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RELATIONSHIP BETWEEN MATERNAL METABOLISM AND ALTERATIONS IN THE DEVELOPMENT OF THE OFFSPRINGS OF DIABETIC AND NON-DIABETIC RATS.

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ABSTRACT

Summary

We used 99 Rats of the Sprague-Dawley strain that weighed between 250 and 350g. They were randomly divided into 4 groups, using diferent dose of STZ. In serum chemistry, diabetic rats showed higher glucose levels than the control ($p < 0.001$). Cholesterol showed no significant difference between the groups. The levels of triglycerides and VLDL were higher in EI than in EII ($P = 0.014$), CI ($P = 0.005$) and CII ($p = 0.001$). Insulin was lower in EI than in EII ($P = 0.004$), CI ($P = 0.007$), and CII ($P = 0.018$). The percentage of HbA1c was higher in diabetic rats than in non-diabetics ($p < 0.001$).

The mortality of the rats of the lot EI was greater than those of the rats of the lot EII ($P < 0.001$). None of the rats EI was able to finish their gestation. Only 23.64% of EI rats had offspring while 86% of the CI and 100% of the CII did so in a normal way ($p < 0.001$). The EII rats, had longer gestations (20.5 h) than those of the control groups ($P = 0.006$). There was no difference in the number of broods per litter between the 3 groups. Diabetic rats had higher levels of glycosuria than controls. The gruoup EI had significantly higher levels of ketonuria than rats in the batches EII, CI and CII ($p < 0.001$). However, EII rats showed higher levels of ketonuria than control rats

KEYWORDS : Streptozotocin, Offsprings, diabetic rats

INTRODUCTION.

Diabetes mellitus is a multifactorial disease given by absolute or relative deficiency of insulin, which manifests itself through a wide variety of clinical and biochemical alterations that will depend on the underlying pathogenesis, environmental factors, metabolic decontrol and the progressive damage of the different tissues [1,2].

Diabetes affects practically all the systems of the organism, making the reproductive not an exception. In men, it causes impotence, retrograde ejaculation, loss of seminal emission and alterations in the spermatogenesis [3,4]. In women, it produces alterations in the follicle genesis, anovulation, corpus luteum insufficiency, uterine involution and reduction of the levels of progesterone, as well as alterations in the retention of the gestation and the development of the products [5,6,7].

Maternal diabetes has long been associated with delayed fetal growth and increased incidence of congenital malformations, as well as increased maternal-fetal morbidity and mortality. Although there has been a positive impact on morbidity through strict control based on diet and treatment with insulin, there are reports in which it is observed that the damage is not fully controlled [8]. The incidence of congenital malformations is 2.3 times higher in fetuses of diabetic mothers than in those of non-diabetics and cause up to 40% of perinatal mortality [10,11].

The mechanisms by which diabetes affects the development have not yet been fully identified, but some factors such as genetic and/or environmental are related to the pathogenesis of this disease and have recently been given a leading role in the transmission of the disease [53].

The environmental factors that impact most of the embryonic development are the increase in glucose and the production of ketone in the mother [12].

Because of the ethical impossibility of studying the underlying mechanisms of diabetes in humans, a wide variety of animal models of diabetes have been developed. These range from those who have spontaneous diabetes, those induced by chemical agents and animals with mutations for such effects. One of the most used models in the study of embryonic development is that of diabetes induced to rodents with chemical agents (Aloxana (AL) and Streptozotocina (STZ)). In these animals, the diabetic condition mimics-present hyperglycemia-, although other factors that occur in diabetes are left aside, so the comparison between these and what happens in humans should be done with caution [13].

In rats, diabetes causes alterations in the estrus cycle, reduces fertility and severely affects gestation [14]. Diabetic rats have fewer sites of uterine implantation, increased frequency of reabsorptions (which would be the equivalent of abortions in humans), lower number of broods per litter with lower viability and increased frequency of congenital malformations [15, 16]. In rats as in humans, the damage is produced during early embryogenesis presenting irreversible effects in the development of the embryos [15,17].

Embryos and fetuses of diabetic rats present signs of delay in their development such as low weight and low size (head-rump length, head and tail) [17, 18]. During early embryogenesis, glucose can cause DNA damage, leading to mutations that unable the expression of critical genes of the development for normal embryogenesis to take place [19]. This damage can delay the duplication time of essential DNA and cell division in the organogenesis. In fact, low doses of glucose in a short period of time produce mutations in the DNA [19].

It has been shown that the teratogenic effect depends on multiple factors such as hyperglycemia and ketonemia. The excessive production of ketone [20, 21] in diabetes- especially the B-hydroxybutyrate [22, 23]-, alters the intrauterine environment and when it's combined with hyperglycemia, synergizes its effect, causing fetal growth retardation and increasing the incidence of congenital malformations [24]. Another proposed mechanism for the genesis of congenital malformations is the damage to different tissues dependent on the increase in the formation of oxygen-free radicals. In fact, it has been observed that supplementing the diet with high doses of antioxidants such as vitamin E and C and gamma-linoleic acid, alone or combined, significantly decrease the dysmorphogenesis and defects of ossification in fetuses of 20 days of gestation [50, 52]. There has also been an increase in the incidence of flow regression syndrome (very common in offspring of diabetic rats) in embryos of diabetic mice treated with retinoic acid (vitamin A) [51].

Thus, it appears that the abnormal intrauterine environment causes morphological and functional changes in developing fetuses, with consequences for adult life. Females that develop in a diabetic environment may present glucose intolerance and gestational diabetes during adulthood [44].

It has also been reported that the establishment of the hypothalamic-pituitary-gonad axis is delayed in adolescent offspring of diabetic rats and decreased LH levels up to 1.5 times. However, they seem to be able to compensate for these alterations, as they do not present any serious problems during reproduction [45,46].

The objective of this study is to determine the effect of maternal diabetes on the extra uterine development of the offsprings until birth and during the first 30 days of life.

ANIMALS AND METHODOLOGY.

We used 99 Rats of the Sprague-Dawley strain that weighed between 250 and 350g. They were kept in individual boxes with temperature control and 12/12 darkness light cycles. They were allowed ad libitum access to food and water. They mated in proportion to 3:1 all night long. The presence of vaginal stopper and/or sperm in the vaginal smear the following morning, determined this as day 1 of gestation. All the rats were weighed twice a week and glucose from the tail vein, glycosuria and ketonuria was measured, once a week. They were randomly divided into 4 groups:

Group 1 (EI).-19 rats who were injected via intraperitoneal (IP), a single dose of 60 mg/kg of streptozotocin (STZ), diluted in acetate buffer 0.1 M pH 4.3 the 6th day of gestation.

Group 2 (IBD).-56 rats who were injected via IP a single dose of 50 mg/kg of STZ the 6th day of gestation.

Group 3 (CI).-15 rats injected with the acetate buffer used to dissolve the STZ.

Goup 4 (CII).-9 rats injected with a needle of the same caliber used in the previous groups.

All rats were allowed to arrive at spontaneous birth and was recorded: gestation days, number of broods per litter, weight and size (head-rump length + head length + tail length) at birth, viability (ability to survive the first 6 hours of extra uterine life (EUL) and the presence of macroscopic congenital malformations. The offsprings remained with their mothers until weaning (at 30 days of EUL), day when the mother was slaughtered by cervical excision. Total blood was obtained from the aorta and the determination of glycated hemoglobin fraction 1c (A1C) was made immediately. The rest of the blood was centrifuged at 3.500 rpm for 10 minutes and the serum was separated, which was stored at-70 º C until be processed later. Determinations were made of glucose, cholesterol, triglycerides, VLDL and insulin. All the offsprings were weighed and measured once a week, taking monthly glucose determinations of the caudal vein on day 30 of EUL. The survival of the offsprings (ability to survive through time) was recorded during the entire study period. All results are expressed in average ± standard deviation. The ANOVA test was performed with a Tukey posttest and the significant difference is considered when drops the value of $P < 0.05$.

RESULTS

The mortality of the rats of the lot EI was greater than those of the rats of the lot EII ($P < 0.001$). None of the rats EI was able to finish their gestation. Only 23.64% of EI rats had offspring while 86% of the CI and 100% of the CII did so in a normal way ($p < 0.001$). The EII rats, had longer gestations (20.5 h) than those of the control groups ($P = 0.006$). There was no difference in the number of broods per litter between the 3 groups (table 1).

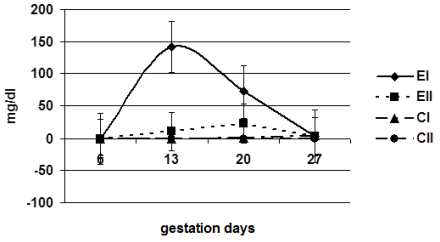
TABLE 1. *Group Comparison*

GROUP	% rats with 11.12 mM	glycemia mortality (%)	% of rats that end gestation	duration of gestation (días)	Broods per Litter
EI (n=19)	57.89*	38.18*º	0*º	-	-
EII (n=56)	62.5*	7.27^{NS}	23.64*	24.23 0.89*	9.31 2.43^{NS}
CI (n=15)	0	0	86	23.16 1.02	9.17 3.38
CII (n=9)	0	0	100	23.62 1.06	8.66 3.08

Data expressed in average ± DE. * $p < 0.01$ vs CI/CII, º $p = 0.007$ vs EII; NSp = Non-significant vs CI/CII Diabetic rats had less body weight during gestation than control rats ($p < 0.001$). Serum glucose levels during gestation were similar in EI and EII and higher than that of CI and CII ($p < 0.001$) (Data not presented).

Diabetic rats had higher levels of glycosuria than controls. The gruoup EI had significantly higher levels of ketonuria than rats in the batches EII, CI and CII ($p < 0.001$). However, EII rats showed higher levels of ketonuria than control rats (Figure 1).

FIGURE 1. *Ketonuria during gestation*



In serum chemistry, diabetic rats showed higher glucose levels than the control ($p < 0.001$). Cholesterol showed no significant difference between the groups. The levels of triglycerides and VLDL were higher in EI than in EII ($P = 0.014$), CI ($P = 0.005$) and CII ($p = 0.001$). Insulin was lower in EI than in EII ($P = 0.004$), CI ($P = 0.007$), and CII ($P = 0.018$). The percentage of HbA1c was higher in diabetic rats than in non-diabetics ($p < 0.001$) (table 2).

Table 2. *Serum analysis of diabetic and non-diabetic rats.*

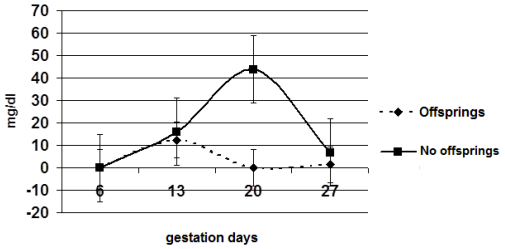
GROUP	Glucose (mM/l)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	HbA_{1c} (%)	Insulin U/dl
EI (n=6)	29.52 7.09*	76.25 12^{NS}	353.4 202.58*º	71 40.36*º	6.23 0.68*	11.10 3.1*º
EII (n=29)	25.59 9.02*	61.7 16.13^{NS}	153.82 114.4^{NS}	30.71 22.9^{NS}	7.96 1.9*	17.85 5.35^{NS}
CI (n=15)	5.78 1.43	55.86 8.12	124.93 29.02	25 5.79	3.64 0.43	18.11 5.39
CII (n=9)	5.33 1.80	48.33 9.89	72.78 28.87	14.67 5.66	3.49 0.31	17.80 4.75

Data on average ± of. * $p < 0.01$ vs CI/CII, º $p < 0.01$ vs EII, NS p = non-significant

Analysis of the EII group

In order to determine what was the most relevant factor in the poor development of the products in diabetic rats, it was analyzed separately from the EII group. Of the 56 rats injected with 50 mg/kg of STZ, only 29 survived or showed glucose levels at 48 hrs. 200 mg/dl. Of these, 13 completed gestation (44.83%). The rats with offspring weighed more on days 20 and 22 than those that did not have offspring ($p < 0.001$); Both groups had similar levels of blood glucose and glycosuria during gestation (data not shown). Rats without offspring showed significantly higher levels of ketonuria on the 20th day of gestation ($P = 0.002$) (Figure 2).

FIGURE 2. Ketonuria of EII rats during gestation.



In the serum chemistry done to slaughter, rats that did not have offsprings had higher levels of cholesterol ($P = 0.036$), triglycerides ($P = 0.042$) and VLDL ($P = 0.042$) as well as lower levels of insulin ($P = 0.023$) than the rats that did have offsprings. Glucose and HbA1c levels showed no significant difference between rats with or without calves (table 3).

Table 3. *Blood Test in EII rats.*

GROUP EII	glucose (mm/l)	cholesterol (mg/dl)	triglycerides (mg/dl)	VLDL (mg/dl)	HbA_{1c} (%)	Insulin (U/dl)
Con crías (n=13)	26.78 10.61	58.92 16.32	108.9 57.80	25.87 11,.62	9.06 2,5	20.07 3.9
Sin crías (n=16)	25.09 7.52	74.06 19.98	197.47 133.6	39.4 26.78	7.6 1.07	16.12 4.3
p=	0.559	0.036	0.042	0.043	0.394	0.023

Data expressed in average ± DE.

OFFSPRINGS:

The offsprings of EII rats presented weight and size at birth similar to the control rats. Of the 119 newborn diabetics only 66.13% were viable ($P < 0.01$ vs CI and CII). The 30-day survival was higher in control groups than in the experimental group. No newborns showed major congenital malformations. The offsprings of diabetic rats weighed and measured less during the first month of life than the controls ($p < 0.01$) (data not shown).

Data expressed in average ± DE.

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DISCUSSION:

STZ is widely used to induce diabetes in rats. The affinity of this substance by B-pancreatic cells is greater than that of Alloxan and its effect is permanent and less toxic to other organs different from the pancreas (i.e. kidney), allowing rats to survive longer without treatment [13].

Non-lethal doses of STZ range from 45 to 100 mg/dl, which can be administered intraperitoneally or intravenously, in single dose or fractionated in three consecutive days. The doses that are used most frequently range from 50 to 70 mg/kg of weight. This dose causes a permanent hyperglycemia with serum glucose levels of 30mm/L and without ketogenesis. Higher doses produce ketogenesis and death of the animal if it is not controlled with insulin therapy [13]. The study showed that STZ at doses of 50mg/kg (EII group) was as effective as 60 mg/kg (EI lot) to induce diabetes, but significantly reduces the mortality of rats, allowing some of them to successfully terminate gestation.

It has been seen that the teratogenic effect of hyperglycemia will depend on the levels of the same and the gestation stage in which the embryo is found [17]. Chronic hyperglycemia alters embryonic development from very early stages, in which a large part of important events are taking place, such as gastrulation, neural induction, symmetry determination, etc.-in fact, at this stage, most of the genes that control the development are activated by [11, 13]- what affects different organs and systems, causing the increase in the incidence of congenital malformations or intrauterine death. The mechanisms are not yet known so this occurs, but recently it has been associated with alterations in cellular communications that manifests with damage to the membranes [48]. The most common alterations range from minor malformations to major malformations that are incompatible with life and result in intrauterine death of fetuses [13-15]. Another environmental factor that alters the intrauterine medium is the presence of ketone. Some researchers consider that the ketone have more influence in the teratogenic processes than the hyperglycemia per se [18], although when their effect is combined it makes synergy. This has been experienced in vitro, combining a minimum amount of glucose with a minimum amount of B-hidroxibutyrato in cultures of embryos of rats, finding a reduction in the number of somites, head-rump length and protein content in these embryos than in those cultivated in any of these single metabolites [14]. We found that rats that did not have offsprings showed higher levels of ketonuria during gestation, while controls do not have ketones in urine. This factor, combined with severe hyperglycemia, may have been the cause of fetal death and subsequent reabsorption of fetuses. Other authors have reported similar findings [17].

There are other factors that may be indirectly related to teratogenic processes such as hypertriglyceridemia and the increase in VLDL [14,17] as well as hypoinsulinemia [17]. We observed that rats had very high levels of both triglycerides and VLDL and very low insulin, compared with rats EII, CI and CII. What's more, EII rats without offspring behaved in a similar way than EII rats. This has been observed previously. In fact, Hypertriglyceridemia is related to ketogenesis.

The relative or absolute absence of insulin and glucose available in the striated muscle increases lipolysis and free fatty acid levels. The free fatty acids are subsequently metabolized in the liver by increasing the production of ketone (especially B-hydroxybutyrate), Ketonuria and acid PH. It has been shown that relatively low levels of insulin (less than what is needed for blood sugar control) are needed to produce a protective effect against the formation of ketone, we found that the rats that could not finish their gestation presented a combination of these factors: hyperglycemia, hyperketonemia, hypertriglyceridemia, increase of VLDL and hipoinsulinemia. So the combination of these factors may have negatively influenced the development of the offsprings.

Thus, the combination of the presence of low levels of insulin, hyperglycemia (or increased of A1c), Hyperketonemia and hypertriglyceridemia can be used to predict the development of the products of diabetic mothers and the strict control of these factors could prevent intrauterine death [14].

As for the effect of diabetes on rats that could conclude pregnancy, EII rats had almost one day more of gestation than control rats (20.05 hrs), although this did not change the weight and size at birth if compared with the controls. It has been found that the extension in the duration of gestation is a compensatory mechanism that allows the offspring of diabetic rats to reach the weight and size similar to that of non-diabetic rats [14].

On the other hand, our group has observed that the fetuses of diabetic rats of 20 days of gestation, weight significantly less than those of the non-diabetic rats, but if it is allowed to progress the gestation, until the spontaneous delivery, the offspring of diabetic rats recover weight until matching with the control rats. The decrease in the viability of diabetic rats has also been reported previously, even if their weight is similar to that of the controls [15].

All of the above leads us to conclude that the development of fetuses in an unfavorable intrauterine environment, such as that given in diabetes; This is, hyperglycemia, hypertriglyceridemia, increased VLDL, and decreased levels of insulin, produces in the offspring serious alterations at birth and during the first 30 days of life, significantly increasing perinatal death and diminishing the chances of a lifetime (21). Of course, further research on the subject should be investigated with future studies.

REFERENCES

1.- Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world A-growing challenge. N Engl J Med 2007; 356: 213-215.
2.- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
3.- NIH Consensus development panel on Impotence JAMA 1993: 270: 83-90
4.- Jarrett R.J. Gestational diabetes: a non-entity?. Br Med J. 1993; 306: 37–38
5.- Wen S.W., Liu S., Kramer M.S. et al. Impact of prenatal glucose screening on the diagnosis of gestational diabetes and on pregnancy outcomes. Am J Epidemiol. 2000; 152:1009–1014
6.- Arbuckle T.E., Wilkins R., Sherman G.J. Birthweight percentiles by gestational age in Canada. Obstet Gynecol. 1993; 81: 39–48
7.- Bailey, C. and Flatt, P.: Animal syndromes of non-insulin-dependent diabetes mellitus. In: Textbook of Diabetes, ed. by John Pickup and Gareth Williams, Blackwell Science. London, 1997, pp 23.1- 23.5.
8.- Bone, A.J. and Gwilliam, D.J. Animal models of insulin-dependent diabetes mellitus. In Textbook of Diabetes, ed by Pickup J and Williams G. Blackwell Science. 2nd Edition, 1997, pp. 16.1-16.16.
9.- Coustan D.R., Nelson C., Caprpenter M.W., Carr S., Rotondo L., Widness J.A. Maternal age and screening for gestational diabetes: a population-based study. Obstet Gynecol. 1989; 73: 557–561
10.- McMahon M.J., Ananth C.V., Liston R.M. Gestational diabetes mellitus: risk factors, obstetric complications and infant outcomes. J Reprod Med. 1998; 43: 372–378
11.- Berkowitz G.S., Lapinski R.H., Wein R., Lee D. Race/ethnicity and other risk factors for gestational diabetes. Am J Epidemiol. 1992; 135: 965–973
12.- Islas Andrade, S., Frati Munari, A., González Angulo, J., Iturralde, P. and Llanos Vega, L.M.: Aumento de los gránulos de secreción neuroendócrina enlas glándulas submaxilares y parótidas en pacientes con diabetes mellitus no dependiente de insulina. Gac. Med. Mex. 128: 411-414, 1992
13.- Islas-Andrade, S., Revilla-Monsalve, M.C., Escobedo de la Peña, J., Polanco- Ponce, A., Palomino-Garibay, A. and Feria-Velasco, A.: Streptozotocin and alloxan in experimental diabetes. Comparison of the two models in rats. Acta Histochemica et Cytochemica 2000: 33: 201-208
14.- Polanco Ponce AC1, Revilla Monsalve MC, Palomino Garibay MA, Islas Andrade S. Effect of maternal diabetes on human and rat fetal development

Ginecol Obstet Mex. 2005;73: (10):544-52.
15.- Triadou N, Portha B, Picon L, Rosselin G. Experimental chemical diabetes and pregnancy in the rat. Evolution of glucose tolerance and insulin response Diabetes. 1982;31:75–79. doi: 10.2337/diabetes.31.1.75
16.- Heinze E, Vetter U. Skeletal growth of fetuses from streptozotocin diabetic rat mothers: in vivo and in vitro studies. Diabetologia. 1987;30:100–10
17.- Holemans K, Aerts L, Van Assche FA. Fetal growth restriction and consequences for the offspring in animal models. J Soc Gynecol Investig. 2003;10:392–399. doi: 10.1016/S1071-5576(03)00134-5.
18.- Turk, J., Corbett, J.A., Ramanadham, S., Borher, A.and Mc Daniel, M.L.: Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets, Biochem. Biophys. Res. Commun. 1993; 197: 1468,
19.- Oh W, Gelardi NL, Cha CJ. Maternal hyperglycemia in pregnant rats: its effect on growth and carbohydrate metabolism in the offspring. Metabolism. 1988;37:1146–1151. doi: 10.1016/0026-0495(88)90192-8.
20.- Plagemann A, Harder T, Janert U, Rake A, Rittel F, Rohde W, Dorner G. Malformations of hypothalamic nuclei in hyperinsulinemic offspring of rats with gestational diabetes. Dev Neurosci. 1999;21:58–67. doi: 10.1159/000017367.
24. Bar-On, H., Roheim, P.S. and Eder, H.E.: Hyperlipoproteinemia in streptozotocin-treated Rats. Diabetes 25: 509, 1976
21.- Hand, A.R. and Weiss, R,E.: Effects of streptozotocin-induced diabetes on the rat parotid gland. Lab. Invest. 1984: 51: 429-440