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BIOMEDICAL SCIENCES

Effects of diabetes between generations on the pre-embryos of rats

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Abstract: Pregestational hyperglycemia cause adverse effects on mothers and their offspring. We aimed to evaluate the maternal hyperglycemia influence on pre-embryos from diabetic rats and on their generations (daughters and granddaughters). Diabetes was induced in *Sprague-Dawley* rats. The mothers and their female pups were submitted to oral glucose tolerance test in adulthood. In day 4 of pregnancy, pre-embryos were collected for morphological analysis. The diabetic mother, daughter and granddaughter rats showed glucose intolerance and their pre-embryos presented developmental delay, degeneration and losses compared to the nondiabetic group. Thus, maternal diabetes transgenerationally affects embryos at early development, which contributes for embryofetal losses.

Key words: hyperglycemia, offspring, preimplantation, pregnancy, rodents.

INTRODUCTION

Pregestational hyperglycemia can cause adverse effects on mothers and their offspring. High rate of pregnancy loss, maternal morbidity, fetal macrosomia and neonatal death are related to maternal diabetic complications (Szmuilowicz et al. 2019). Studies have demonstrated that hyperglycemia damages embryogenesis as early as the preimplantation phase (Bueno et al. 2014, 2020), indicating that normal development requires a narrow glucose concentration range. Maternal hyperglycemia causes lower number of cells, developmental delay, and higher apoptosis rate in rat preimplantation embryos (Bueno et al. 2014). Furthermore, clinical, epidemiological and experimental studies confirm the intrauterine transmission of diabetes (Alejandro et al. 2020, Paula et al. 2022). These studies suggest that offspring exposed to maternal diabetes can develop metabolic disorders in adulthood, which can lead to a vicious cycle of metabolic diseases for several generations (Tozour et al. 2020). More studies are necessary to clear if these impairments can be prevented or comprise the future generations. Thus, our objective was to evaluate the maternal hyperglycemic influence on pre-embryos of diabetic rats and on their generations (daughters and granddaughters).

MATERIALS AND METHODS

All procedures and handling of animals used in this study were authorized by the Ethics Committee on the Use of Animals (CEUA) of our Institution (Protocol Number 1375/2021). 30-days-old male and female *Sprague-Dawley* rats, weighing around 100 g were maintained in our vivarium under controlled conditions of temperature (22±2º C), humidity (60 ± 10 %) and light/dark cycle (12 h). The animals were housed in groups of three in polypropylene boxes (41 x 34 x 16 cm) with autoclaved wood shavings and

with environmental enrichment (paper balls). Filtered water and feed were offered *ad libitum*.

The adult animals (90-days-old) were mated to obtain offspring, and their female pups were randomly distributed into two experimental groups. For the mild diabetes (MD) group, beta-cytotoxic drug (single dose of streptozotocin - 100 mg/kg, subcutaneously) was used at postnatal day 5 for diabetes induction. For the nondiabetic group (Control-C), only citrate buffer (vehicle) was used. At day 75 of life, the oral glucose tolerance test (OGTT) was made as inclusion criteria to C and MD groups according to Sinzato et al. (2021). At 110 days, all the C and MD rats were mated. The pregnant rats (n min = 6/group) in each group were killed on gestational day 4 (GD4) for analysis of their blastocysts. Another MD rats continued the pregnancy to obtain offspring (OMD). The adult OMD rats were similarly mated, and half was killed on GD4 for analysis of their blastocysts and another half were mated to obtain adult female offspring (granddaughters– GMD). These were also mated to obtain their pre-implantation embryos on GD4. All the generations were submitted to OGTT performed by a blinded evaluator in day 90 of life. The total areas under the curve (AUC) obtained by glycemic values were calculated for each rat (Tai 1994).

To study the transgenerational effects of maternal diabetes on preimplantation embryos, the pregnant rats (C, MD, OMD and GMD) were anesthetized by overdose with sodium thiopental and killed for decapitation at GD4. Following, the uterine horns were removed and flushed with 1mL saline/horn for collection of the preimplantation embryos. Pre-embryos were examined by a blinded evaluator using a phase-contrast microscope (DMR, Leica®, Brazil) to determine cell numbers and morphology. Preimplantation embryos were classified as abnormal when displaying blastomeres anomalies such as disarrangement, failure of compaction, irregular shape and large variation in size (Bueno et al. 2014).

The results were presented as the mean ± standard deviation. For the asymmetric distribution of the data, a Poisson distribution followed by Wald's multiple comparison test was used. $P< 0.05$ was considered as confidence limit.

RESULTS

The glycemia obtained during the OGTT were increased in MD, OMD and GMD adult rats (Figure 1a) and, consequently the AUC values were also increased compared to the C group (Figure 1b).

There was no difference in the corpora lutea numbers among the groups. The mean number of normal pre-embryos (blastocysts) was reduced in OMD and GMD compared to the other groups. A higher number of degenerating pre-embryos was observed in the rats MD and OMD compared to the C and GMD dams. In the groups OMD and GMD, the pre-embryo losses and with developmental delay was higher than in the groups C and MD (Table I). The illustrative figures of the pre-embryonic structural changes are shown in Figure S1 – Supplementary Material.

DISCUSSION

This study relates how maternal hyperglycemia influences on pre-embryos from diabetic rats and on their generations. Several structural complications on the pre-embryo and implantation process difficult the attachment in the endometrial epithelium (Bueno et al. 2020). MD group presented higher number of morphological alterations, while the OMD and GMD rats presented a higher number of pre-embryos with developmental delay. Furthermore, GMD group increased the embryonic losses

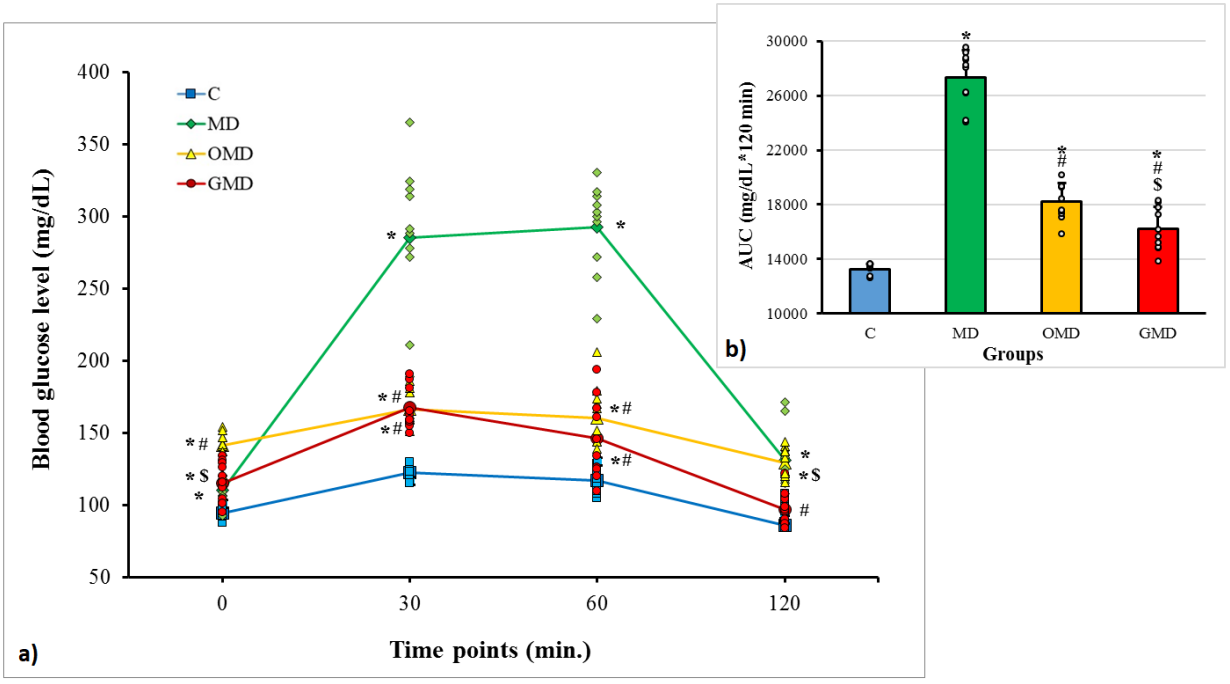

Figure 1. a) Oral Glucose Tolerance Test (OGTT) and **b)** Area under the curve (AUC) in 90 days of life of control (C) and mildly diabetic (MD) dams; and offspring (OMD) and granddaughters (GMD) exposed to transgenerational diabetes. Data reported as mean ± standard deviation. *p<0.05 – compared to the Control group; #p<0.05 - compared to the MD group; $p<0.05 – compared to the OMD group (Poisson distribution followed by Wald's multiple comparison test).

before implantation. In general, we suggest that hyperglycemia impaired oocyte quality due to changes in mitochondrial distribution and activity possibly by mitochondrial DNA mutations (Li et al. 2018) and might be related to the preimplantation embryo changes and losses. Besides, maternal oxidative stress can modify the redox state of preimplantation embryos, leading to an impaired growth and development of these offspring (Bueno et al. 2020). Consequently, the number of fetuses of diabetic rats are lower (Sinzato et al. 2021) due to these structural changes in the preimplantation embryos.

In human pregnancy, the newborns are macrosomic at birth. During pregnancy, diabetes-induced lipid transfer to the fetus occurs due to an increased maternal–fetal gradient, which may contribute to a larger body fat mass in newborns of diabetic women. In contrast, the fetuses from diabetic rats are classified as small for gestational age caused by fetal growth restriction during intrauterine milieu. The body fat increment occurs during the last trimester of intrauterine life. However, fat deposition in rat fetuses is lower than in humans, and the rats present a shorter pregnancy period and may not favor sufficient fat accumulation to cause macrosomia (Herrera & Amusquivar 2000).

In conclusion, this model of transgenerational diabetes corroborates that maternal hyperglycemia can disturb the implantation process due to changes in rat pre-embryos along generations. These structural alterations in pre-implantation embryos might explain why women with pregestational diabetes have higher rates of pregnancy losses, indicating that is necessary a tight glycemic control previously and in the course of pregnancy.

Table I. Pre-embryo (blastocyst) analysis on gestational day 4 of control (C) and mildly diabetic (MD) dams; and offspring (OMD) and granddaughters (GMD) exposed to transgenerational diabetes. Data reported as mean ± standard deviation. p<0.05 - different letters indicate significant statistically difference (Poisson Distribution followed by Wald's Multiple Comparison Test).

	C (n=12)	MD (n=8)	OMD (n=6)	GMD (n=10)
Corpora lutea	11.75 ± 2.26a	15.5 ± 4.11a	13.00 ± 3.69a	12.33 ± 3.87a
Normal pre-embryo (Blastocyst)	9.83 ± 2.48a	9.13 ± 3.04a	2.33 ± 2.73b	0.78 ± 1.56c
Degenerating pre-embryos	0.17 ± 0.39 a	5.13 ± 3.18b	0.17 ± 0.41a	0.78 ± 1.39c
Pre-embryos with developmental delay	0.83 ± 0.72a	1.00 ± 1.07a	5.67 ± 6.53b	3.89 ± 5.28b
Pre-embryonic loss before implantation	0.92 ± 1.00a	1.75 ± 2.87a	4.83 ± 4.75b	6.89 ± 4.78b

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SUPPLEMENTARY MATERIAL

Figure S1

How to cite

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