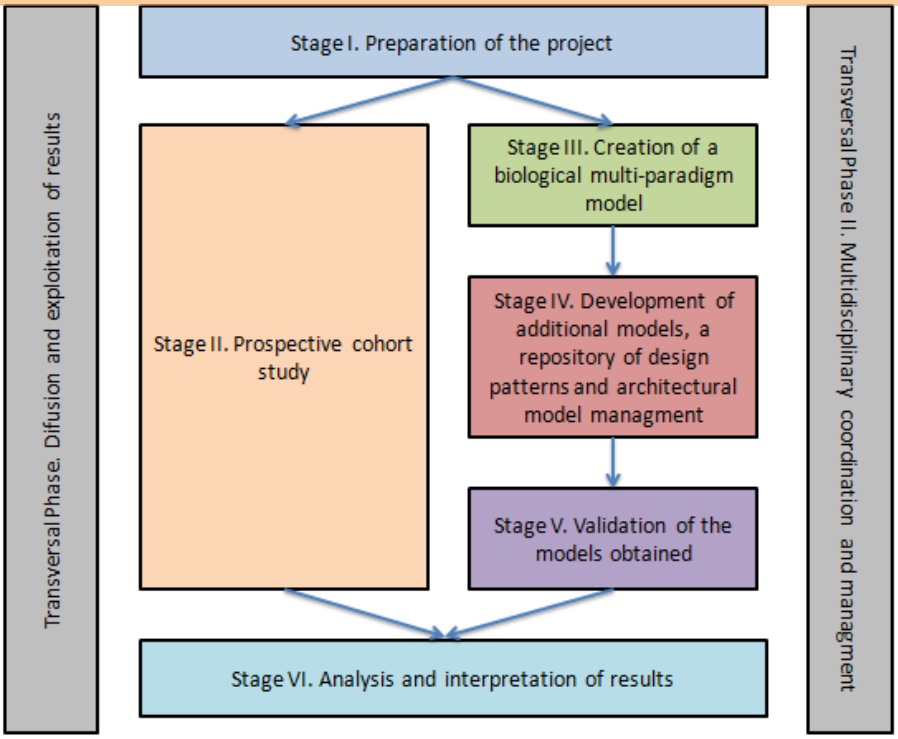
After the finalization of these stages, the last task consists in the interpretation of the results where the experts can confirm the hypothesis trough a developed model.

By the other hand, there are considered two additional transversal stages dedicated to the follow of evolution and management of the project, and the publication of the results obtained during the implementation of the project.

Following each stage is detail in picture below.

Each stage includes tasks that indicate the activities to perform and the instruments thatare used to the evaluation of each one.

Figure 2: *Methodology in stages of Project.*



Project stages and instruments of evaluation

Stage I: Preparation of the project.

In this stage it is prepare the environment to implement the research in women during pregnancy and lactation. The tasks are the followings:

Task 1.1 Training staff.

A formation course of 40 hours is carried out. The aim is to acquire knowledge about pregnancy, lipid behavior, human milk and the biological multiparadigm model.

Task 1.2 Acquisition and preparation of the material.

Acquisition of material used by taking biological samples is carry out by a specialized laboratory that provides reactive to obtain the biomarkers and their posterior analysis. With the samples a database is performed.

Stage II: A prospective cohort study.

In this stage the study is carry out in population of pregnant women included in the research, since 12 weeks of gestation until 6 months after delivery.

Task 2.1 Pregnant women recruitment.

The pregnant women are recruiting in Granada's hospitals according inclusion criteria, the project is explained and if they agree on participate sign the informed consent.

The calculation of the size of the necessary sample will be as follow: to get a power of 80 % in the detection of differences when contrasting hypothesis trough an X^2 test bilateral for two independents samples with a level of signification of 5 % and assuming that the difference of lipid profile levels should be at least of 20 %.

Task 2.2 Clinical history and nutritional evaluation.

In the first interview to the pregnant woman and after the sign of the informed consent the medical record is performed. Nutritional evaluation is carry out through a validate survey that was published in 2012 by our research team (González Jiménez et al., 2012).

Bioelectrical impedance was also performed. In clinical history socio demographics variables personal and familiars were collected and anxiety and depressive test were performed (Snaith & Zigmond, 1983).

Task 2.3 Laboratory test in pregnant women.

Laboratory test were performed to pregnant women during the first, second and thirst quarter.

First quarter: 8-12 weeks of pregnancy. Complete blood count, Rh group, Coombs test, Serology and urine culture.

Second quarter: 24-28 weeks of pregnancy. O'Sullivan test, Coombs test, complete blood count.

Thirst quarter: 35-37 weeks of pregnancy. Complete blood count, Coagulation study, Streptococcus B.

Task 2.4 Collection of samples. Laboratory test of the mother.

Each pregnant woman is trained to obtain their own lipid profile and they receive an agenda where there is indicated the date of the test with a weekly frequency.

A lipid profile is performed in both groups of pregnant women, a test that measure the lipids or fats in blood such as total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It also includes the prolactin levels. This collection of samples begins in the 20 weeks and ended 20 days after delivery. In figure number 2 there are included all the controls to pregnant women, the time of the test and the kind of alimentation in the week of the sample. Since the 37 week the lipid profile is taken each 72 hours and then daily when an increase of the values occurs and it's followed until 20 days after delivery.

Table 1. Protocol of registration of the level of biomarkers and nutritional evaluation.
It is described the week of pregnancy of the pregnant woman, the day and the hour of the test and the kind of food consumed the day before.

Week of pregnancy	Day and hour	Triglycerides, prolactin, glucose and cholesterol level	Breakfast during the week	Lunch during the week	Dinner during the week	Drinks
20	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread ,oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
24	Tuesday 9:00	TGL: Prol: Glucose: HDL	Bread ,oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and	Soup, vegetables, salad, fish	Water, soft drink,

		cholesterol: LDL cholesterol:		vegetables.		milk, coffee, tea, juice
28	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread ,oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
30	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread ,oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
31	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread ,oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
32	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
33	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice

34	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
35	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
36	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
37	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
38- 42	Tuesday 9:00	TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea, juice
20 days after delivery		TGL: Prol: Glucose: HDL cholesterol: LDL cholesterol:	Bread, oil, ham, tomato, bread roll	Meat, fish, fruit, legumes and vegetables.	Soup, vegetables, salad, fish	Water, soft drink, milk, coffee, tea,

						juice

Description of the reagents

Measures during this stage are performed with the reagent disk Piccolo®, using the blood analyzer Piccolo express™. It is designed for using in quantitative determination "in vitro" of the total cholesterol, High Density Lipoproteins and triglycerides in a clinic laboratory (Scafoglieri et al., 2012; Barrett et al., 2014).

Obtaining and preparing of samples.

To determine the values of total cholesterol, HDL, LDL and triglycerides the samples should be taken after 8 or 12 hours without eating.

The test begins after 10 minutes of transferring the sample to the disk. The levels of normality have been stablished to the following biomarkers:

Total cholesterol: under 200 mg/dl. LDL cholesterol: 40-60 mg/dl, values under 40 mg/dl indicate a higher risk of cardiovascular disease.

Prolactin: 10- 209 ng/ml.

Triglycerides: under 150 mg/dl, with a high limit between 155 and 199 mg/dl and higher between 200 and 499 mg/dl. During pregnancy it is recommended to keep it under 300 mg/dl.

Gestational diabetes: it is diagnose when in the O'Sullivan test the values are equal or superior to the following: blood sugar 105 mg/dl; 1 hour,190 mg/dl; 2 hours, 165 mg/dl and 3 hours, 145 mg/dl.

Task 2.5 Collection of samples: mother's milk.

It is about to follow the evolution of the process of lactation and the quality of mother's milk. In each group of study three samples are collected: colostrum (2 to 6 days after delivery); transition milk (7 to 21 days after delivery) and maturing milk (after 21 days of delivery).

Lactogenesis is the process that begins with milk secretion. It is stablished between 24 hours and the sixth day after delivery and is a consequence of the action of prolactin and the low estrogenic level. Before delivery, PRL levels increase but the estrogens of placenta blocked the secretion of glands. The maintaining of lactation requires a continuous suction of the nipple. The estrogenic fall after delivery allows unblock or mammary tissue.

Task 2.6 Following of lactation during the first six months.

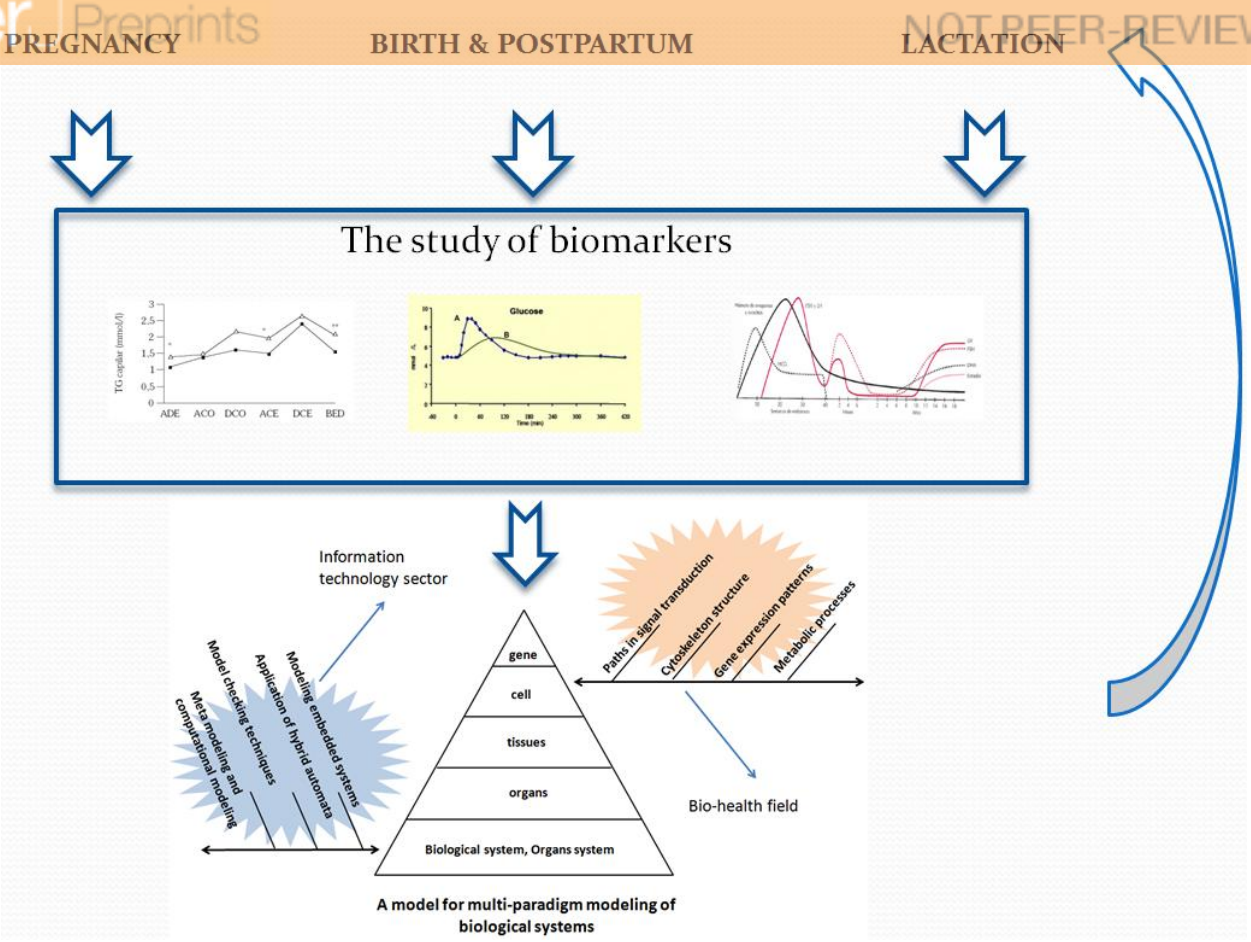
According the WHO it is recommended exclusive lactation during the first six months and with other food during two years. During the following of mothers, six workshops are performed about lactation and its importance.

STAGE III. Creation of a biological multiparadigm model.

With this protocol it is hoped to reflex graphically through a multiparadgim model, the levels of triglycerides, cholesterol, glucose and prolactin and their influence in the beginning of lactation and the synthesis of mother's milk. This allow to adjust physiological, genomic, metabolic and biochemical changes that experienced a mother when begin lactation. These changes are coordinated in multiples tissues and in different levels of organism that work as an integrated biological system.

The figure 3 describe an example of a multiparadigm model of biological systems of biomarkers.

Figure 3. In the diagram it is described a multiparadigm model that includes all the areas of development of a biological system from organs to genes.



Task 3.1: Definition of a biological model.

The purpose of this task consist in characterize the biochemical variables that influence in a biological/mathematical model that describe the dynamic of biomarkers and other factors at the beginning of lactation and taken in consideration the role that played estrogens and progesterone regulating the activity of human placental lactogen (HPL).

Task 3.2: Development of a simulator of the model with a graphic that allow performing continuous and discontinuous blocks in complex system of control in real time.

The model obtained in the previous task should be adapted to an environment of control system simulation with characteristics in real time, besides a simulator of the model with the graphic tool to select (HyVisual, MatLab). This is ended with compilation of initial clinical data of pregnant woman to perform graphics of biomarkers in different tissues.

Task 3.3: Statistical and biophysical and analysis of pregnant women data.

In this stage it is carry out a compilation, preparation, description and visualization of obtained data according the variables of interest to research. Data model using in inferences, proving and future prognosis are created.

Stage IV. Development of additional models, design patterns and architecture of models management.

In this stage and non-continuous models of control (stochastic process, Petri net) will be developed in discreet time of the dynamic of triglycerides in different tissues during the last weeks of pregnancy and the beginning of lactation.

Task 4.1 Development of specific non-continuous- models (discrete time).

During this task different models are studied and valued: stochastic process, queuing networks, Petri net, until select the most adequate model. Besides, it is necessary a preliminary evaluation of performance and costs. Finally the selected modes are implemented.

Task 4.2 Development in a repository of patterns of design and software components.

With this information it is developed a minimal, compositional and consistent group that keep the quality of requirements of simulation graphic tools of biological process.

Task 4.3 Implementation of models with automatic hybrids.

It is about performance a change of biomarkers in pregnant woman when she has hypertriglyceridemia, a few days before delivery. Software tools (HyVisual) of automatic hybrids were used with this purpose to obtain evolution graphics of different concentration of biochemical parameters, integrating differential equations models with a high level of detail.

Task 4.4 Software architecture of management and importation of models **(**GIM-EE**).**

In this last task a software architecture and language of architectonic description of metamodeling is designed (AMDL) it is also defined the rules to score and representing models and the software tool based on architecture GIM-EE is implemented.

Stage V: Validation of obtained multiparadigm model .

In this stage the aim is to validate all models of metabolism of fats during the last quarter of gestation and the beginning of lactation in pregnant women. Two methods are implemented: the use of advanced techniques of model checking to determine if functional requirement relative to biomarkers dynamic are satisfied for each one model and also the nonfunctional relatives to temporal restriction of each change of stage (gestation, hypertriglyceridemia, lactation). In second place with simulation of a visual tool (HyVisual) that obtains the graphic results using the same models but implemented in automatic hybrids.

Stage VI: Analysis and interpretation of results.

In pregnant women levels of triglycerides are irregular with increases during the first and the thirst quarter and a new increase before delivery. The analysis of the results allows analyze biomarkers in pregnant women, determining the behavior in different groups of women and the regulation after delivery. It is studied their relation with lactogenesis and the quality of human milk to determine their influence in development of lactation. All these with a graphic representation in healthy women and women with associated risk.

Task 6.1 Statistical analysis of getting results.

Statistical analysis to correlate biomarkers in healthy women and women with risk and their relation with nutritional state, physical activity and life style.

Task 6.2 Studying of regulation of lipid profile after delivery in both groups.

Pregnant women are valued since 20 weeks of gestation until 20 days after delivery. Previous days before delivery occurs and abrupt increase of triglycerides levels, that's why the aim is study the normalization of those levels in both groups of pregnant women. It is also analyzed the influence of biomarkers in milk quality and duration of lactation.

CHRONOLOGICAL SEQUENCE

The research includes the fallowing stages: (Figure 4)

- Stage 0. Bibliographical research and preparation of the study: 3 months.

- Stage 1. Recruitment. 12 weeks of pregancy 12, First echography. Project information. Informed consent: 2 months.

- Stage 2. 12 – 20 weeks of pregnancy. Initial evaluation. Medical history. Nutritional assessment. Physical activity and stress level: 2 months.

- Stage 3. 20 weeks of pregnancy: explaining the procedure to puncture the reactive strip. Beginning to collect samples to know the lipid profile and prolactin level: 4 months.

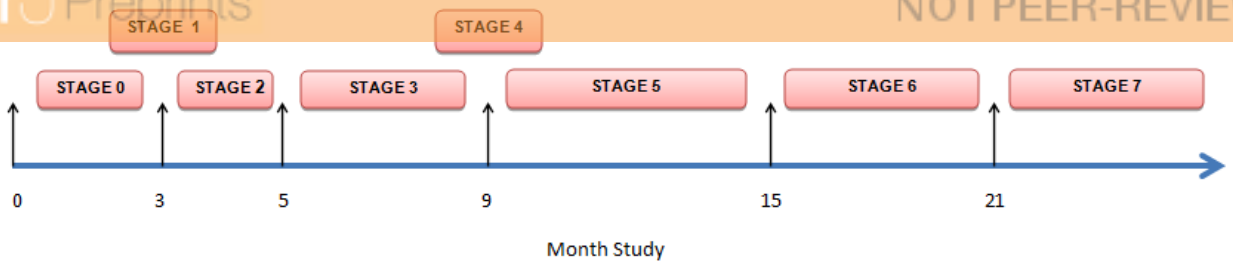
-Stage 4. 37 weeks of pregnancy: to develop tests each 72 hours until get the peak of triglycerides levels when daily extraction start.

-Stage 5. First week after delivery. Determining the lipid profile.To collect samples of mother's milk to evaluate makers during the synthesis of fatty acids.

-Stage 6. Following of lactation during 6 months after delivery.

-Stage 7. Interpretation and publication of the results: 6 months.

Figure 4: *Chronological sequence*



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571

572 **ETHICAL ASPECTS DURING INVESTIGATION**

573 The welfare and respect to the privacy of patients that participate in the investigation is
574 responsibility of the research and it is necessary the approval of the Ethical Committee.
575 It is developed the document of the informed consent and finally it is mentioned the
576 ethical regulation proposed by the Committee of investigation and Clinical Trial in
577 Helsinki Declaration of 1964 and review in Fortaleza, Brazil in 2013.

578

579 **LIMITATION OF PROJECT. RISK TABLE.**

580 During the development of the project it may present risk situation that should be
581 considered:

582 **Table 2.** Following the possible risks and the contingency plan is described.

583

Risk	Probability	Impact	Contingency plan
Since 24 weeks healthy women becomes diabetics.	5%	Low	According the calculation of the sample "n" should be 86 and 112 solutions have been taken to solve the possible risks.
Since 24 weeks healthy women becomes hypertensive.	6%	Meddle	According the size of the sample "n" should be 86 and 112 solutions have been taken to solve the possible risks.
Leaving of the research by women.	10%	High	A 20 % of women to solve problems were added to the "n" calculatedin the sample.

Premature delivery (from 24 week to 37 weeks of gestation)	8%	High	Women with premature deliveries are evaluated until the moment of delivery just like the other group to watch the behavior of biomarkers in previous days of delivery. they are also analyzed the lipid profile and composition of colostrum, transition milk and maturing milk.
Biological model do not represent reality	15%	High	It will be find techniques of alternative representation that allow solving problems of the research.

Women that have changed of clinical situation during the study are evaluated in an independent way, as a new additional group and the obtained results are compared with the other two groups.

DISCUSSION

The change in metabolism of pregnant woman is necessaries to the maintaining of pregnancy and for a correct fetal development. One of the parameters affected is the level of triglycerides that experienced an initial decrease, followed by a progressive increase until the moment of delivery. Through the present review the relation with some of the most frequent pathologies during pregnancy was determined and the increase of triglycerides is higher than a normal pregnancy.

In this context the biological model can have a special relevance, allowing the follow of the changes in biomarkers and ended appointed those situation that become in predictors of a potential pathological situation. In this way interventions will be carry out more quickly and a better primary prevention.

It is necessary to emphasize the role of lactation in the normalization of metabolic parameters after delivery. This aspect should be taken in consideration to provide advice and support to pregnant woman.

Finally, it is important emphasize in the importance of the creation of a mathematical model with computer simulators of the level of biomarkers during the different months of pregnancy and the different pathologies of a pregnant woman.

This informatics tools will be very effective to know the risk of the alteration of the level of biomarkers for the health of the mother and the baby. The prevention of these alterations is basic to decrease the risk during and after delivery.

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