STANDARDIZATION OF AN EFFICIENT EXPERIMENTAL GESTATIONAL DIABETES PROTOCOL

PADRONIZAÇÃO DE UM PROTOCOLO EXPERIMENTAL EFICIENTE DE DIABETES GESTACIONAL

ESTANDARIZACIÓN DE UN PROTOCOLO EFICAZ DE DIABETES GESTACIONAL EXPERIMENTAL

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ABSTRACT
Diabetes mellitus (DM) is characterized by a dysfunction in insulin secretion and/or action which causes hyperglycemia. When the disease occurs during pregnancy, it is called gestational diabetes mellitus (GDM), and hyperglycemia affects the fetus's development. The present study aimed to standardize an efficient model of GDM induction in Wistar rats using streptozotocin (STZ). Therefore, the animals are placed for mating overnight, and the next day the presence of sperm is verified in the vaginal wash, indicating the first day of gestation (G1), and after five days (G5), the pregnant rats were induced to diabetes, intraperitoneally, using STZ, at a dose of 50 mg/kg. The animals were weighed, and their blood glucose levels were measured (pre-mating, G7, and G17), being considered diabetics when blood glucose ≥ 200 mg/dL. After birth, the puppies were evaluated for the number of individuals, gender, and stillbirths. The blood of mothers was collected to assess oxidative damage caused by diabetes. The results showed efficacy in the use of the alternative dye gentian violet for sperm and cell

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identification. Weight gain, hyperglycemia, and the presence of oxidative damage showed an efficient GDM induction for 55.6% of total animals, considered a successful protocol superior to those commonly observed by those researchers in the GDM study area. Also, we observed a higher number of stillbirths in the GDM group, confirming data from the literature that characterize the hyperglycemic environment as hostile to the development of the fetus.

**Keywords:** Standardization; gestational diabetes; hyperglycemia; gestation.

**RESUMO**

O diabetes mellitus (DM) é caracterizado por uma disfunção na secreção e/ou ação da insulina que causa hiperiglicemia. Quando a doença ocorre durante a gravidez, é chamada de diabetes mellitus gestacional (DMG), e a hiperiglicemia afeta o desenvolvimento do feto. O presente estudo teve como objetivo padronizar um modelo eficiente de indução de DMG em ratos Wistar utilizando estreptozotocina (STZ). Portanto, os animais são colocados para acasalamento durante a noite, e no dia seguinte é verificada a presença de espermatozoides no lavado vaginal, indicando o primeiro dia de gestação (G1), e após cinco dias (G5), as ratas prenhes foram induzidas ao diabetes, por via intraperitoneal, utilizando STZ, na dose de 50 mg/kg. Os animais foram pesados e mensurados os níveis de glicemia (pré-acasalamento, em G7 e G17), sendo considerados diabéticas quando glicemia ≥ 200 mg/dL. Após o nascimento, os filhotes foram avaliados quanto ao número de indivíduos, sexo e natimortos. O sangue das mães foi coletado para avaliar o dano oxidativo causado pelo diabetes. Os resultados mostraram eficácia no uso do corante alternativo violeta genciana para identificação de espermatozoides e células. O ganho de peso, a hiperiglicemia e a presença de dano oxidativo mostraram uma indução eficiente do DMG em 55,6% do total de animais, considerado um protocolo de sucesso superior aos comumente observados pelos pesquisadores da área de estudo do DMG. Além disso, observamos maior número de natimortos no grupo com DMG, confirmando dados da literatura que caracterizam o ambiente hiperiglicêmico como hostil ao desenvolvimento do feto.

**Palavras-chave:** Padronização; diabetes gestacional; hiperiglicemia; gestação.

**RESUMEN**

La diabetes mellitus (DM) se caracteriza por una disfunción en la secreción y/o acción de la insulina que provoca hiperiglicemia. Cuando la enfermedad aparece durante el embarazo, se denomina diabetes mellitus gestacional (DMG), y la hiperiglicemia afecta al desarrollo del feto. El presente estudio tenía como objetivo estandarizar un modelo eficaz de inducción de DMG en ratas Wistar utilizando estreptozotocina (STZ). Para ello, los animales se colocan para el apareamiento durante la noche, y al día siguiente se verifica la presencia de espermatozoides en el lavado vaginal, lo que indica el primer día de gestación (G1), y al cabo de cinco días (G5), se indujo la diabetes a las ratas gestantes, por vía intraperitoneal, utilizando STZ, a una dosis de 50 mg/kg. Se pesaron los animales y se midieron sus niveles de glucosa en sangre (antes del apareamiento, G7 y G17), considerándose diabéticos cuando la glucosa en sangre ≥ 200 mg/dL. Tras el nacimiento, se evaluó el número de individuos, el sexo y los nacidos muertos de los cachorros. Se recogió sangre de las madres para evaluar el daño oxidativo causado por la diabetes. Los resultados mostraron eficacia en el uso del colorante alternativo violeta de genciana para la identificación de espermatozoides y células. El aumento de peso, la hiperiglicemia y la presencia de daño oxidativo mostraron una inducción eficaz de la DMG para el 55,6% del total de animales, considerándose un protocolo exitoso superior a los observados habitualmente por los investigadores de la zona de estudio de la DMG. Además, observamos un mayor número de mortinatos en el grupo con DMG, confirmando datos de la literatura que caracterizan el ambiente hiperiglicémico como hostil para el desarrollo del feto.
Palabras clave: Normalización; diabetes gestacional; hiperglucemia; gestación.

1. Introduction

Gestational diabetes mellitus (GDM) is characterized by glucose intolerance that begins during pregnancy. Its worldwide prevalence is 15.2% among pregnant women (Sun et al., 2022), it generally resolves postpartum but returns years later in most cases. The recommendation for treatment and diagnosis is the same parameters used outside of pregnancy (Milech et al., 2007; Golbert & Campos, 2008; Rudge et al., 2005). GDM is responsible for high rates of perinatal mortality, especially due to very large fetuses, the presence of malformations, and congenital anomalies, related to the presence of increased glucose levels at the beginning of pregnancy (García, 2008; Silva et al., 2009).

The study of diabetes and therapeutic attempts is carried out in animal models, mainly in rats and mice (Silva & Presgrave, 2008). A distinction is made between animals that spontaneously develop the disease, due to a genetic predisposition, and animals that are subjected to different techniques for inducing the disease, such as pancreatectomy, exposure to specific viruses, or intravenous (IV) or intraperitoneal (IP) administration of cytotoxic agents, such as streptozotocin (STZ) and alloxan (ALX) (Gispen & Biessel, 2000). The agent ALX presents high mortality after induction and ketosis. STZ has greater stability and cellular selectivity, as it is absorbed via the glucose transporter GLUT-2, and pancreatic beta cells express this transporter, making them more sensitive to STZ. At the same time, the extrapancreatic parenchyma remains intact (Lenzen, 2008).

Rodents have been widely used due to their advantages of easy handling (feeding, cleaning, and allocation), high resistance to infection, ease of dissection, and tissue extraction, in addition to developing pathophysiological similarities with human diabetes (Lerco et al., 2003). However, the lack of standardization in mating protocols combined with diabetogenic induction leads to large losses of animals, unnecessary expenses, and inexhaustible repetitions, which culminate in a major delay in the development of study in this area. In this context, the present study aimed to develop...
and standardize an experimental model for inducing GDM in rats that is highly efficient.

2. Methods

2.1 Animals

All procedures performed with the animals were approved by the Animal Use Ethics Committee of the Federal University of Uberlandia (UFU), protocol 056/18. We used 51 Wistar rats (*Rattus novergicus*), 39 females and 12 males, that were kept in polysulfone boxes, with adequate air circulation, controlled temperature between 18-22°C, a humidity of 45 to 55%, with an autoclaved wood shavings bed, and a 12/12-hour light-dark cycle. For feeding, commercial feed and drinking water were used in drinking fountains, *ad libitum*.

2.2 Mating

Rats reach puberty at 30 days of age, but it is suggested that, for the ability to maintain pregnancy to be adequate, they wait longer. Therefore, the animals used were 60 days old, and during this period, the males weighed around 200-250g and females 150-180g.

Nulliparous females were previously evaluated in relation to weight (g), using an electronic scale for weighing with a precision of 0.1 gram (Kern®), and fasting blood glucose (mg/dL) by obtaining a drop of blood from the animal's tail, with the aid of a scalpel, and the reading was taken using a glucometer (Descarpack Plus®). Therefore, females were transferred to the male boxes in the late afternoon only for mating, in a ratio of two females to one male (polygamous system). On the morning of the following day, the females were returned to their original boxes, and the vaginal fluid was collected to control copulation.

In the vaginal washing technique, the rats were restrained manually, then a Pasteur pipette containing saline solution (0.9%) was introduced superficially into the vaginal canal, and the collected material was deposited on the identified glass slides. Subsequently, the slides were dried in an oven for histological staining, using gentian
violet, washing in 70% alcohol and water, mounting the slide, using mounting medium and a coverslip. Then, the reading and capture were carried out under an optical microscope, with a 20x objective. Thus, it was possible to identify with greater precision the presence of sperm in the vaginal wash (Figure 2), indicating the first day of gestation (G1), or its absence, and in this case, it was used to characterize which phase of the reproductive cycle the rat was in what is known as the estrous cycle, which lasts 4 to 5 days, estimating the female’s fertile day, to determine the best day for re-mating (Figure 3).

2.3 Induction to GDM

On the fourth day of gestation (G4), the rats were fasted at the end of the day for 24 hours. In G5, the diabetes induction procedure was performed. Induction occurred through an intraperitoneal (IP) injection of streptozotocin (STZ) (Santa Cruz Biotechnologies®).

The drug was diluted at the time of induction, at a dose of 50 mg of STZ per kg of animal weight, diluted in citrate buffer (0.01M and pH 4.5), as STZ is only active in an acidic environment. After dilution, the animals were mobilized manually, and with a sterilized syringe with a fixed 6mm needle, the drug was applied to the animals intraperitoneally, in the third abdominal quadrant (Figure 1). After application, the animals remained fasting for one hour, as the mechanism of action used by STZ includes its binding to GLUT-2 receptors in the intestine, the same ones used by dietary glucose, so fasting guarantees absorption exclusive to the drug.

Figure 1 – Abdominal quadrants for intraperitoneal application. STZ was applied in the third quadrant.
STANDARDIZATION OF AN EFFICIENT EXPERIMENTAL GESTATIONAL DIABETES PROTOCOL

After this time, commercial food and drinking fountains containing water with 1.5% glucose were offered, and this remained for 24 hours, with the aim of avoiding death from hypoglycemia. Which is common to occur in the first 24 hours post-induction.

A parallel group of pregnant rats, determined as the control group (CT), were induced under the same conditions, using only the citrate buffer diluent (without the drug) for comparison.

After 48 hours of induction, exactly on the seventh day of gestation (G7), the females were fasted for eight hours. At the end of the day, body weight and blood glucose assessments were carried out, aiming to verify weight gain resulting from pregnancy and the success of GDM induction, with rats that presented fasting blood glucose ≥ 200 mg/dL being considered diabetic. In G17, the same assessments were repeated.

After approximately 21 days, the puppies began to be born. At this time, the following were measured: total number of puppies per mother, sex, and number of live and dead births. After this time, mothers who did not go into labor were counted as a failed pregnancy.

2.4 Biochemical Analysis

To check the damage caused by GDM in the mothers, they were anesthetized using Ketamine and Xylazine (1:1, 0.2g/100ml), and then 1.5 ml of blood was collected via intracardiac puncture. Subsequently, the blood was centrifuged at 1500 rpm for 10 minutes at 25 °C to obtain serum. This is stored in an ultrafreezer at -80 °C, to wait for biochemical tests: Fluorescent AGE assay and Advanced Oxidation Protein Products Assay (AOPP).

Measurement of fluorescent AGEs (F-AGEs) concentrations was based on the spectrofluorimetric detection (Kalousevá, Skrha & Zima, 2002). The serum (250 μL) was transferred into black 96-well plates and phosphate buffer saline (PBS) solution was used as blank. The excitation and emission wavelengths were 320 nm and 440 nm, respectively (Molecular Devices, Menlo Park, CA, USA). The fluorescence intensity was expressed in arbitrary units per milliliter of serum (AU/mL) and in AU/g of...
total protein. The Bradford method measured the total protein (Bradford, 1976). Absorbance was measured at 570 nm in a 96-well plate reader spectrophotometer (brand®).

The AOPPs levels as a marker of protein oxidative damage were measured by the action of ROS (chlorinated compounds) on proteins, leading to the formation of dityrosine residues and protein cross-linking. AOPP was determined according to the method of Witko-Sarsat and collaborators (1996) using a spectrophotometer (brand®) and calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The 10 μL of serum sample was mixed with 170 μL of PBS and 20 μL of citric acid. The curve was made using chloramine and potassium iodide (1.16M), 200 μL of the standards were mixed with 10μL of potassium iodide and 20 μL of citric acid, and the plate was shaken for six minutes before reading. The absorbance of the reaction mixture was read at 340 nm. AOPP concentrations were expressed as μ mol•L⁻¹ of chloramine-T equivalents.

3. Results

3.1 Copulation Control

To verify successful copulation, we used gentian violet staining of vaginal washings, as this dye is easily accessible and cheap, it brought better visibility of all the cells present in the slide, being essential for identifying sperm (Figure 2B) and characterization of the estrous cycle, which allowed predicting an ideal moment for a new mating attempt (Figure 3). After the end of the gestational period, we achieved 100% pregnancy success in the CT group, and 55.6% success in the GDM group (Figure 4C, p = 0.005).
STANDARDIZATION OF AN EFFICIENT EXPERIMENTAL GESTATIONAL DIABETES PROTOCOL

Figure 2 – Vaginal washes from rats stained with gentian violet. A: Fertile phase absent of sperm (estrus, see figure 2); B: Fertile phase combined with the presence of sperm (arrows), indicating G1. Scale = 10μm.

Source: Author.

Figure 3 – Phases of the estrous cycle observed in vaginal washes from Wistar rats stained with gentian violet. A: Proestrus, characterized by a large number of round and polynucleated cells, found scattered or grouped, and keratinized cells; B: Estrus, presents only keratinized and anucleated cells; C: Metestrus, presence of few leukocytes, little mucus, and some keratinized cells; D: Diestrus, numerous leukocytes (very small) and mucus filaments are visualized. Scale = 10μm.

Source: Author.
3.2 Evolution of Body Weight and Blood Glucose

With the data on weight and blood glucose evolution, in the pre-gestational, seventh, and seventeenth gestational days, we evaluated the success of induction of GDM (Figure 4).

After administration of STZ, we obtained a group of non-pregnant and diabetic rats, characterized by weight maintenance (Figure 4A) and an increase in glycemic rate (Figure 4B, G7 and G17 p < 0.00001). We had a GDM group, of pregnant and diabetic rats, all of which showed progress in weight gain (Figure 4A, G7 p = 0.0026, G17 p = 0.00001), and hyperglycemia (Figure 4B, G7 and G17 p < 0.00001).

Figure 4 – Assessment of rats, before pregnancy, in G7 and G17, in the different comparison groups, non-diabetic (CT), gestational diabetes (GDM), and diabetic (D). A: Evolution of body weight in grams: the dotted line represents animals that did not gain weight in G7 and G17. B: Evolution of blood glucose in mg/dL: Before (p > 0.9999), G7 and G17 (**p < 0.0001). C: Percentage of pregnancy success: shows 100% gestational success in the CT group, and 55.6% for the GDM group (**p = 0.005). Holm-Sidak test (A and B) and Binomial test (C).

3.3 Offspring Assessment

Regarding the number of puppies born alive (Figure 5A, p = 0.41) and the ratio of males (p = 0.32) and females (p = 0.32) per mother (Figure 5B), there were no statistically significant differences between the CT and GDM groups. However, in relation to the rate of stillborn and dead puppies after birth (Figure 5C), the GDM group presented 38% of stillborn (p < 0.0001), 16.3% of puppies that died between 1 and 7 days (p < 0.0001), and 6.5% of puppies that died from 8 to 30 days (p = 0.02), while...
the CT group had a rate of 1.8% of stillbirths and 0.9% of dead pups between 1 and 7 days.

Figure 5 – Assessment of gestational quality. A: Number of pups born alive per rat (p = 0.41); B: Sex ratio of offspring (male and female p = 0.32); C: Rate of stillborn puppies, dead from 1 to 7 days, and from 8 to 30 days. A higher percentage of stillborn and dead puppies after birth (***p < 0.0001 and **p = 0.02) was noted in the gestational diabetes group. Mann Whitney U test (A), t-test (B), and Binomial test (C).

3.4 Maternal Blood Analysis

When evaluating oxidative stress through the determination of advanced glycation end products (AGEs) and advanced oxidation protein products (AOPP), we saw that both components were increased in diabetic animals (Figure 6).
Figure 6 – Assessment of oxidative stress in the serum of mothers in the control (CT) and gestational diabetes (GDM) groups. A, B, C, D, and E: quantification of advanced glycation products (AGEs). A higher concentration of AGEs was noted in the GDM group when compared to the CT, (A, B, C, and D: p < 0.0001; E: p = 0.0085). F: advanced protein oxidation products (AOPP), showing a higher concentration of AOPPs in the GDM group (p < 0.0001). Mann Whitney U test.

4. Discussion

The gestational diabetes (GDM) induction protocol must be initiated by mating the animals, in this way, the mating was carried out in the proportion of two females for each male, a method called temporary polygamous, and after mating the rats are separated from the males for evaluation of successful pregnancies. In the study by Mattaraia & Moura (2012), the authors verified the reproductivity of Wistar rats in different mating systems and concluded that the temporary polygamous system was the most efficient, guaranteeing animal welfare, as it respects the biology of the females, and, the reproductive capacity of males is maximized, which represents a saving in physical space and resources used.

To accurately determine the beginning of pregnancy, which was important for inducing diabetes on the correct days of pregnancy, copulation was performed at night,
and in the morning the vaginal washes obtained were stained for better identification of cells (Barril et al., 2016). The slides were stained with an alternative dye, gentian violet, facilitating the identification of sperm and the determination of the phase of the female's estrous cycle, an essential method for correctly determining the first day of pregnancy (G1). The use of alternative dyes demonstrates effectiveness for staining animal and plant cells and can be easily acquired for histological preparations and visualization under a microscope (Da Silva, De Araújo & Bezerra, 2019; Da Rocha et al., 2021). Gentian violet, used in the present study, is an alternative dye with antiseptic activity, bacteriostatic and bactericidal power, low cost, and ease of use and access.

Many authors who study GDM report the difficulty of obtaining pregnant and diabetic animals, and the consequent use of a large number of animals to obtain a significant sample (Rudge et al., 2013). In most experimental studies, a lot of variation in the methodology for inducing GDM is observed, which consequently creates difficulty in replicating the procedures by other researchers. In the studies seen, the authors carry out vaginal washings at non-standard times, they do not stain the slides, only a few studies monitor the estrous cycle, not all of them specify the use of temporary or permanent polygamous methods and the proportions of females per male, other studies induce diabetes prior to pregnancy, among other divergences, or do not clearly describe this in their methodologies (Caluwaerts et al., 2003; Damasceno et al., 2004; Lira et al., 2016; França, Oliveira & Balbi, 2016). All these details were studied and, in this work, we bring the most efficient procedures from the moment of copulation.

The gestational period of Wistar rats lasts from 19 to 22 days, and after the tenth day, an increase in abdominal volume begins to be noticed. Cases of dystocia are rare, and birth lasts around 1 to 2 hours, with an average of 8 puppies per birth (Andrade, Pinto & Oliveira, 2006). However, diabetes during pregnancy can impact gestation time, weight gain, and the number of stillborn and live-born puppies.

The drug of choice for inducing diabetes was streptozotocin (STZ). It causes DNA damage by inhibiting insulin synthesis and secretion, culminating in the process of death of pancreatic beta cells (Lerco et al., 2003; Kirsten, Sesterheim & Saitovitch, 2010). Its action can be influenced by concentration, infusion speed, dose, route of administration, diet, fasting time, and animal weight (Santos, 2017). STZ has a glucose
molecule in its chemical structure and is therefore able to use GLUT-2 transporters to enter the cell. As pancreatic beta cells have many GLUT-2 type glucose transporters, they become more affected by the drug, with preservation of the extra-pancreatic parenchyma (Elsner et al., 2007; Lenzen, 2008). In a systematic review, Ferreira & Nicolau (2011) carried out a survey of articles on diabetes induction and concluded that STZ is the most used drug (70% of studies), but with variable doses and routes, of 35 to 65 mg/kg and intraperitoneal (IP) and intravenous (IV), respectively.

To begin the induction of GDM, on the fourth day of gestation, the females were kept fasting for 24 hours, to be induced to diabetes on the fifth gestational day, using the drug STZ, via IP, considered easier to manage than the IV (Deeds et al., 2011), and uses smaller doses than the subcutaneous via. Fasting prior to induction aims to inhibit the competition of glucose arising from food with the glucose molecule present in the drug. The half-life of STZ is short, ranging from 5 to 15 minutes, therefore, it must be diluted before induction. An acidic buffer must be used for the best activity, such as citrate buffer with pH 4.4 - 4.5 (Eleazu et al., 2013). The dose will depend on several factors such as animal species, administration, age, weight, and nutritional and hydration status (Etuk & Muhammed, 2010). The time of day in which STZ is administered also affects the success of the induction, the study by Candela, Hernandez & Gagliardino (1979) showed that inductions carried out in the late afternoon were more successful when compared to those carried out in the morning. Therefore, in our work, the animals were induced at 5 p.m., and we achieved 100% success in inducing diabetes. The study of Eleazu, Iroaganachi & Eleazu (2013) concluded that effective doses for inducing diabetes in rats range from 55 to 75 mg/kg, using the via IP, in a single dose. When very low doses are applied, the tendency is to lead to unstable and reversible diabetes. In our study, we used a dose of 50 mg/kg, a slightly lower dose, but which proved to be effective, leading to stable and irreversible diabetes.

One of the concerns of the GDM protocol is the possibility of the STZ drug crossing the placental barrier, and acting directly on embryonic development and organogenesis, or even on the fetal pancreas. In this context, it is known that induction before conception or in the first gestational days can affect early development and
cause miscarriages (Fraser et al., 2007). Our choice of induction in G5 has based on the information that the period of organogenesis in rats begins from G6 and extends to G15 (Medeiros et al., 2014), and the development of beta-pancreatic cells in rats occurs during the last week of gestation (Blondeau et al., 2011), thus, the endocrine pancreas of fetuses would not be affected by STZ. In the literature, we find inductions at the most different gestational ages, from pre-conception to G10 (Blondeau et al., 2011; Caluwaerts et al., 2003; Ramos-Alves et al., 2012; Natif et al., 2003), also, induction in G5 (Merzouk et al., 2002).

Our success rate for inducing GDM was 55.6%, considered very efficient compared to reports from researchers who study diabetes. Some studies report on how unfavorable the maternal environment becomes when mothers are in hyperglycemia. In severe GDM, severe hyperglycemia alters placental maturation, making it insufficient in its fetal nutrition function, causing developmental harm and leading to a higher incidence of newborns with restricted body weight. On the other hand, a mildly hyperglycemic environment results in fetuses with macrosomia and hypoinsulinemia (Calderon et al., 1999). One of the factors involved in these complications caused by GDM is the oxidative damage caused by excess glucose in maternal-fetal blood (Jakuš et al., 2014).

The formation and accumulation of advanced glycation end products (AGEs) and AOPP in groups of mothers with hyperglycemia reflect the damage caused by the induction of GDM. AGEs are generated by the non-enzymatic reaction of sugar with the free amino groups of proteins and amino acids under conditions of hyperglycemia, altering their structure and making them dysfunctional (Barbosa, Oliveira & Seara, 2008; Vlassara & Uribarri, 2014; Lobo et al., 2017). A variety of toxic α-oxoaldehydes are produced under hyperglycemia in diabetes mellitus, with methylglyoxal (MGO) being one of the most potent glycating agents, playing a key role in endothelial dysfunction that leads to insulin resistance, hypertension, and nephropathy in diabetic patients (Reyaz et al., 2020). The plasma level of MGO, among others, was significantly increased in diabetic rats compared to healthy controls, demonstrating that the protocol applied was capable of generating hyperglycemia during pregnancy by increasing the presence of glycoxided products in plasma serum.

The increase in the oxidation of proteins, lipids, and carbohydrates occurs when
the production of reactive oxygen species exceeds the local antioxidant capacity, playing an essential role in the pathogenesis of many diseases (Kalousova, Skrh & Zima, 2002). AOPPs are cross-linked protein products containing di-tyrosine that result in the oxidation of proteins and are generated as a secondary response to oxidative stress, which occurs due to the reaction of chlorinated oxidants with plasma proteins. These AOPPs are elevated in diabetes, they have a structure similar to AGEs and have the ability to induce the production of pro-inflammatory cytokines and adhesive molecules (Tsukahara et al., 2003; Conti et al., 2019; Zhou et al., 2021).

The puppies from the group of diabetic mothers had higher mortality rates. Many studies report how unfavorable the maternal environment becomes when mothers are in hyperglycemia, which can alter placental maturation, making it insufficient in its function of fetal nutrition, causing harm to the development of the offspring (Ejdesjö, Wentzel & Eriksson, 2012; Da Cruz et al., 2023). One of the factors involved in these complications caused by GDM is the oxidative damage caused by excess glucose in maternal-fetal blood (Jakuš et al., 2014).

The objective of this study was to standardize an efficient protocol for inducing GDM, from copulation to proof of the disease in mothers during pregnancy and childbirth. In this sense, we were able to show a sequence of essential care to better obtain positive results in the experimental model, as well as confirm the established disease not only by measuring blood glucose, but also by studying the oxidative damage commonly linked to diabetes mellitus.

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STANDARDIZATION OF AN EFFICIENT EXPERIMENTAL GESTATIONAL DIABETES PROTOCOL


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