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Maternal and fetal outcomes of rats submitted to a high-fat diet and micronutrient mixture

Resultados maternos e fetais de ratos submetidos à dieta rica em gordura e mistura de micronutrientes

Resultados maternos y fetales de ratas sometidas a dieta rica en grasa y mezcla de micronutrientes

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Abstract
The consumption of high-fat diets is a global problem. The use of micronutrients, such as calcium and vitamin D, has positive effects on glucose metabolism during pregnancy, reproductive performance, and embryofetal development. This study aims to evaluate the effects of a mixture of micronutrients on glucose metabolism and perinatal outcomes in rats exposed to a high-fat diet (HFD). The female rats received a standard diet (SD) or high-fat diet (HFD) from weaning to the end of pregnancy. The pregnant rats were treated with the micronutrient mixture (Mix) only during pregnancy, and were distributed into 4 groups: SD, SD+Mix, HFD, and HFD+Mix. At the end of pregnancy, the rats were euthanized for collection of maternal adipose tissue, maternal and fetal blood samples, and placental samples. The HFD dams presented a higher number of fetuses with low weight at birth compared to the SD groups. Fetuses from SD+Mix and HFD+Mix had higher serum concentrations of vitamin D compared to the control respective groups. The HFD+mix group had higher maternal serum concentrations of vitamin D and increased rate of fetal viability, lower maternal visceral fat weight and lower percentage of embryonic deaths compared to the HFD group. Thus, the Mix treatment causes benefits to mothers and offspring exposed to HFD intake because of improved maternal glycemia, lower adiposity index, increased serum vitamin D, and embryofetal viability. This study shows the importance of prioritizing a healthy diet to achieve maternal physical well-being and enable the healthy development of offspring.
Keywords: Rodents; Pregnancy; High-Fat Diet; Micronutrients; Fetal Development.

Resumo
O consumo de dietas ricas em gordura é um problema global. Micronutrientes como cálcio e vitamina D trazem benefícios ao metabolismo da glicose, desempenho reprodutivo e desenvolvimento embriofetal. Este estudo tem como objetivo avaliar os efeitos de uma mistura de micronutrientes no metabolismo da glicose e nos resultados perinatais em ratos expostos a uma dieta rica em gordura (HFD). Ratas fêmeas receberam dieta padrão (DP) ou rica em gordura (DG) do desmame até o final da prenhez. Durante a gestação, um grupo recebeu a mistura de micronutrientes (Mix), formando quatro grupos: DP, DP+Mix, DG e DG+Mix. No final da prenhez, foram coletadas amostras de tecido

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adiposo, sangue materno e fetal e placenta. Ratas DG apresentaram maior número de fetos com baixo peso ao nascer em comparação às DP. Fetos de mães DP+Mix e DG+Mix tiveram concentrações séricas mais altas de vitamina D. O grupo DG+Mix apresentou maior concentração sérica materna de vitamina D, maior viabilidade fetal, menor peso de gordura visceral e menor taxa de mortes embrionárias em relação ao grupo DG. Assim, o tratamento com Mix trouxe benefícios para mães e filhos expostos à DG, promovendo melhor controle glicêmico materno, menor adiposidade, aumento da vitamina D e maior viabilidade embriofetal. Esse estudo reforça a importância de uma dieta saudável para o bem-estar materno e o desenvolvimento fetal adequado.
Palavras-chave: Roedores; Gravidez; Dieta Hiperlipídica; Micronutrientes; Desenvolvimento Fetal.

Resumen
El consumo de dietas ricas en grasas es un problema global. El uso de micronutrientes, como el calcio y la vitamina D, tiene efectos positivos sobre el metabolismo de la glucosa durante el embarazo, rendimiento reproductivo y el desarrollo embriofetal. Este estudio tiene como objetivo evaluar los efectos de una mezcla de micronutrientes sobre el metabolismo de la glucosa y los resultados perinatales en ratas expuestas a una dieta alta en grasas (HFD). Las ratas hembras recibieron una dieta estándar (SD) ó una dieta rica en grasa (HFD) desde el destete hasta el final del embarazo. Las ratas preñadas fueron tratadas con la mezcla de micronutrientes (Mix) solo durante el embarazo y se distribuyeron en 4 grupos: SD, SD+Mix, HFD y HFD+Mix. Al final del embarazo, las ratas fueron sacrificadas para la recolección de tejido adiposo materno, sangre materna y fetal y muestras de placenta. Las madres HFD presentaron mayor número de fetos con bajo peso al nacer en comparación con los grupos SD. Los fetos de madres SD+Mix y HFD+Mix tuvieron concentraciones séricas más altas de vitamina D en comparación con los respectivos grupos de control. El grupo HFD+Mix tuvo concentraciones séricas maternas más altas de vitamina D, mayor tasa de viabilidad fetal, menor peso de la grasa visceral materna y menor porcentaje de muertes embrionarias en comparación con el grupo HFD. Por tanto, el tratamiento Mix aporta beneficios a las madres y a los hijos expuestos a la ingesta de grasa debido a una mejor glucemia materna, menor índice de adiposidad, aumento de la vitamina D sérica y de la viabilidad embriofetal. Este estudio muestra la importancia de priorizar una alimentación saludable para lograr el bienestar físico materno y permitir el desarrollo saludable de la descendencia.
Palabras clave: Roedores; Embarazo; Dieta Alta En Grasa; Micronutrientes; Desarrollo Fetal.

1. Introduction

A healthy lifestyle during pregnancy is significant for the prevention of gestational diseases and is considered an essential requirement for a successful pregnancy (Phelan et al., 2020). Inadequate nutritional conditions during pregnancy can permanently change the structure and function of specific organs of the offspring (Langley-Evans & McMullen, 2010), leading to metabolic and functional diseases at adulthood. This concept, currently known as Developmental Origins of Health and Disease (DOHaD), addresses how changes occurring in critical windows of development can program offspring for health conditions or diseases later in life (Hanson, 2015). The DOHaD concept is not restricted only to the fetal phase but also includes other periods before and after pregnancy such as conception, embryonic period, breastfeeding, and infant period (Fleming et al., 2021). Offspring exposed to an unfavorable intrauterine environment such as malnutrition, diabetes, hypertension, and other maternal diseases may develop short- and long-term complications. DOHaD advocates strategies to reverse the processes that can cause fetal programming (Paauw et al., 2017).

Maternal high-fat diet intake is one of the insults that can compromise the embryofetal development. The consumption of high-fat diets (HFD) and decreased physical activity are factors that negatively influence the intrauterine environment, which also contribute significantly to the increased prevalence of diseases in the adult life of these offspring (Kayser et al., 2015; Sanchez-Garrido et al., 2015; Stevanović-Silva et al., 2021). Experimental models have been used to evaluate the consequences of consuming a high-fat diet. Studies have found that HFD alters metabolism, causing glucose intolerance (Paula et al., 2022), insulin resistance, and increased adiposity (Saullo et al., 2022) in rats in adulthood. Rats fed a high-fat diet from weaning until adulthood showed lower fertility rates due to ovarian dysfunction, negatively impacting reproduction (Paula et al., 2022), with a larger rate of fetuses classified as small for gestational age (Sinzato et al., 2022).

Adequate nutritional health is important before and during pregnancy and is essential for the maternal organism and the health of the offspring (Bull, 2021; Likhar & Patil, 2022; Soofi et al., 2022). Several interventions, such as physical exercise and supplementation with vitamins and antioxidants, before and during pregnancy have been evaluated to prevent short, medium, and long-term consequences in adult offspring (Lassi et al., 2014; ACOG, 2020; Ota et al., 2020; Ralston & Leuthner, 2022; Sebastiani et al., 2022). During pregnancy, vitamin and mineral supplements are widely used among women to reduce the risks of premature birth and low birth weight, which are especially effective when started during the first trimester (Karamali et al., 2018). Some micronutrients that are used include calcium, vitamin D, zinc, and magnesium, which have positive effects on glucose metabolism, metabolic control during pregnancy, reproductive outcomes, and embryofetal development (Asemi et al., 2015; Karamali et al., 2018). The most important contribution of vitamin D during pregnancy is to increase calcium absorption and placental calcium transport (Olmos-Ortiz et al., 2015). Furthermore, vitamin D regulates the immune system to control inflammation by inhibiting the action of pro-inflammatory cytokines. It also plays an important role in the placenta by stimulating the decidualization of the endometrium and the synthesis of progesterone and estradiol (Agarwal et al., 2018). Micronutrients are also important for insulin synthesis, secretion, and sensitivity, acting to increase insulin gene expression, depolarization of pancreatic cells, and increasing the expression of the insulin receptor (Asemi et al., 2015; Zhang et al., 2016; Karamali et al., 2016; Kilinc et al., 2018).

Studies showing the benefits of micronutrients against the high-fat diet-induced damage during pregnancy are still scarce. Therefore, we hypothesized that treatment with a mixture of calcium, vitamin D3, magnesium, and zinc reduces maternal glycemia, contributing to lower percentages of embryonic losses in rats exposed to HFD. Additionally, this maternal treatment might normalize the number of newborns with adequate weight. Therefore, the objective of this study is to evaluate the effects of the micronutrient mixture on glucose metabolism and perinatal outcomes in rats exposed to a high-fat diet (HFD).

2. Methodology

An experimental, laboratory research of a quantitative nature was carried out (Tossi & Petry, 2021; Pereira et al., 2018) using descriptive statistics using mean values, standard deviation, absolute frequency and relative percentage frequency (Shitsuka et al., 2014) and statistical analysis (Vieira, 2021).

Ethics Committee on the Use of Animals

The research project registered by the number 1218/2017 agrees with the Ethical Principles for Animal Research established by the National Council for Control of Animal Experimentation (CONCEA) and was approved by the Committee on Ethics in the Use of Animals of the Botucatu Medical School – São Paulo State University (UNESP).

Animals

Twenty-two days-old Sprague-Dawley rats were acquired from the Vivarium of the State University of Campinas (CEMIB-UNICAMP) and maintained in the Vivarium of the Experimental Research Laboratory of Gynecology and Obstetrics – Experimental Research Unit (UNIPEX) of the Faculty of Medicine of Botucatu-UNESP under controlled conditions of temperature (22±2ºC), humidity (70±10%), and light/dark cycles (12 h). Filtered water and food were offered *ad libitum*. As a form of environmental enrichment, paper balls were put in the cages (Simpson & Kelly, 2011).

Sample size calculation

Considering the four experimental groups in the pregnancy period and based on previous experiments conducted in our laboratory about the area under the curve using a power of 90% and an error of 5%, the calculated sample size was 10 animals per group.

Diet composition

At 22 post-natal days of life (after weaning), the female rats were distributed into the respective groups according to the diet types: a standard diet (SD, Kcal content: 28.54% protein, 62.65% carbohydrate, 8.7% fat, Purina, Brazil) or HFD (Kcal content: 23.43% protein, 46.63% carbohydrate, 30% fat, using lard as fat source) (Paula et al., 2022). The rats were subjected to an SD or HFD diet from weaning to the end of pregnancy.

Mating and experimental groups

At adulthood, male (only SD) and female (SD or HFD) Sprague-Dawley rats (200–250 g) were mated (1 male: 2 females) overnight. The following morning, when spermatozoa were found in the vaginal smear, it was designated as day 0 of pregnancy. After 15 consecutive days, the nonmated female rats (infertile) were anesthetized with overdose sodium thiopental (Thiopentax®, Cristália, Brazil, 120 mg/kg dose), euthanized and excluded from this study (Moraes-Souza et al., 2017). The mated rats were once more distributed to form the experimental groups that received the vehicle or the mixture containing Vitamin D and micronutrients during pregnancy: SD - Pregnant rats that received a standard diet + vehicle from weaning to the end of pregnancy; SD + Mix - Pregnant rats that received a standard diet and were treated with the mixture; HFD - Pregnant rats given a high-fat diet + vehicle from weaning until the end of pregnancy, and HFD + Mix - Pregnant rats that received a high-fat diet (HFD) and were treated with the mixture.

Treatment

Depending on the experimental group, each animal intragastrically (*gavage*) received the vehicle or the mixture in the morning throughout the pregnancy period (from day 0 to day 20 of pregnancy), except in the morning of laparotomy. The animals received 500 µL of vehicle (propylene glycol) or the mixture (containing 1 IU/kg of Vitamin D, 126 mg calcium, 12 mg magnesium, and 0.5 mg zinc). The composition of the mixture considered the daily recommendations for female rats of reproductive age specified by the National Research Council (US) Subcommittee on Laboratory Animal Nutrition (1995).

Oral glucose tolerance test (OGTT)

On the day 17 of pregnancy, the OGTT was performed. After six hours of fasting, a drop of blood was collected from the distal part of the tail of each rat for glycemic determination (time 0) using a conventional glucometer, and the values were expressed in milligrams per deciliter (mg/dL). Shortly after, the rats intragastrically (*gavage*) received a glucose solution (0.2 g/mL) at a dose of 2.0 g/kg of body weight. After 30, 60, and 120 minutes of glucose administration, blood glucose levels were determined again using a conventional glucometer (Sinzato et al., 2022). The total glucose response was assessed by estimating the area under the curve (AUC) using the trapezoidal method (Tai, 1994).

Blood Collection and Laparotomy

On the morning of the day 21 of pregnancy, blood glucose levels were measured using a conventional glucometer and the rats were intraperitoneally anesthetized with sodium thiopental (Thiopentax®, Cristália, Brazil, 120 mg/kg dose) and subjected to decapitation for blood collection to determine the serum concentrations of vitamin D (Code #80987) using commercial kits (Crystal Chemicals, USA). Afterward, a laparotomy was performed to expose the uterine horns, ovaries, fetuses, and placentas, which were removed to analyze the maternal reproductive outcomes. The fetuses were sexed and weighed with their respective placentas. The fetal weight and respective placenta [fetal weight (g)/placental weight (g)] were used for the determination of placental efficiency. After weighing, the fetuses were anesthetized and euthanized for blood collection (pool) to determine the serum concentrations of vitamin D using the same commercial kits (Crystal Chemicals, USA).

Maternal reproductive outcomes

Mothers who did not have live fetuses at the end of pregnancy were not included in the reproductive analyses. The pregnant uterus was dissected to count the number of live and dead fetuses and reabsorptions (embryonic deaths). The percentage of embryonic deaths was calculated as (Number of corpora lutea/implantation number) x 100. The percentage of fetal viability was calculated as (Number of alive fetuses/corpora lutea number) x 100.

Statistical analysis

For the comparative analysis of glycemia in the OGTT and AUC, the Gamma Distribution Test was used, while the Poisson Distribution and Wald tests were used for reproductive outcomes due to the high variability of the data. To compare the serum concentrations of vitamin D and periovarian fat weight, the Analysis of Variance (ANOVA) was used, followed by Tukey's Multiple Comparison Test. For the parameters of the number of male and female pups, fetal weight classification, and placental weight and efficiency, Fisher's Exact Test was used. For all statistical comparisons, a minimum confidence limit of 95% ($p < 0.05$) was considered.

3. Results and Discussion

Periovarian fat weight

Table 1 shows the weight of periovarian fat in rats intragastrically treated with vehicle or micronutrient mixture. Treatment with Mix reduced the weight of periovarian fat in the HFD/mix group compared to the HFD and SD groups.

Table 1 - Maternal periovarian fat weight (grams –g) on day 21 of pregnancy of rats exposed to high-fat diet from weaning to the end of pregnancy and treated with the vehicle or micronutrient mixture during pregnancy.

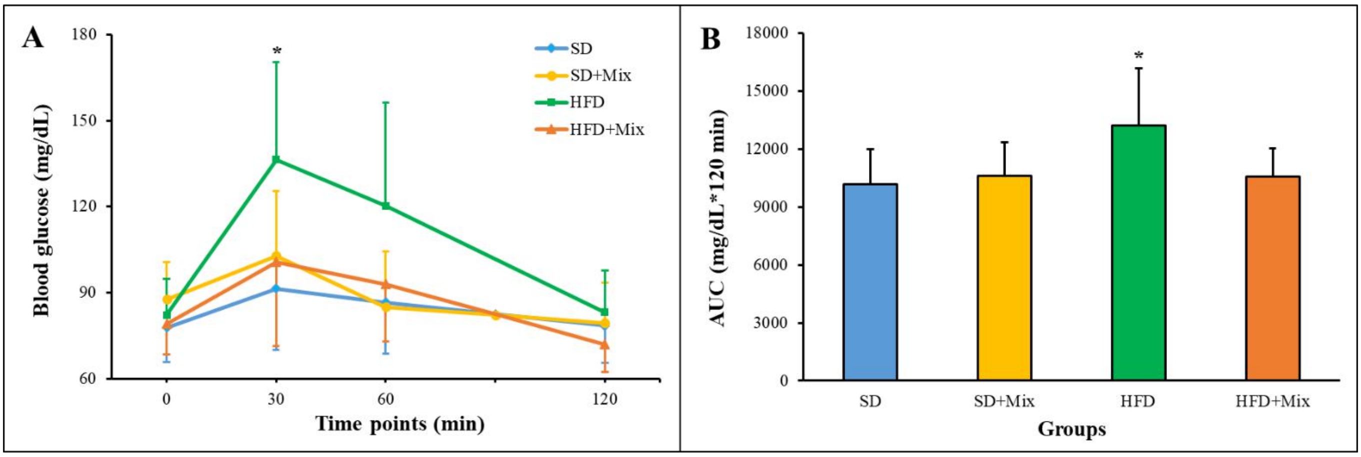
Variable	SD	SD + Mix	HFD	HFD + Mix
Periovarian fat (g)	0.75 ± 0.19	0.66 ± 0.17	0.84 ± 0.18*	0.39 ± 0.31$^{*\$}$

Values expressed as mean ± standard deviation (SD). n = 10 rats/group.
* *p< 0.05 – compared to the SD group;* $^{\$}$*p< 0.05 – compared to the HFD group (ANOVA followed by Tukey´s Multiple Comparison Test).*
Source: Survey Data (2025).

Oral glucose tolerance test (OGTT) and area under the curve (AUC)

During the OGTT, the HFD group presented higher blood glucose levels as compared to the SD group (Figure 1).

Figure 1 - Glucose tolerance test (OGTT) (A) and area under the curve (AUC) (B) on day 17 of pregnancy in rats given a standard diet (SD) or a high-fat diet (HFD) from weaning to the end of pregnancy and treated with a mixture of Vitamin D and micronutrients during pregnancy.



Values expressed as mean ± standard deviation (SD). n = 10 rats/group.
** p< 0.05 – compared to the SD group (Gamma Distribution and Wald Test). Source: Survey Data (2025).*

Serum concentrations of vitamin D

Table 2 shows the maternal and fetal serum concentrations of vitamin D of full-term pregnant rats treated with the vehicle or mixture. All pregnant rats treated with the mixture and their respective fetuses showed a significant increase in serum vitamin D as compared to HFD and SD groups.

Table 2 - Serum maternal and fetal concentrations of vitamin D on day 21 of pregnancy of rats given a standard diet (SD) or a high-fat diet (HFD) from weaning to the end of pregnancy and treated with a mixture of vitamin D and micronutrients during pregnancy.

Variable	SD	SD + Mix	HFD	HFD + Mix
Vitamin D (ng/mL) (maternal serum)	5.27±2.47	15.96±8.24*	1.76±0.95	21.53±13.10*$
Vitamin D (ng/mL) (fetal serum)	12.94±4.92	55.26±7.97*	15.54±3.68	65.64±11.27*$

Values are expressed as mean ± standard deviation (SD). n = 10 rats/group.
** p< 0.05 – compared to the SD group; $p< 0.05 – compared to the HFD group (ANOVA followed by Tukey´s Multiple Comparison Test). Source: Survey Data (2025).*

Embryonic deaths and fetal viability

The HFD rats had about 15% of embryonic deaths and 82% of fetal viability in relation to the SD group (7.5% and 96%, respectively). The HFD+Mix group presented a lower percentage of embryonic deaths and higher percentage of fetal viability as compared to the HFD group and in contrast than the SD rats (Figure 2).

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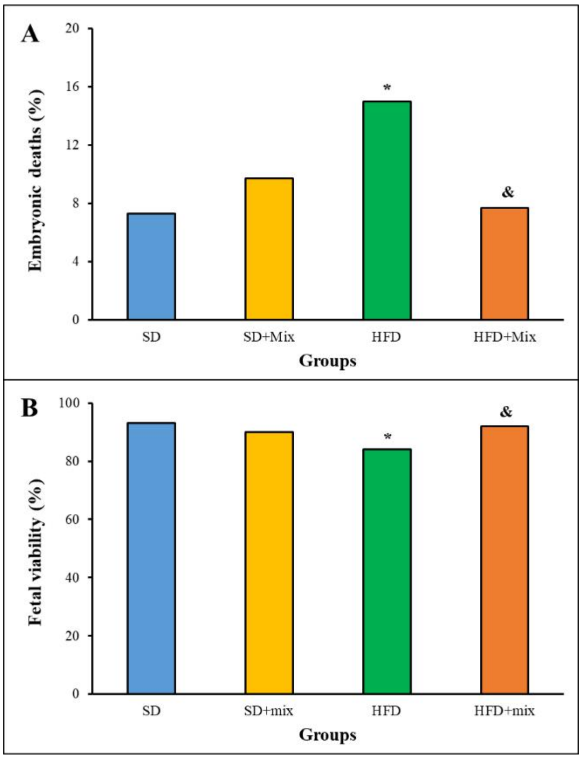
Figure 2 - Percentage of embryonic deaths (A) and fetal viability (B) of rats exposed to the standard diet (SD) or a high-fat diet (HFD) from weaning to the end of pregnancy and treated with a mixture of Vitamin D and micronutrients during pregnancy.



Values expressed as percentage (%). n = 10 rats/group.
* p< 0.05 – compared to the SD group, $^{\&}$p< 0.05 – compared to the HFD group (Fisher´s Exact test). Source: Survey Data (2025).

Fetal weight and placental efficiency

The fetal mean weight from HFD+Mix dams showed no difference (p>0.05) compared to those from HFD dams. There was lower fetal weight, regardless of the gender, in the HFD and HFD+Mix groups compared to the SD and SD+Mix groups. The male newborns presented higher weights than the females newborns in all the groups, except the male newborns from HFD+Mix dams because they showed no difference than female offspring of the same group. There was no significant difference related to placental weight and efficiency of male newborns among the groups. The placental weights of male newborns were greater than female one from the SD and SD+Mix dams at the end of pregnancy, however no gender difference was observed between male and female newborns from HFD and HFD+Mix dams. Additionally, the placental weight and efficiency of female newborns were lower and higher, respectively, in the HFD+Mix dams as compared to those from the HFD dams. The placental efficiency of male and female newborns from the HFD dams was lower compared those from the SD dams, but the female newborns from HFD+Mix presented greater placental efficiency compared to those from HFD rats. No difference on the placental efficiency was verified between male and female newborns from different dams (p>0.05) (Table 3).

Table 3 - Fetal weight, and placental weight and efficiency on day 21 of pregnancy of rats exposed to a standard (SD) or high-fat diet (HFD) from weaning to the end of pregnancy and treated with a mixture of vitamin D and micronutrients during pregnancy.

Variables	SD	SD+Mix	HFD	HFD+Mix
	Female, n= 39 Male, n= 49	**Female, n= 32 Male, n= 32**	**Female, n= 46 Male, n= 52**	**Female, n= 40 Male, n= 43**
Mean fetal weight (g)[a]	5.84±0.33	5.69±0.58	5.14±0,66*	5.14±0.64*#
Male fetal weight (g)[a]	5.93±0.42A	5.88±0.54A	5.33±0.67A*#	5.22±0.59A*#
Female fetal weight (g)[a]	5.60±0.44B	5.49±0.55B	4.99±0.59B*#	5.05±0.69A*#
Mean placental weight (g)[a]	0.56±0.10	0.51±0.05	0.53±0.08	0.50±0.08
Placental weight (male) (g)[a]	0.54±0.07A	0.52±0.05A	0.53±0.08A	0.51±0.09A
Placental weight (female) (g)[a]	0.53±0.09B	0.50±0.05B	0.53±0.08A	0.48±0.07A$
Mean placental efficiency	10.74±1.43	11.22±1.26	9.89±1.57*	10.58±2.04$
Placental efficiency (male)	11.06±1.30A	11.31±1.26A	10.11±1.61A*#	10.41±1.94A
Placental efficiency (female)	10.81±1.46A	11.13±1.28A	9.64±1.52A*#	10.77±2.16A$

Values expressed as mean ± standard deviation (SD). n = 10 rats/group.
**p< 0.05 – compared to the SD group; #p<0.05 – compared to the SD/mix group; &p<0.05 – compared to the HFD group ([a]Poison Distribution and Wald test; Fisher´s Exact test).*
Different capital letters: difference between male and female offspring. Source: Survey Data (2025).

4. Discussion

To minimize the HFD-induced damage, such as glucose intolerance (Paula et al., 2022), increased visceral adiposity (Gomes et al., 2023), lower fetal weight due to decreased placental efficiency, and increased embryonic deaths (Sinzato et al., 2022), we proposed to offer a mixture of vitamin D and micronutrients (calcium, zinc and magnesium) to the HFD rats during pregnancy. Our main findings showed that dams and their respective fetuses presented higher maternal serum levels of vitamin D after mixture intervention of these dams, and there was a reduction of the blood glucose levels, contributing to a decline of the embryonic deaths, increased fetal viability and placental efficiency in HFD+Mix rats.

There is evidence that vitamin D improves energy metabolism by several pathways. Vitamin D supplementation increases fatty acid oxidation (Marcotorchino et al., 2014). Lower adipose tissue mass gain was observed in mice fed HFD and supplemented with vitamin D due to increased energy expenditure and the preferential consumption of lipids as an energy source (Marcotorchino et al., 2014). Karamali et al. (2018) observed the benefit of a mixture of magnesium, zinc, calcium, and vitamin D in the glycemic control of gestational diabetes.

Literature shows conflicting results regarding specific markers for obesity induced by a high-fat diet (Férézou-Viala et al., 2007; Couvreur et al., 2011; Elahi et al., 2009; Dearden et al., 2014; King et al., 2014). Although many experimental animals exposed to high-fat diets present higher pre-pregnancy weight and/or impaired glucose metabolism, the resulting obesity may not accurately reflect human overweight phenotypes (Christians et al., 2019). The fat percentage used in these experimental studies varies from 45 to 60% (Assis et al., 2006; Srinivasan et al., 2006; Christians et al., 2019), which does not reproduce human consumption that ranges from 30 to 40% (Christians et al., 2019). In our study, HFD corresponded to 30% of lipids (Paula et al., 2022), which caused an augmented weight of periovarian adipose tissue (Saullo et al., 2022). The HFD group that received the mixture had a reduction in the absolute weight of periovarian adipose tissue at the end of pregnancy. Observational studies have demonstrated that calcium intake is inversely associated with body weight (Gonzalez et al., 2006; Bueno et al., 2008), dyslipidemia (Jacqmain et al., 2003), and type 2 diabetes (Pittas et al., 2007). In the same context, it has been reported that higher vitamin D intake and higher serum 25(OH)D levels are related to lower adiposity (Caron-Jobin et al., 2011; Sulistyoningrum et al., 2012) and metabolic health (Tai et al., 2008). These findings corroborate our results, suggesting that the treatment with micronutrients contributed to the decreased weight of the periovarian fat in the HFD group.

HFD exposure makes rodents more susceptible to metabolic complications (Sanchez-Garrido et al., 2015), like as HFD-fed animal models have presented hyperglycemia, glucose intolerance, and insulin resistance (Park et al., 2011; Wu et al., 2019). In our study, rats that received a high-fat diet showed higher blood glucose levels during OGTT, leading to the enlarged availability of glucose in the bloodstream per minute, confirmed by higher AUC values. In contrast, the HFD rats that received treatment with a mixture of vitamin D and micronutrients showed lower glycemic levels during OGTT. These were similar values to groups that were fed the standard diet, indicating that the treatment contributes to the glycemic homeostasis that was impaired by the high-fat diet. Both groups that received the micronutrient mixture had a significant increase in serum 25 (OH)D levels. Vitamin D is necessary for normal insulin secretion and glucose tolerance (Amirasgari et al., 2019) and improves insulin sensitivity by attenuating the expression of pro-inflammatory cytokines and oxidative stress (Tanaka et al., 1984; Jamilian et al., 2019). Clinical studies showed that a combined therapy of vitamin D and calcium (Batra et al., 2019) and the association of micronutrients (Jamilian et al., 2019) were significantly effective in controlling the glycemic profile of patients, corroborating our results using a model of experimental animals.

In this study, the HFD dams had a higher weight in adipose tissue, which might increase the inflammatory processes in the placenta, leading to damaged maternal-fetal exchanges (Johns et al., 2020), irregular estrous cycles, decreased fertility rates, and impaired follicular development that contributed to a decreased ovarian reserve induced by larger inflammation in the ovarian region (Hohos & Skaznik-Wikiel, 2017; Paula et al., 2022), contributing to high rates of embryofetal losses. The HFD rat mothers that were supplemented with vitamin D during pregnancy presented 12 times more this vitamin than HFD rats, confirming a positive supplementation. In addition to the increase of serum 25 (OH)D levels in treated dams, fetal serum levels showed higher concentrations of 25 (OH)D than in their mothers, regardless of diet. The fetus is completely dependent on the maternal supply of 25(OH)D. 25(OH)D readily crosses the placenta and is activated to 1,25(OH)2D by the fetal kidneys (Rodda et al., 2015). After treatment with the supplementation of vitamin D, calcium, zinc, and magnesium, the HFD rats had a decreased adiposity and, consequently, an attenuation of the inflammatory process (Caron-Jobin et al., 2011; Sulistyoningrum et al., 2012; Jamilian et al., 2019), which contributed to increase the fetal viability and to reduce the embryonic deaths.

Additionally, the groups fed a high-fat diet showed a high rate of low-birth-weight fetuses. A high-fat diet administered during pregnancy changes several maternal plasma/serum components and causes fetal maldevelopment of rats (Wentzel et al., 2019). There is controversial data regarding fetal weight in HFD models, as the increase in weight gain depends on the percentage of fat and the exposure time. Furthermore, studies show that differences in species are also factors that contribute to this variability, showing that there is a tendency for higher fetal weight in mice and lower weight in rats at birth (Ribaroff et al., 2017; Christians et al., 2019). This corroborates our results since newborns from HFD dams were born with low fetal weight. The placenta is the main regulator of fetal growth, and placental structural changes might explain poor fetal growth. HFD models have shown that there is a marked inflammatory process in the placenta related to a reduced supply of nutrients and oxygen to the fetus (Loardi et al., 2015; Challier et al., 2017; Johns et al., 2020), although these pathophysiological mechanisms are not well understood. Then, we suggest that the decreased fetal growth in our study might be related to the lower placental efficiency of the rats exposed to HFD, as confirmed in our findings.

Vitamin D acts as a regulator of calcium, balancing the parathyroid hormone in the body, and can significantly impact fetal growth (Jakubiec-Wisniewska et al., 2022). Some studies have shown that vitamin D improves fetal weight at birth (Ibrahim et al., 2019; Jakubiec-Wisniewska et al., 2022). However, studies state the relationship between increased fetal weight and vitamin D treatment is at doses above 500 IU/day (Henry & Norman, 1984; Jakubiec-Wisniewska et al., 2022). Our findings showed that the supplementation with the mixture showed no improvement in these parameters. Therefore, we might suggest that the dose used in this study was not able to influence fetal growth. In the present study, the male fetuses had greater weights than the female one. Multiple factors influence fetal growth during pregnancy (Pedersen, 1980). Studies evaluated sexual dimorphism and showed that sex is also a factor that influences fetal growth, and in general the higher weight has been observed in male newborns than in females at birth (Clarke, 1788; Cooperstock & Campbell, 1996; Alur, 2019). The especial data observed in this study was HFD+Mix dams presented a reduction of the female placental weights, but higher placental efficiency, suggesting that the mixture of vitamin D, calcium and zinc was favorable to change the placenta. This positive effect contributed to increase the weight of the female newborns, which presented no difference of the male ones. Furthermore, there are sex-specific anthropometric, hormonal, and placental influences, and several of them are yet unknown and appear to interact in many complex ways, which impairs the fetal growth (Alur, 2019).

As possible limitations of this study, we must mention the importance of the dosage of calcium to associate with vitamin D levels. In addition, the measurement of serum insulin and pro-inflammatory cytokine levels, and morphological analysis of the placenta could contribute to integrate the findings.

5. Conclusion

Thus, the rats fed a high-fat diet and treated with a mixture of micronutrients presented better maternal outcomes such as normoglycemia, lower adiposity index, and increased serum vitamin D, as well as benefits on fetal outcomes (increased levels of serum vitamin D, greater fetal viability, and lower embryonic deaths), and higher female placental efficiency.This suggests that treatment with a mixture of nutrients during pregnancy may be an effective alternative to reduce the maternal high-fat diet-induced damage. However, it does not exclude the fact that pregnant women should prioritize a healthy diet and lifestyle.

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