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Analysis of the endocrine pancreas in adulthood in rats born with intrauterine growth restriction

Análise do pâncreas endócrino na idade adulta de ratas nascidas com restrição de crescimento intrauterino

Análisis del páncreas endocrino en la edad adulta en ratas nacidas con restricción del crecimiento intrauterino

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Abstract
Uncontrolled maternal hyperglycemia can negatively program fetal development, resulting in growth restriction and increased risk of metabolic diseases in adulthood. Severe diabetes (uncontrolled) was induced by streptozotocin (a beta-cytotoxic drug) as a model to cause fetal growth restriction. The aim of this study was to evaluate the functional characteristics of the pancreatic islets of these adult SGA rats at the end of pregnancy, to understand the role of major endocrine–pancreatic hormones in reproductive dysfunctions. For this purpose, non-diabetic and severely diabetic Sprague Dawley rats were used to obtain female offspring (F1), which were classified as appropriate (AGA) or small (SGA) for gestational age, generating two groups: AGA from non-diabetic mothers (Control) and SGA from severely diabetic mothers (SGA group) (n = 10 animals/group. No differences were observed in insulin-, glucagon-, proliferation-, or apoptosis-positive cells. Decompensated maternal diabetes led to intrauterine growth restriction in the offspring and is associated with glucose intolerance, hyperinsulinemia, and oxidative stress in adult females. Although no significant changes were observed in the proportion of insulin-positive cells, the increase in somatostatin-immunoreactive cells suggests that glycemic dysfunction may involve alterations in non-β cells, affecting insulin signaling and secretion. These findings highlight the need for further studies to explore the cellular mechanisms involved in this process.
Keywords: Intrauterine growth restriction; Pancreatic islets; Oxidative stress; Diabetes.

Resumo
A hiperglicemia materna não controlada pode programar negativamente o desenvolvimento fetal, resultando em restrição de crescimento e aumento do risco de doenças metabólicas na vida adulta. O diabete materno não controlado (diabete grave) foi o modelo para causar restrição de crescimento fetal. O objetivo deste estudo foi avaliar as características funcionais das ilhotas pancreáticas desses ratos SGA adultos no final da gestação, para entender o papel dos principais hormônios endócrino-pancreáticos nas disfunções reprodutivas. Para isso, ratas *Sprague Dawley* não

diabéticas e com diabete grave induzido quimicamente foram utilizadas para obtenção de descendentes fêmeas (F1), classificadas como adequadas (AIP) ou pequenas (PIP) para a idade de prenhez, distribuídas em: AIP de mães não diabéticas (Controle) e PIP de mães com diabete grave (PIP) (n=10 animais/grupo). Não houve diferenças nas células marcadas para insulina, glucagon, proliferação e morte (apoptose) celular. O diabete materno descompensado levou à restrição de crescimento intrauterino na prole, está associado à intolerância à glicose, hiperinsulinemia e estresse oxidativo na vida adulta das fêmeas. Embora não tenham sido observadas alterações significativas na proporção de células positivas para insulina, o aumento de células imunomarcadas para somatostatina sugere que a hiperglicemia pode prejudicar células não-β, afetando a sinalização e secreção de insulina. Esses resultados ressaltam a necessidade de estudos adicionais que explorem os mecanismos celulares envolvidos neste processo.
Palavras-chave: Restrição de crescimento intrauterino; Ilhotas pancreáticas; Estresse oxidativo; Diabete.

Resumen
La hiperglucemia materna no controlada puede programar negativamente el desarrollo fetal, resultando en restricción del crecimiento y un mayor riesgo de enfermedades metabólicas en la vida adulta. Diabetes materna no controlada (grave) fue inducida quimicamente para tener restricción del crecimiento fetal. El objetivo de este estudio fue evaluar las características funcionales de los islotes pancreáticos de estas ratas adultas SGA al final de la gestación, para comprender el papel de las principales hormonas endocrino-pancreáticas en las disfunciones reproductivas. Ratas Sprague Dawley no diabéticas y con diabetes grave tubieron crías hembras (F1), que fueron clasificadas como adecuadas (AEG) o pequeñas (PEG) para la edad gestacional: AEG de madres no diabéticas (Control) y PEG de madres con diabetes grave (n=10 animales/grupo). Sin embargo, no se observaron diferencias en las células marcadas para insulina, glucagón, proliferación y muerte (apoptosis) celular. La diabetes materna descompensada conduce a restricción del crecimiento intrauterino en la descendencia y se asocia con intolerancia a la glucosa, hiperinsulinemia y estrés oxidativo en la vida adulta de las hembras. El aumento de células inmunomarcadas para somatostatina sugiere que la disfunción glucémica puede involucrar alteraciones en células no β, afectando la señalización y secreción de insulina, destacando la necesidad de estudios adicionales sobre los mecanismos celulares involucrados.
Palabras clave: Restricción del crecimiento intrauterino; Islotes pancreáticos; Estrés oxidativo; Diabetes.

1. Introduction

Human epidemiological studies (Ravelli et al., 1999; Roseboom et al., 1999) and experimental animal studies (Ozanne et al., 2004; Guzman et al., 2006; Nathanielsz, 2006) have demonstrated that an adverse environment in utero or in early neonatal life alters growth and predisposes individuals to health problems throughout life. An unfavorable intrauterine environment can lead to impaired development of organs and tissues across successive generations (Aerts et al., 2006; Fernandez-Twinn et al., 2006; Zambrano et al., 2009), a phenomenon that characterizes the Developmental Origins of Health and Disease (DOHaD) (Gluckman et al., 2010). According to the DOHaD hypothesis, exposure to adverse condition, such as undernutrition, uncontrolled diabetes, or hypertension, elicits adaptive responses that may promote short-term survival but, depending on the context, may also increase the risk of developing noncommunicable chronic diseases later in life.

One of the most characteristic examples of fetal programming is fetal growth restriction (FGR), previously referred to as intrauterine growth restriction (IUGR). FGR is defined as birth weight below the 10th percentile for gestational age or less than 2.5 kg at term (Melamed et al., 2021). FGR is a public health concern that contributes to neonatal mortality and multiple complications that can severely affect quality of life (Chew et al., 2021). Epidemiological studies have demonstrated various consequences of low birth weight, including type 2 diabetes, hyperlipidemia, obesity, and increased risk of cardiovascular diseases in adulthood (Barker et al., 1989; Hales et al., 2001; Holemans et al., 2003; Gluckman et al., 2007).

Experimental animal models are often used because they can reproduce conditions similar to those in humans. Interventions used to generate newborns with FGR include ligation (Wigglesworth, 1974) or occlusion (Tanaka et al., 1994) of the uterine arteries, administration of corticosteroids (Benediktsson et al., 1993), protein restriction (Resnick et al., 1982), overall nutrient restriction (Woodall et al., 1996; Girard et al., 1977; Woodall et al., 1999), induced hypertension (Nassar et al., 2012), or maternal diabetes (Cruz et al., 2023; Ravishankar et al., 2007; Corvino et al., 2015a). When adult rats (from 75 days of age) are administered streptozotocin (a beta cell-specific cytotoxic drug), these animals develop blood glucose levels above 300 mg/dL, characterizing a uncontrolled or severe diabetes. Severe diabetes in rats mimics the hyperglycemic profile

observed in human type 1 Diabetes mellitus (Corvino et al., 2015b). Female rats with severe diabetes have a high proportion of offspring classified as small for gestational age (SGA), indicating that these pups experienced FGR due to maternal hyperglycemia (Corvino et al., 2015b; Cruz et al., 2023; Volpato et al., 2015).

In our laboratory, it has already been demonstrated that adult SGA females presented insulin resistance and glucose intolerance prior to pregnancy. These SGA rats exhibited oxidative stress, insulin resistance, and impaired fetal development when evaluated at the end of pregnancy, showing the impact of fetal programming induced by severe maternal diabetes (Cruz et al., 2023).

Insulin resistance is characterized by a state of hyperinsulinemia resulting from reduced sensitivity of peripheral tissues to insulin. Consequently, hyperglycemia develops, and depending on blood glucose levels, the condition may be diagnosed as a glucose intolerance or diabetes. In response to persistent hyperglycemia, the pâncreas, specifically the β-cells, continues to secrete insulin in an attempt to restore normoglycemia (Petersen et al., 2017). The pancreas consists of two main regions: the exocrine portion, which is responsible for the secretion of digestive enzymes, and the endocrine portion, composed of α-pancreatic cells, β-pancreatic cells, δ-pancreatic cells, and pancreatic polypeptide (PP) cells (Atkinson et al., 2020). All endocrine pancreatic cells, except for the PP cells, form the pancreatic islets, which are responsible for the secretion of hormones that work together to maintain glucose homeostasis (Kordowich et al., 2009). α-cells secrete glucagon, a hormone that raises blood glucose levels by promoting hepatic glycogen breakdown. In contrast, elevated blood glucose levels stimulate β-cells to synthesize and secrete insulin (Muller et al., 2017). The main function of insulin is to facilitate glucose uptake from the blood into other tissues for utilization (Petersen & Shulman, 2018). δ-cells secrete somatostatin, which inhibits both insulin and glucagon secretion, providing a fine-tuning mechanism between these two hormones to ensure glucose homeostasis (Brereton et al., 2015).

Given that adult SGA rats (small for gestational age, SGA) exhibited impaired glucose and insulin tolerance both before and at the end of pregnancy (Cruz et al., 2023), we hypothesize that pancreatic islet cells may have impaired synthesis of their respective hormones prior to gestation, which could negatively impact the course of pregnancy. Considering the reproductive performance outcomes observed in the offspring of severely diabetic dams, the aim of this study was to evaluate the functional characteristics of the pancreatic islets of these adult SGA rats at the end of pregnancy, to understand the role of major endocrine–pancreatic hormones in reproductive dysfunctions.

2. Methodology

An experimental research was carried out, partly in the field and partly in the laboratory, and in a quantitative study (Pereira et al., 2018) using simple descriptive statistics using mean values, standard deviations (Shitsuka et al., 2014; Akamine & Yamamoto, 2009) statistical analysis (Bekman & Costa Neto, 2009)

2.1 Animals

This study was conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Male and female Sprague Dawley rats were obtained from the Multidisciplinary Center for Biological Investigation (CEMIB) at the University of Campinas (UNICAMP) and housed in the Animal Facility of the Experimental Research Laboratory in Gynecology and Obstetrics – UNIPEX, School of Medicine of Botucatu, São Paulo State University (UNESP), under controlled conditions of temperature (22 ± 2°C), humidity (60 ± 10%), and a 12-hour light/dark cycle. Filtered water and standard chow were provided ad libitum. The study protocol was approved by the Institutional Animal Care and Use Committee (CEUA) of the Botucatu

Medical School – FMB/UNESP (Protocol No. 1341/2019).

2.2 Induction of Severe Maternal Diabetes

Severe diabetes (SD) was induced in adult female rats (90 days old, n = 12) via intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich®, USA), a β-cell-specific cytotoxic agente (dose of 40 mg/kg). Non-diabetic control females (90 days old, n = 7) received an equivalent volume of 0.01 M citrate buffer (pH 4.5) by the same route. Seven days after STZ or buffer administration, blood samples were collected from the tail vein for blood glucose measurement using a standard glucometer (OneTouch Ultra, Johnson & Johnson®, USA). Inclusion in the diabetic group was confirmed by blood glucose levels >300 mg/dL, which characterizes severe diabetes in rats and mimics the uncontrolled hyperglycemia of human type 1 Diabetes mellitus. Non-diabetic (control) rats had glucose levels below 120 mg/dL (Corvino et al., 2015a).

2.3 Mating and Pregnancy

From day 110 of life (adulthood), severely diabetic and non-diabetic female rats were mated overnight with male rats (n = 9). Every three females were placed in a cage with one male of similar age in the late afternoon. The next morning, males were removed, and vaginal smears were performed on all females. Rats with positive vaginal smears for sperm were considered pregnant, and this day was designated as gestational day 0 (Sinzato et al., 2020). The pregnant rats were transferred to individual cages enriched with paper balls (Simpson & Kelly, 2011), with food and water provided ad libitum. At the end of pregnancy (around gestational day 22), delivery occurred via vaginal birth, and the litter (First generation) was obtained.

2.4 Offspring of Severely Diabetic Dams

After birth, live newborns from severely diabetic and non-diabetic dams were weighed and classified based on body weight using the control group's mean ± 1.0 standard deviation (SD) as a reference. Newborns were classified as small for gestational age (SGA) if their weight was below the control mean − 1.0 × SD; appropriate for gestational age (AGA) if their weight was within the control mean ± 1.0 × SD; and large for gestational age (LGA) if their weight was above the control mean + 1.0 × SD (Corvino et al., 2015a). Female newborns classified as AGA from non-diabetic/control mothers were included in the control group, while female newborns classified as SGA from severely diabetic mothers were included in the fetal growth restriction group (restricted group).

2.5 Oral Glucose Tolerance Test (OGTT), Area Under the Curve (AUC), and Serum Insulin Concentration

On day 115 of life (adulthood), the animals (n = 10 rats/group from different litters) were subjected to an OGTT, a procedure routinely used in clinical settings for diagnosing diabetes (Paula et al., 2022). Blood glucose levels were measured at 30, 60, and 120 minutes after oral administration of a D-glucose overload using a conventional glucometer (Sinzato et al., 2021). The total area under the glycemic curve (AUC) was calculated and analyzed as previously described (Tai, 1994). During the same test, blood samples were also collected in the fasting state to determine serum insulin levels using a sandwich ELISA assay.

2.6 Mating, pregnancy and laparotomy

The adult rats (after 115 days of life) of the control and diabetic groups were submitted to mating for 15 consecutive days, comprising three estrous cycles. For this, three females were placed in the presence of a normoglycemic male in the overnight period. The next morning (7–9 a.m.), the males were removed and the vaginal smear was performed. The presence

of spermatozoa on the slides confirmed the diagnosis of pregnancy, which was considered zero pregnancy day (D0) (Moraes-Souza et al., 2017).

On the day 21 of pregnancy, the female rats (Control and SGA) were anesthetized with sodium thiopental (Thiopentax® - intraperitoneal route - 120 mg/kg according to Ethical Committee's protocols) and, after confirming the signs that showed the anesthetic procedure according to the instructions of the Institutional Veterinarian, the animals were decapitated according to the experimental groups to obtain blood samples. The animals were then submitted to laparotomy for the collection of the pancreas.

2.7 Analysis of Oxidative Stress

At the end of pregnancy, blood was collected for analysis of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) and for the Thiobarbituric acid reactive substances (TBARS). The blood samples in heparinized tubes were kept under refrigeration immediately after collection and then were centrifuged at 1200 rpm (185 x g) for 10 min at 4°C. Next, the plasma was discarded and the erythrocytes were washed in 2 mL of phosphate saline buffer (PBS, pH 7.4) and centrifuged at 3500 rpm (1575 x g) for 1 min at 4°C (Sinzato et al., 2022). The supernatant was discarded. This procedure was repeated three times, and then the washed erythrocyte samples were divided into two aliquots: an aliquot was diluted (1:20) into purified water for the measurement of TBARS level and another aliquot was diluted into stabilizing solution of 2.7 mM EDTA and 0.7 mM 2-mercaptoethanol (1:20) for SOD, and GPx, (Sinzato et al., 2022). The hemoglobin (Hb) concentration was determined using Drabkin's solution according to the diluent used (purified water or stabilizing solution) (Tentor & Salvati, 1981).

2.8 Morphological Analysis of the Endocrine Pancreas

At the end of pregnancy, pancreatic samples were collected to assess the immunolabeling of insulin, glucagon, and somatostatin. Pancreases (n = 5/group) were excised and used for routine morphological analysis with hematoxylin and eosin (H&E) staining and immunohistochemical analysis. The pancreas samples were immunolabeled with antibodies against insulin, glucagon, and somatostatin (endocrine–pancreatic hormones), Ki-67 (cell proliferation), and cleaved caspase-3 (apoptotic cell death). For this purpose, pancreas samples were fixed in 4% formaldehyde for 24 hours and subsequently stored in 70% ethanol. Tissue fragments were processed and embedded in paraffin. Paraffin blocks containing pancreatic tissue were sectioned in a microtome at a thickness of 5 µm and mounted on silanized slides. The sections were then deparaffinized and rehydrated through descending ethanol gradients. Antigen retrieval was performed using a pressure cooker (Elite Bistro®). Endogenous peroxidase activity was blocked by incubating the slides with a universal peroxide block (Hydrogen Peroxide Block) at 27°C for 30 minutes. Nonspecific protein binding was blocked using a ready-to-use protein blocking solution (Protein Block®). After incubation with the primary antibodies (Table 1), slides were incubated with a secondary antibody (Histofine®), and the reaction was developed using a chromogen (3,3-diaminobenzidine – DAB). Samples were counterstained with Mayer's hematoxylin and mounted with resin. As a negative control, samples from each group were processed with the omission of the primary antibody, which was replaced with 1% bovine serum albumin (BSA). Immunohistochemical evaluation was performed by counting positive and negative cells and analyzing the immunolocalization of each antibody using ImageJ software (NIH, USA) (Gallego et al., 2018).

Table 1 - Description of primary antibodies, antigen retrieval steps, dilutions, and incubation conditions used for immunohistochemical analysis during the experiment.

Primary Antibody	Code	Dilution	Antigenic recovery	Incubation
Anti-Insulin	Abcam®, Code: ab8304	1:10.000	30 minutes	2 hours at 27°C
Anti-Glucagon	Abcam®, Code: ab8055	1:500	30minutes	1 hour at 27°C
Anti-Somatostatin	DAKO®, Code: a0566	1:2.000	Free of recovery	Overnight at 4°C
Anti-Ki-67	Spring®, SP6	1:100	30 minutes	Overnight at 4°C
Anti-Cleaved Caspase-3	Elabsciense, E-AB-63510	1:200	30 minutes	Overnight at 4°C

Source: Research data (2025).

2.9 Statistical Analysis

All analyses were performed with the assistance of the Biostatistics specialist from the Research Support Office (EAP) of the Botucatu School of Medicine, Unesp. Data were presented as mean ± standard deviation and percentage. Comparisons between groups were performed using the Student's t test, Fisher's exact test, Gamma distribution for proportion data. The minimum confidence limit of 95% ($p<0.05$) was used. All analyses were performed using the SAS for Windows program, v.9.4.

3. Results

In this study, SGA rats exhibited an increased area under the curve (AUC) during the oral glucose tolerance test, elevated serum insulin concentrations, and higher levels of thiobarbituric acid reactive substances (TBARS). Additionally, there was a reduction in the antioxidant enzyme superoxide dismutase (SOD) (Table 2).

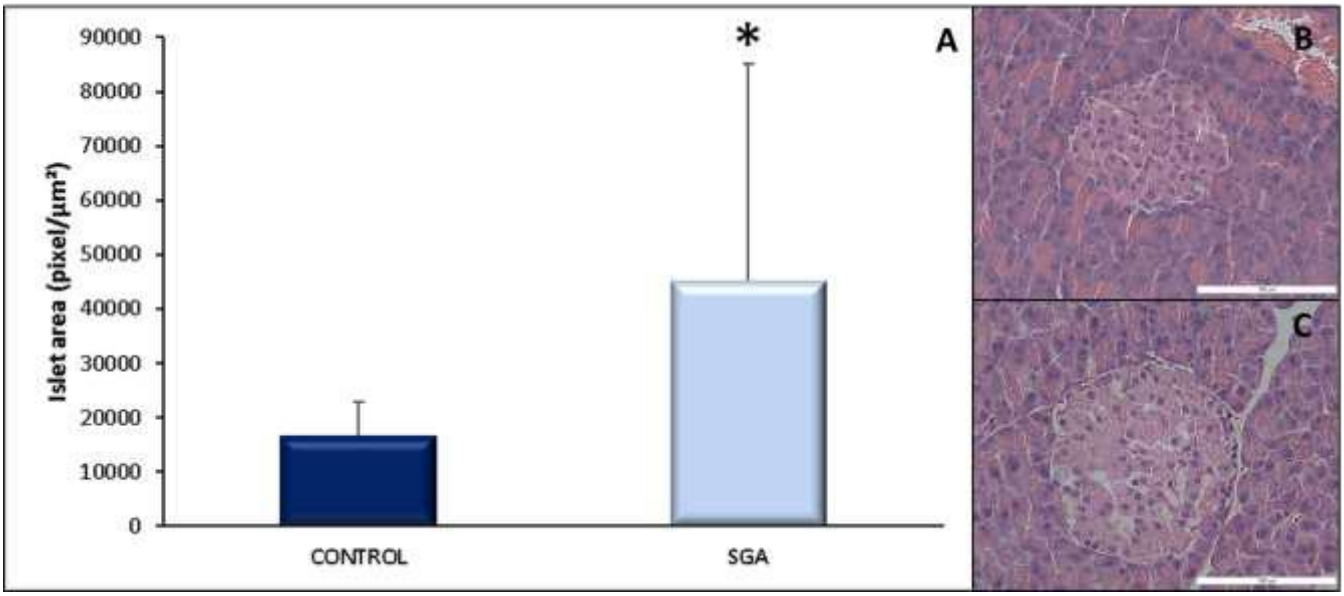
Table 2 - Oral glucose tolerance test at different times, area under the curve and biomarkers at adulthood (day 115 of life) of female pups coming from nondiabetic dams (Control) or fetal growth restricted from severely diabetic dams.

	AUC (mg/dL*120 min)	Insulin (ng/mL)	TBARS (nM/mg Hb)	SOD (U/g Hb)	GPx (mM/min/mg Hb)
CONTROL	10160.0±1832.0	0.8±0.21	58.24±6.07	17.11±1.51	4.25±0.43
SGA	16633.5±1212.5*	1.66±0.7*	75.54±21.98*	11.6±2.22*	5.43±1.22

Legend: AUC = area under the curve; TBARS = thiobarbituric acid reactive substances; SOD = superoxide dismutase; GPx = glutathione peroxidase; Hb = hemoglobin; SGA = small for gestational age.
Data are shown as mean ± standard deviation (SD). n = 10 animals/group.
*$p<0.05$ - compared to the Control group (Student's t test for AUC and serum insulin; Gamma distribution for TBARS and SOD). Source: Research data (2025).

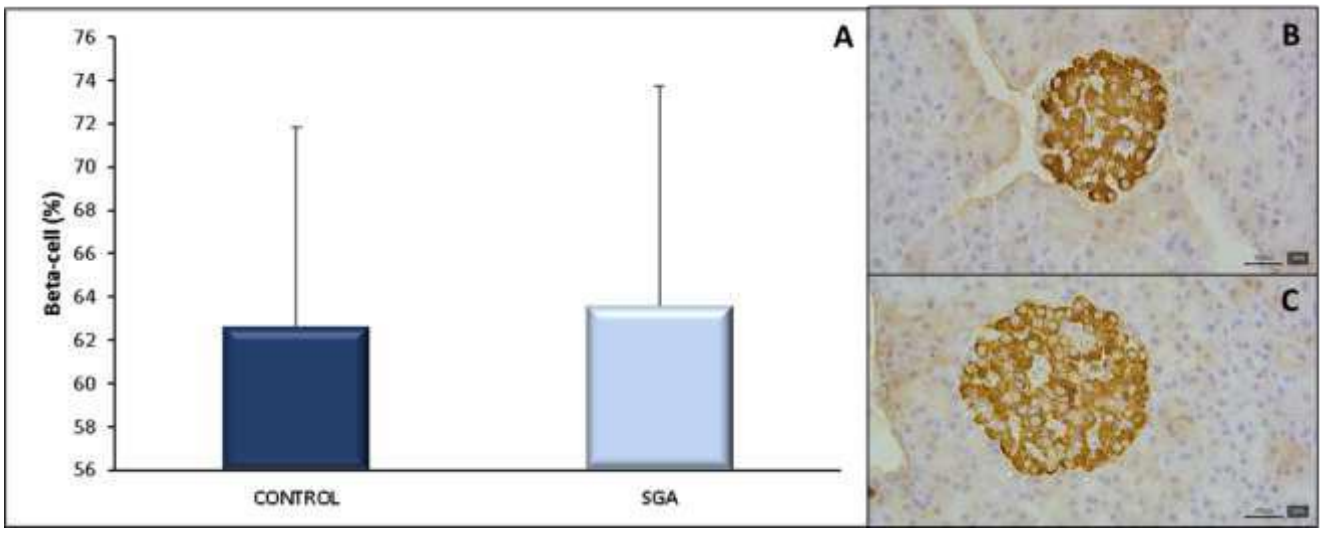
Regarding the morphological data of the endocrine pancreas, there was an increase in pancreatic islet area (Figure 1) and in the percentage of somatostatin-positive cells (Figure 4) in SGA rats compared to the control group. No differences were observed in the insulin-positive cells (Figure 2) or glucagon-positive cells (Figure 3). Likewise, no changes were found in the markers for cell proliferation (Figure 5) or apoptotic cell death (Figure 6).

Figure 1 - Pancreatic islet area on gestational day 21. A. Photomicrograph of the pancreas showing the pancreatic islet from non-diabetic rats (Control); B. Photomicrograph of the pancreas showing the pancreatic islet from rats born small for gestational age (SGA); C. Graph representing pancreatic islet area (pixels/µm²) in the different study groups.



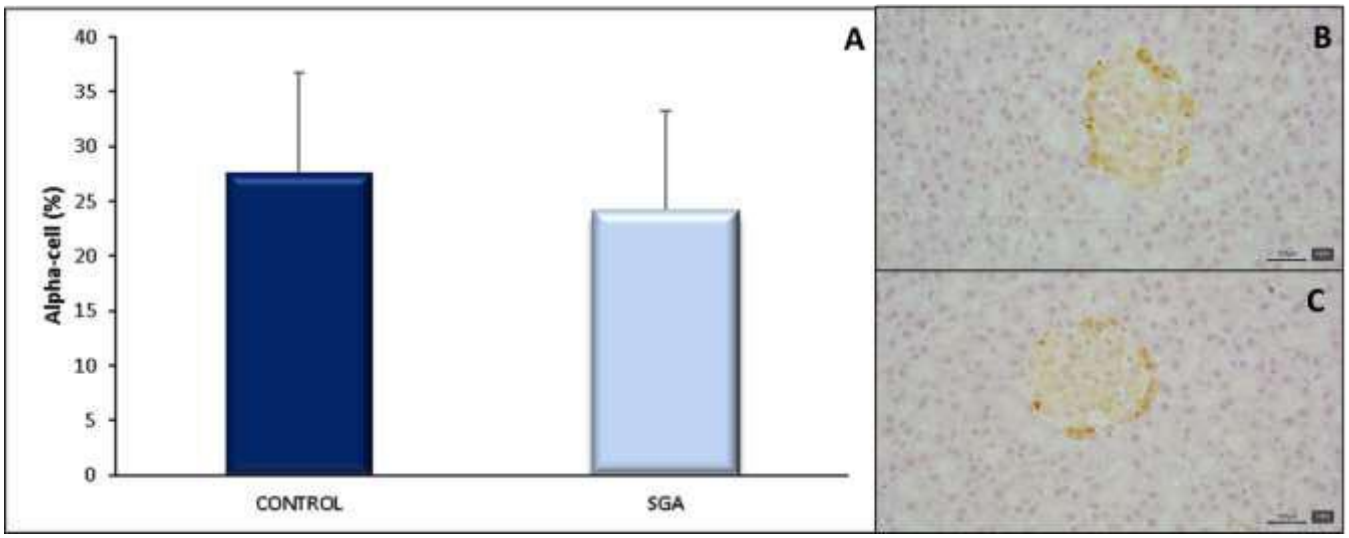
Data are expressed as mean ± standard deviation. *p < 0.05 – compared to the control group (Student's t-test). Source: Authors.

Figure 2 - Insulin immunolabeling (β-pancreatic cells) in pancreatic islets on gestational day 21. A. Graph representing the percentage (%) of insulin-positive cells in relation to pancreatic islet cells; B. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for insulin (brown cytoplasmic staining) in the Control group; C. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for insulin (brown cytoplasmic staining) in the SGA group.



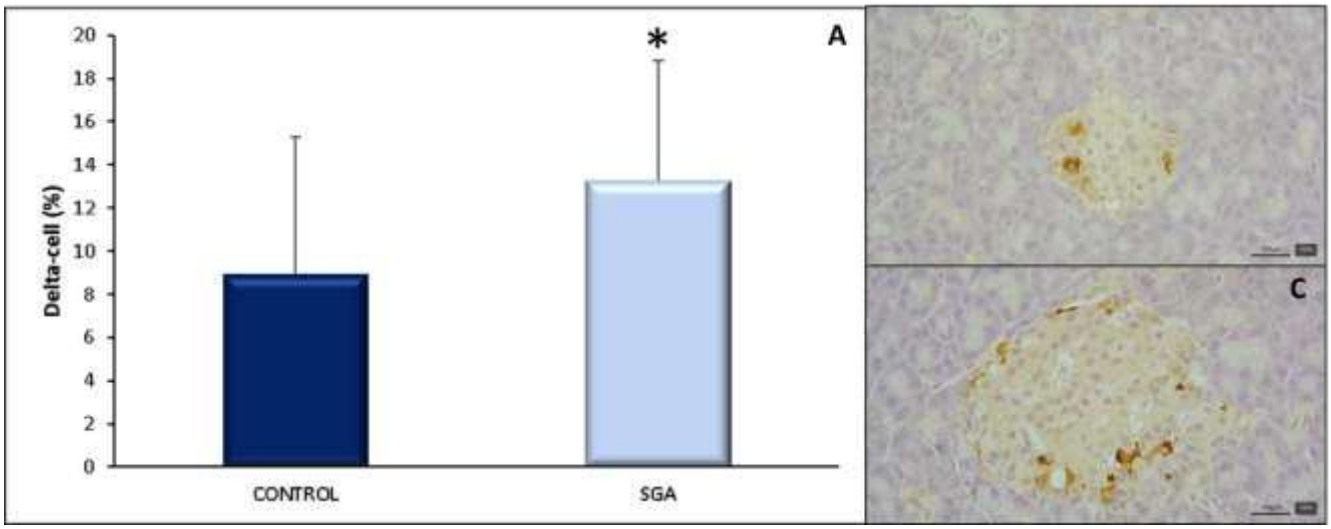
Data are expressed as mean ± standard deviation. p > 0.05 – no statistically significant difference. Source: Authors.

Figure 3 - Glucagon immunolabeling (α-pancreatic cells) in pancreatic islets on gestational day 21. A. Graph representing the percentage (%) of glucagon-positive cells in relation to total number of pancreactic islet cell; B. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for glucagon (α-pancreatic cells) (brown cytoplasmic staining) in the control group; C. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for glucagon (α-pancreatic cells) (brown cytoplasmic staining) in the offspring of SGA dams.



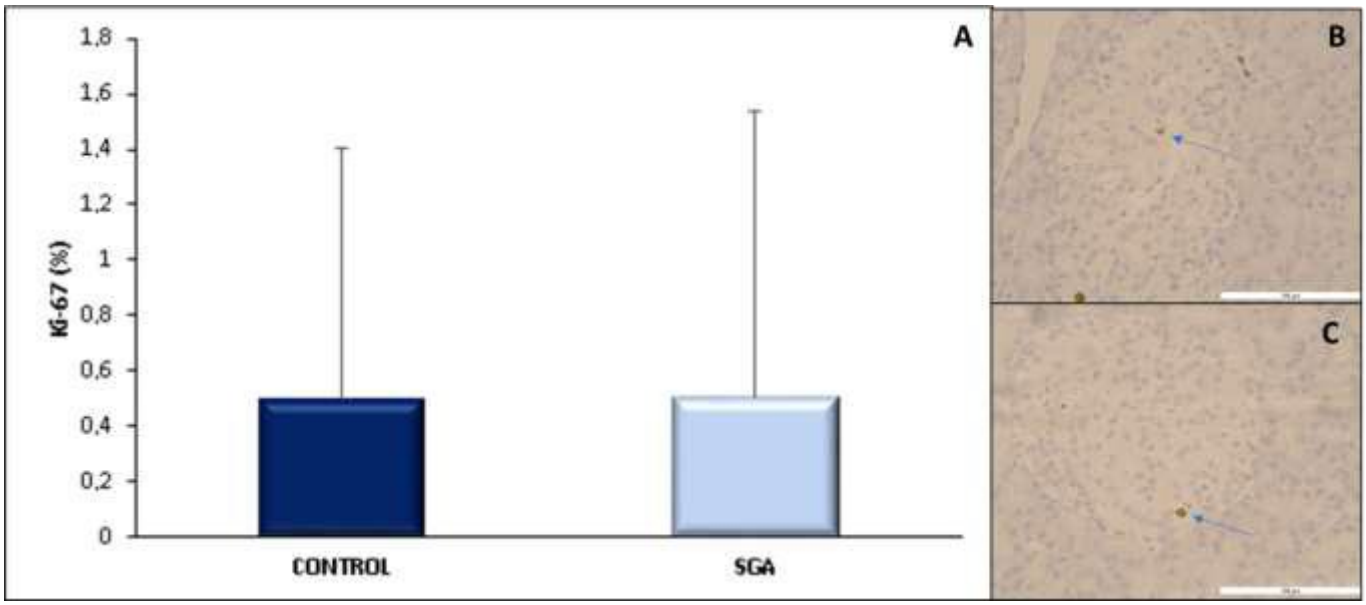
Data are expressed as mean ± standard deviation. p > 0.05 – No statistically significant difference (Student's t-test). Source: Authors.

Figure 4 - Somatostatin immunolabeling (δ-pancreatic cells) in pancreatic islets on gestational day 21. A. Graph representing the percentage (%) of somatostatin-positive cells in relation to the total number of pancreatic islet cells; B. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for somatostatin (δ-pancreatic cells) (brown cytoplasmic staining) in the control group; C. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for somatostatin (δ-pancreatic cells) (brown cytoplasmic staining) in the offspring of SGA dams.



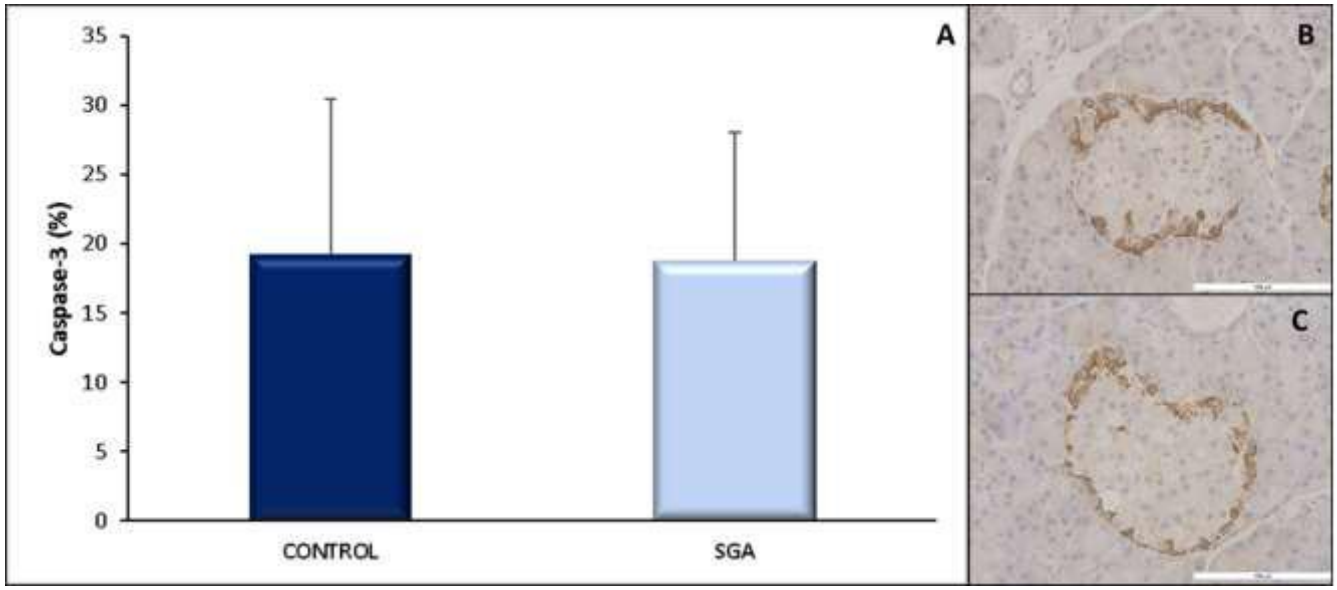
Data are expressed as mean ± standard deviation. *p < 0.05 – compared to the control group (Student's t-test). Source: Authors.

Figure 5 - Ki-67 immunolabeling. A. Graph representing the percentage (%) of Ki-67-positive cells (cell proliferation marker) in pancreatic islets on gestational day 21; B. Representative photomicrograph of a pancreatic islet with nuclear Ki-67 immunolabeling (nuclear staining) (blue arrow) in the Control group; C. Representative photomicrograph of a pancreatic islet with nuclear Ki-67 immunolabeling (nuclear staining) (blue arrow) in the SGA group.



Data are expressed as mean ± standard deviation. p > 0.05 – No statistically significant difference (Student's t-test). Source: Authors.

Figure 6 - Cleaved caspase-3 immunolabeling in pancreatic islets on gestational day 21. A. Graph representing the percentage (%) of cleaved caspase-3-positive cells in relation to the total number of pancreatic islet cells; B. Photomicrograph of the pancreas showing a pancreatic islet immunolabeled for cleaved caspase-3 (brown cytoplasmic staining – arrow) in the Control group; C. Photomicrograph of the pancreas showing a pancreatic islet (outlined in black) immunolabeled for cleaved caspase-3 (brown cytoplasmic staining) in the SGA group.



Data are expressed as mean ± standard deviation. p > 0.05 – No statistically significant difference (Student's t-test). Source: Authors.

4. Discussion

Our findings show that severe maternal diabetes led to lower offspring weight during the perinatal period. In adulthood, female offspring with fetal growth restriction exhibited a greater area under the curve (AUC) based on blood glucose measurements taken at different time points during the oral glucose tolerance test (OGTT). Harris (1998) observed that higher glycemic levels may result from reduced glucose uptake in peripheral tissues (skeletal muscle and adipose tissue). These findings support the Developmental Origins of Health and Disease (DOHaD) hypothesis, indicating a higher risk of metabolic disease (such as insulin resistance, impaired glucose tolerance, and type 2 diabetes) in growth-restricted females born to severely diabetic dams, even in the absence of any intervention.

This investigation demonstrated that female offspring born to severely diabetic mothers and classified as SGA (small for gestational age) accounted for more than 70% at birth, corroborating previous results from our laboratory (Corvino et al., 2015). Maternal hyperglycemia leads to overstimulation of fetal pancreatic islets, resulting in disorganized fetal beta cells that are unable to respond to stimuli (Aerts & Van Assche, 1977). When offspring from severely diabetic dams were evaluated one day before vaginal delivery, they showed hypoinsulinemia and were consequently born with low birth weight (approximately 20% lesser than controls) (Corvino et al., 2015), which occurred because the hyperglycemia affects the fetal insulin/insulin-like growth factor (IGF) system, impairing development and growth regulation (White et al., 2015).

The SGA rats showed elevated serum insulin concentrations, suggesting a state of insulin resistance. Growth-restricted newborns often undergo accelerated postnatal catch-up growth, which favors the development of insulin resistance and eventually diabetes (Berends et al., 2018). Furthermore, adult rats considered as SGA showed increased AUC and abnormal responses during the OGTT, indicating that maternal diabetes-induced growth restriction contributes to the development of hyperglycemia (Cruz et al., 2023).

Hyperglycemia observed in SGA rats led to a decrease in superoxide dismutase (SOD) and an increase in thiobarbituric acid reactive substances (TBARS) in the blood of these animals. There is evidence that hyperglycemia induces increased production of reactive oxygen species (ROS) due to the enhanced entry of reducing equivalents into the mitochondrial electron transport chain (Burgos-Morón et al., 2019). Oxidant agents are generated as a result of intracellular metabolism in mitochondria, peroxisomes, and various cytosolic enzymatic systems. Additionally, external factors can trigger ROS formation. To counteract this, organisms possess a sophisticated enzymatic and non-enzymatic antioxidant defense system, including enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). This defense system neutralizes and regulates ROS levels to maintain physiological homeostasis. Therefore, an imbalance between oxidant and antioxidant levels may act as a stress signal that activates specific redox-sensitive signaling pathways, leading to oxidative stress (Yaribeygi et al., 2019). Our SGA rats showed hyperglycemia and an imbalance in the redox homeostasis between antioxidants and oxidants, resulting in blood oxidative stress (Cruz et al., 2023). To determine whether glucose homeostasis alterations and oxidative stress were caused by changes in the endocrine pancreas of SGA rats, the cellular composition of the pancreatic islets was evaluated. No significant difference was found in the percentage of β-cells, suggesting that glucose intolerance in these animals may be due to defects in both insulin secretion and sensitivity, as also noted by Gatford et al. (2014).

In contrast, an increase in islet area and in the percentage of somatostatin-immunolabeled δ-cells was observed. These non-β cells play a key role in the glycemic response by transmitting regulatory signals to β-cells to modulate insulin secretion (Pfeifer et al., 2014). The relative increase in δ-cells reinforces the hypothesis that changes in non-β cells may also contribute to the glycemic dysregulation observed. No significant differences were found in proliferation or apoptosis indices in pancreatic islets. Conversely, Nazari et al. (2017) reported increased β-cell apoptosis in offspring of diabetic rats compared to

controls. This discrepancy may be attributed to the fact that Nazari et al. focused specifically on β-cells, whereas the present study evaluated pancreatic islets as a whole, without distinguishing between cell types. These findings highlight the need for further research to explore the relationship between specific cell types and the processes of apoptosis and proliferation in the context of diabetes-induced pancreatic alterations.

5. Conclusion

The present study demonstrated that uncontrolled maternal diabetes caused an intrauterine growth restriction in offspring. The altered growth trajectory is associated with glucose intolerance in adult females, as evidenced by increased area under the curve (AUC) in the oral glucose tolerance test (OGTT) and hyperinsulinemia. Although no significant changes were observed in the proportion of pancreatic β-cells, the increase in somatostatin-immunolabeled δ-cells suggests that glycemic dysfunction may involve alterations in non-β cells, affecting insulin signaling and secretion. These findings reinforce the Developmental Origins of Health and Disease (DOHaD) hypothesis, indicating that intrauterine insults, such as maternal diabetes can program long-lasting metabolic alterations, increasing the risk of insulin resistance and type 2 diabetes in adulthood, and underscore the need for additional studies to further elucidate the cellular mechanisms involved in this process.

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Conflict of interest

The authors declare no conflict of interest.

References

Aerts, L., & Van Assche, F. A. (1977). Rat foetal endocrine pancreas in experimental diabetes. J Endocrinol.73, 339-46.

Aerts, L., & Van Assche, F. A. (2006). Animal evidence for the transgenerational development of diabetes mellitus. Int J Biochem Cell Biol. 38(5-6), 894-903.

Akamine, C T & Yamamoto, R K (2009). Estudo dirigido: estatística descritiva. (3ed). Editora Érica. Bekman, OR & Costa Neto, PLO (2009). Análise estatística da decisão. (2ed). Ed. Edgar Blucher.

Atkinson, M .A., Campbell-Thompson, M., Kusmartseva, I., & Kaestner, K. H. (2020). Organisation of the human pancreas in health and in diabetes. Diabetologia. 63(10), 1966-1973.

Barker, D. J. P., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. J. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ.298, 564–7.

Benediktsson, R., Lindsay, R. S., Noble, J., Seckl, J. R., & Edwards, C. R. (1993) Glucocorticoid exposure in utero: new model for adult hypertension. Lancet. 341(8841), 339e41.

Berends, L. M., Dearden, L., Tung, Y. C. L., Voshol, P., Fernandez-Twinn, D. S., & Ozanne, S. E. (2018). Programming of central and peripheral insulin resistance by low birthweight and postnatal catch-up growth in male mice. Diabetologia. 61, 2225–34.

Brereton, M. F., Vergari, E., Zhang, Q., & Clark, A. (2015). Alpha-, Delta- and PP-cells: Are They the Architectural Cornerstones of Islet Structure and Co-ordination? J Histochem Cytochem. 63(8), 575-91.

Burgos-Morón, E., Abad-Jiménez, Z., Marañón, A. M., Iannantuoni, F., Escribano-López, I., López-Domènech, S., Salom, C., Jover, A., Mora, V., Roldan, I., Solá, E., Rocha, M., & Víctor, V. M. (2019). Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. J Clin Med. 4;8(9), 1385.

Chew, L. C., & Verma, R. P. (2021). Fetal growth restriction. In: StatPearls. StatPearls Publishing: Treasure Island (FL). 1–16.

Corvino, S. B., Netto, A. O., Sinzato, Y. K., Campos, K. E., Calderon, I. M., Rudge, M. V., et al. (2015a). Intrauterine growth restricted rats exercised at pregnancy. Reprod Sci. 22, 991–99.

Corvino, S. B., Volpato, G. T., Rudge, M. V., & Damasceno, D. C. (2015b). Intrauterine Growth Restricted Rats Exercised before and during Pregnancy: Maternal and Perinatal Repercussions. Evid Based Complement Alternat Med. 2015, 294850.

Cruz, L. L., Barco, V. S., Paula, V. G., Gallego, F. Q., Souza, M. R., Corrente, J. E., Zambrano, E., Volpato, G. T., & Damasceno, D. C. (2023). Severe Diabetes Induction as a Generational Model for Growth Restriction of Rat. Reprod Sci. 30(8), 2416-2428.

Fernandez-Twinn, D. S., & Ozanne, S. E. (2006). Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Phys Behav. 88(3), 234-243.

Gatford, K. L., Kaur, G., Falcão-Tebas, F., Wadley, G. D., Wlodek, M. E., Laker, R. C., Ebeling, P. R., & McConell, G. K. (2014). Exercise as an intervention to improve metabolic outcomes after intrauterine growth restriction. Am J Physiol Endocrinol Metab. 1;306(9), E999-1012.

Girard, J. R., Ferre, P., Gilbert, M., Kervran, A., Assan, R., & Marliss, E. B. (1977). Fetal metabolic response to maternal fasting in the rat. Am J Physiol. 232(5): E456e63.

Gluckman, P. D., & Hanson, M. A. (2007). Developmental plasticity and human disease: research directions. J Intern Med. 261(5), 461–471.

Gluckman, P. D., Hanson, M. A., & Buklijas, T. (2010). A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis. 1(1), 6-18.

Guzman, C., Cabrera, R., Cardenas, M., Larrea, F., Nathanielsz, P. W., & Zambrano, E. (2006) Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. J Physiol. 572, 97-108.

Hales, C .N., & Barker, D .J. (2001). The thrifty phenotype hypothesis. Br Med Bull. 60, 5–20.

Harris, R. B. S. (1998). Acute and chronic effects of leptin on glucose utilization in lean mice. Biochem Biophys Res Commun. 245, 502–9.

Holemans, K., Aerts, L., & Van Assche, F. A. (2003). Fetal growth restriction and consequences for the offspring in animal models. J Soc Gynecol Investig.10, 392–99.

Holemans, K., Aerts, L., & Van Assche, F. A. (2003). Fetal growth restric tion and consequences for the ofspring in animal models. J Soc Gynecol Investig. 10:392–9.

Kordowich, S., Mansouri A., & Collombat, P. (2009). Reprogramming into pancreatic endocrine cells based on developmental cues Mol. Cell. Endocrinol. 315, 11-18.

Lai, L. L., Lu, H. Q., Li, W. N., Huang, H. P., Zhou, H. Y., Leng, E. N., & Zhang, Y. Y. (2021). Protective effects of quercetin and crocin in the kidneys and liver of obese Sprague-Dawley rats with Type 2 diabetes: Effects of quercetin and crocin on T2DM rats. Hum Exp Toxicol. 40, 661-672.

Melamed, N., Baschat, A., Yinon, Y., Athanasiadis, A., Mecacci, F., Figueras, F., et al. (2021). FIGO (International Federation of Gynecology and obstetrics) initiative on fetal growth: best practice advice for screening, diagnosis, and management of fetal growth restriction. Int J Gynaecol Obstet. 152, 3–57.

Moraes-Souza, R. Q., Soares, T. S., Carmo, N. O., Damasceno, D. C., Campos, K. E., & Volpato, G. T. (2017). Adverse effects of Croton urucurana B. exposure during rat pregnancy. J. Ethnopharmacol. 199, 328–333,

Müller, T. D., Finan, B., Clemmensen, C., DiMarchi, R. D., & Tschöp, M. H. (2017). The New Biology and Pharmacology of Glucagon. Physiol Rev. 97(2), 721-766.

Nassar, A. H., Masrouha, K. Z., Itani, H., Nader, K. A., & Usta, I. M. (2012). Effects of sildenafil in nomega-nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. Am J Perinatol 29(6), 429e34.

Nathanielsz, P. W. (2006). Animal models that elucidate basic principles of the developmental origins of adult diseases. ILAR J. 47(1), 73-82.

Nazari, Z., Nabiuni, M., Ghaffari, S., Saeidi, M., Shahriyari, A., & Golalipour, M .J. (2017). Gestational Diabetes Induces Pancreatic Beta-Cells Apoptosis in Adult Rat Offspring. Int. J. Morphol. 35(1), 16-20, 2017.

Ozanne, S. E., & Hales, C. N. (2004). Lifespan: catch-up growth and obesity in male mice. Nature. 427(6973), 411-412.

Paula, V. G., Sinzato, Y. K., Moraes-Souza, R. Q., Soares, T. S., Souza, F Q G, Karki B, Andrade Paes A M, et al. (2022). Metabolic changes in female rats exposed to intrauterine hyperglycemia and post-weaning consumption of high-fat diet. Biol Reprod. 106, 200–12.

Pereira A. S. et al. (2018). Metodologia da pesquisa científica. [e-book gratuito]. Ed.UAB/NTE/UFSM. Shitsuka et al. (2014). Matemática fundamental para a tecnologia. São Paulo: Ed. Érica.

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance, Physiol. Rev. 98 (4), 2133–2223.

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. Nat Rev Endocrinol. 3(10), 572-587.

Pfeifer, C. R., Shomorony, A., Aronova, M. A., Zhang, G., Cai, T., Xu, H., Notkins, A. L., & Leapman, R. D. (2015). Quantitative analysis of mouse pancreatic islet architecture by serial block-face SEM. J Struct Biol.189(1), 44-52.

Ravelli, A. C., Van Der Meulen, J. H., Osmond, C., Barker, D. J., & Bleker, O. P. (1999). Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr. 70(5), 811-816.

Ravishankar, V., Buhimschi. C. S., Booth, C. J., Bhandari, V., Norwitz, E., Copel, J., et al. (2007). Fetal nucleated red blood cells in a rat model of intrauterine growth restriction induced by hypoxia and nitric oxide synthase inhibition. Am J Obstet Gynecol. 196(5). 482 e1e482 e8.

Resnick, O., Morgane, P. J., Hasson, R., & Miller, M. (1982). Overt and hidden forms of chronic malnutrition in the rat and their relevance to man. Neurosci Biobehav Rev. 6(1), 55e75.

Roseboom, T. J., Van der Meulen, J. H., Ravelli, A. C., et al. (1999). Blood pressure in adults after prenatal exposure to famine. J Hypertens. 17(3), 325-330.

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats-Behavioural and neurochemical aspects. Behav Brain Res. 222(1), 246-64.

Sinzato, Y. K., Gelaleti, R. B., Volpato, G. T., Rudge, M. V. C., Herrera, E., & Damasceno, D. C. (2020). Streptozotocin-induced leukocyte DNA damage in rats. Drug Chem Toxicol. 43(2), 165-168.

Sinzato, Y. K., Klöppel, E., Miranda, C. A., Paula, V. G., Alves, L. F., Nascimento, L. L. S., et al. (2021). Comparison of streptozotocin-induced diabetes at different moments of the life of female rats for translational studies. Lab Anim Res.55, 329–40.

Sinzato, Y. K., Paula, V. G., Gallego, F. Q., Moraes-Souza, R. Q., Corrente, J. E., Volpato, G. T., & Damasceno, D. C. (2022). Maternal diabetes and postnatal high-fat diet on pregnant offspring. Front Cell Dev Biol.10, 818621.

Tai, M. A. (1994). Mathematical model for the determination of total area under glucose tolerance and other metabolic curves. Diabetes Care.17, 152–4.

Tanaka, M., Natori, M., Ishimoto, H., Miyazaki, T., Kobayashi, T., & Nozawa, S. (1994). Experimental growth retardation produced by transient period of uteroplacental ischemia in pregnant Sprague-Dawley rats. Am J Obstet Gynecol. 171(5), 1231e4.

Tentor, L., & Salvati, A. M.(1981). Hemoglobinometry in human blood. Methods Enzymol. 1981;76, 707–15.

Volpato, G. T., Damasceno, D. C., Sinzato, Y. K., Ribeiro, V. M., Rudge, M. V., & Calderon, I. M. (2015). Oxidative stress status and placental implications in diabetic rats undergoing swimming exercise after embryonic implantation. Reprod Sci. 22(5), 602-8.

White, V., Jawerbaum, A., Mazzucco, M. B., Gauster, M., Desoye, G., & Hiden, U. (2015). Diabetes-associated changes in the fetal insulin/insulin-like growth factor system are organ specific in rats. Pediatr Res.77, 48-55.

Wigglesworth, J. S. (1974). Fetal growth retardation. Animal model: uterine vessel ligation in the pregnant rat. Am J Pathol. 77(2), 347e50.

Woodall, S. M., Breier, B. H., Johnston, B. M., Bassett, N. S., Barnard, R., & Gluckman, P. D. (1999). Administration of growth hormone or IGF-I to pregnant rats on a reduced diet throughout pregnancy does not prevent fetal intrauterine growth retardation and elevated blood pressure in adult offspring. J Endocrinol. 163(1), 69e77.

Woodall, S. M., Breier, B. H., Johnston, B. M., & Gluckman, P. D. (1996). A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. J Endocrinol. 150(2), 231e42.

Yaribeygi, H., Atkin, S. L., & Sahebkar, A. (2019). Mitochondrial dysfunction in diabetes and the regulatory roles of antidiabetic agents on the mitochondrial function. Journal of Cellular Physiology. 234 (6), 8402–8410.

Zambrano, E. (2009). The transgenerational mechanisms in developmental programming of metabolic diseases. Rev Invest Clin. 1(1), 41-52.

Zhou, W., & YE, S. (2018). Rapamycin improves insulin resistance and hepatic steatosis in type 2 diabetes rats through activation of autophagy. Cell Biol Int. 42, 1282-1291.