Research Article

Influence of Vitamin E on the Reproductive Capacity and Fetal Development in Obese Rats

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ABSTRACT

Prenatal obesity has been associated with a higher incidence of congenital defects, impairment of reproductive capacity and fetal development of the offspring. The increase of free fatty acids, reactive oxygen species and other mediators of cellular communication mediate the disorders in the evolution and results of pregnancy. Vitamin E for its antioxidant action seems to be a therapeutic candidate that relieves the onset of the disorders discussed above.

Obesity model was induced in newborn Wistar rats by five doses of monosodium glutamate administered subcutaneously (4 mg/g body weight). The rats selected as controls were injected with the vehicle (0.9 % NaCl) with the same administration scheme. At 90 days after birth, the rats were mated with healthy rats. Once gestated they were divided into four experimental groups: two obese and two control, treated with vitamin E (150 mg/kg weight /ml 0.9 % NaCl) or vehicle, all administered by esophageal tube. The pregnancy was interrupted on day 20, proceeding to the analysis of the reproductive capacity, fetal development and alterations in the offspring.

The administration of vitamin E significantly reduced pre-implantation losses in the control group and prevented the in post-implantation losses in obese rats. In the groups with vitamin E there was a significant decrease in growth and fetal development variables compared with the vehicle groups. Obese rats with vitamin E had a frequency of skeletal anomalies considered normal development variations similar to the control groups. No external or visceral malformations were found. Supplementation with vitamin E had no beneficial effect on the reproductive capacity of obese rats and had a negative effect on offspring growth. However, the vitamin improved offspring survival and allowed an adequate balance in fetal bone development. Results suggest the need of experimental designs that enable studying the functional placental capacity in this model of obesity.

INTRODUCTION

Obesity is a relevant health problem worldwide [1], leaving a notable mark on women of reproductive age. The increase of fatty acids in the circulation and the chronic inflammatory state that characterizes obesity leads to increased levels of reactive oxygen species [2], which contributes to the appearance of the adverse

effects associated with the pregnancy of the obese woman, disturbing the health of the mother and her offspring [3].

Epidemiological studies of the last 20 years have shown a strong association between maternal obesity and Neural Tube Closure Defects (NTD) in offspring [4,5]. Some cohort studies have concluded that obesity is not only associated with the DTN, but also favors the appearance of other congenital malformations such as cardiovascular anomalies, cleft lip, anal rectal atresia, hydrocephalus and limb reduction among others [6,7].

The exact cause of this result is unknown, although one of the theories is oxidative damage mediated by reactive species on biomolecules, particularly lipids [8]. In obesity, the increase in non-esterified fatty acids and proinflammatory cytokines stimulate mitochondrial and extra-mitochondrial generation of reactive species with cytotoxic activity [9]. Changes in the concentration of reactive oxygen species modify cellular signaling and therefore there is an altered genetic expression. In addition, the increase of non-esterified fatty acids and reactive oxygen species activates several kinases that phosphorylate serine and threonine residues. Some of them are c-Jun amino terminal kinase (JNK) and isoforms of protein kinase C (PKCα, β2, δ) [10] which leads to apoptosis signaling pathways by the activation of gene transcription that lead to apoptosis, such as Bad (cell death agonist associated with Bcl2), TRADD (death domain that activates caspases 3 and 8) and others [11]. In women with hypertrophied adipose tissue and increased fatty acids in the blood, alterations in folliculogenesis and oogenesis occur, as well as an abnormal development of the blastocyst that leads to infertility [12]. The gestation of obese mothers could cause intrauterine growth retardation and congenital malformations in offspring [13]. The foregoing describes the urgency of preventing maternal overweight and obesity on time [14].

The vitamin E administration has shown beneficial effects in diseases such as atherosclerosis, cardiovascular events, teratogenicity induced by anticonvulsants, cancer [15-16] and in obesity among others etiologies [17]. This antioxidant vitamin limits the spread of lipid peroxidation, a chain reaction that occurs in cell membranes, compromising the cell integrity. It has been shown that vitamin E inhibits the activity of systems that generate free radicals, including the increase in the expression of iNOS and NADPH oxidase [18-19]. Despite all these studies, there are reports in which the intervention with Vitamin E has not been effective, finding contradictory or inconclusive results [20-23].

Especially interesting are the studies carried out in models of diabetic rats gestated where vitamin E was supplied, whose results coincide in showing a considerable reduction of embryonic malformations. These effects have demonstrated the relationship between the oxidative stress of the mother and the etiology of the congenital malformations of the offspring [24-26]. The administration of vitamin E to pregnant women has proven in several studies to be safe and beneficial when was used in low doses. Vitamin E stabilizes cell membranes, exerts an antioxidant power, but inhibits the action of PKC and cell proliferation [27]. With respect to the negative effects, there are some reports that show that in rodent models during pregnancy, high doses can be harmful [21-23]. The models of pregnancy and obesity in rats that reproduce the teratogenic effects are scarce. Rats with obesity induced by Monosodium Glutamate (MSG) maintain a hyperlipidemia during the embryonic period associated with an increase in defects at birth [28]. This model presents a high coincidence with the appearance of congenital malformations as in models of pregestational diabetes. Bearing in mind that diabetes mellitus and obesity have in common an increased risk of oxidative stress and a similar pattern of congenital malformations [29], we propose to evaluate the influence of vitamin E administration on the fetal development of obese rats.

MATERIAL AND METHODS

The female pups of Wistar rats were randomly selected to form two groups; one of the groups was used for the model of obesity with use of MSG and the other group constituted the control.

The day of birth was taken as day zero to induce obesity. Five doses of MSG were administered subcutaneously (4mg/g body weight) alternant from 2 until 10 days of live. The MSG was dissolved in 0.9 % sodium chloride (NaCl) in a solution containing 100 mg of substance per ml of physiological saline solution [30]. The rats selected as controls were injected with the vehicle (0.9 % NaCl) with the same administration scheme. The rats were kept under conventional conditions, with free access to water and food (CMC 1000 formula granulated

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feed produced by CENPALAB), covering their nutritional needs. The room was maintained with controlled temperature between 22 °C to 24 °C, continuous ventilation and constant cycles of 12 h light/ 12 h dark.

After 90 days, the experimental group was used to evaluate the Lee index (LI) to define obesity [31].

$$LI = \sqrt[3]{body\ weight\ (g)/(\ naso-anal)length\ (cm)}$$

The obese animals reach LI values greater than 0.300 g/cm. The obese and control rats were mated with healthy males of the same strain. When was evident the presence of sperm in the vaginal smear in the early morning, it was considered the zero day of gestation. Pregnant rats were randomly divided into four experimental groups: control rats with vehicle (CV), obese rats with vehicle (OV), control rats with vitamin E (CE) and obese rats with vitamin E (OE).

The vitamin E was supplied with a granulated preparation of 50 % alpha tocopherol acetate with silicon oxide (Company of solid medicines of the pharmaceutical industry MEDSOL) 150 mg/kg of body weight diluted in 1 ml of 0.9% NaCl by esophageal tube. The same route supplied the vehicle (0.9% NaCl). Both treatments were administered from day zero to day 19 of pregnancy. During this period, the weight gain of the dams was determined.

The pregnant rats were killed on day 20 of pregnancy by exsanguination under anesthesia with sodium pentobarbital (60 mg/Kg of weight). They underwent laparotomy to obtain the uterine horns and ovaries. Corpus luteum, implantations, reabsorptions, live fetuses and dead fetuses were counted. The pre-implantation losses (IPPr) and post-implantation rates (IPPs) were calculated with the respective formulas [32].

$$\text{IPPr} = \frac{\text{Number of corpus luteum} - \text{Number of implantations}}{\text{Number of corpus luteum}}$$

$$\text{IPPr} = \frac{\text{Number of implantations} - \text{Number of live fetuses}}{\text{Number of implantations}}$$

Abdominal fat was removed and weighed. The placentae of each fetus were extracted, weighed and the placental index was determined as a marker of placental efficacy. For this, the rate between the fetal weight (g) and the weight of the placenta (g) was calculated.

Fetuses were removed from amniotic membranes and externally analyzed, in terms of: shape and size of the skull, implantation of the ears, eyes closed, the integrity of the palate and the upper lip in the mouth, position and size in the upper and lower extremities. The thoracic and abdominal regions were examined in its ventral and dorsal portion in search of bruises, hemorrhages, integrity of the skin or defect of the neural tube. Weight, height, biparietal diameter (DBP) and the anteroposterior diameter (DAP) were determined for all fetuses [33]. After the external analysis, half of the fetuses were placed in Bodian's solution [29] for a month. Later the Wilson cuts with some modifications were carried out [34]. Three frontal cuts were made at level of the head, one in the region of the nasal septum, another at the level of the eyeballs and the third at the height of the cerebral hemispheres. Thoracotomy and laparotomy were performed for the organs examination (Figure 1).

Figure 1: Fetuses processed to detect congenital anomalies. A: Fetus preserved in Bodian's solution for visceral analysis. B: Courts proposed by Wilson (taken from Damasceno DC, 2008 [29]). C: Representation of the modified Wilson cuts made in this investigation. D: Fetus processed for skeletal analysis. Side view. Photography taken with a digital camera (Canon).

The rest of the fetuses were prepared for the skeletal examination by staining with Alizarina red [32]. Each fetus was placed in a Petri dish to analyze under stereoscopic the body structures from the head to the caudal regions (Figure 1).

In each bone, the size, shape and absence or addition of bone were analyzed. The absence of alizarin staining in a portion of the bone was defined as incomplete ossification for their gestational age [35] (Figure 2). The ossification points were counted at the level of: sternum, carpus, metacarpus, tarsus y

metatarsus upper and lower phalanges, and vertebrae of the tail (Figure 2). The ossification points are indicators of fetal development [32].

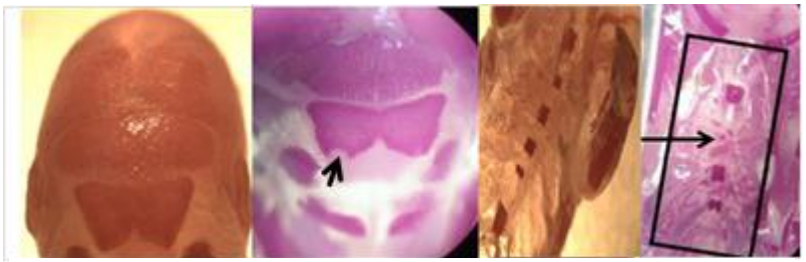

Figure 2: **Fetuses stained with Alizarin red**. A: Normal ossification skull. B: incomplete ossification of supraoccipital. C: Sternum with normal ossification points for gestational age. D: lower number of sternum ossification sites accord to gestational age. Photography taken with digital camera coupled to stereo camera (Motic SMZ-143).

To perform the statistical analysis of results, a database was created in Microsoft Excel program. The results were processed by INFOSTAT program and Statistic 8.0 package. For the comparisons between experimental groups, the non-parametric U Mann-Whitney test was used. Fisher's exact test was used to compare percentage values. Differences will be considered significant when p values equal to or less than 0.05.

RESULTS

The groups of obese rats showed a significant increase in abdominal fat compared to their respective control groups (23 ± 9.2 vs 14.1 ± 4.0 and 24.58 ± 6.7 vs 15.11 ± 2.6). They also presented a lower weight gain during pregnancy with significant differences when compared with their control group (65.44 ± 20.8 vs 98.43 ± 19.8 and 58.57 ± 19.5 vs 98.60 ± 18.0).

When the reproductive variables were analyzed (Table 1), the groups of obese rats had a lower number of corpus luteum, implantations and fetuses than their respective controls (CV vs OV, CE vs OV). No significant differences were found with the administration of vitamin E.

Table 1: Reproductive variables in the groups of obese and control groups.

Variables	CV n =14	OV n =15	CE n =15	OE n =14
Corpus luteum N Mean ± SD	177 12,6 ± 2,4	159 9,9 ± 2,3*	203 13,5 ± 2,3	129 9,2 ± 1,8[b]
Implantations N Mean ± SD	164 11,7 ±2,9	145 9,0 ± 3,2*	197 13,07 ± 2,7	122 8,71 ± 1,7[b]
Pre-implantation losses rates %	7,3	8,8	2,9[e]	5,4
Reabsorptions N (%)	2 (1,22)	2 (1,38)	1 (0,51)	2 (1,67)
Live fetuses N Mean ± SD	161 11,5 ± 3	136 8,50 ± 3,4*	192 12,7 ± 3,1	118 8,4 ± 1,8[b]
Dead fetuses N (%)	1 (0,62)	7 (5,15)[d]	4 (2,04)	2 (1,69)
Post-implantation rates (%)	1,8	6,2[d]	2,5	3,3

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n: number of mother rats; N: number; SD: standard deviation.

* p < 0.05 when comparing obese with vehicle (CV) vs vehicle control (OV) (non-parametric U Mann-Whitney test).

b p < 0.05 when comparing the control with vitamin E (CE) vs obese with vehicle with vitamin E (OE) (non-parametric test U Mann-Whitney).

d p < 0.05 when comparing obese with vehicle (OV) with vehicle control (CV) (Fisher's exact test).

e p < 0.05 when comparing the control with vitamin E (CE) vs obese with vitamin E (EO) (Fisher's exact test).

Vitamin E produced a significant decrease in preimplantation losses in the control group. In the obese rats, decrease of this variable also occurred, although without significant differences. In the untreated obese, there was an increase in fetal deaths compared to their control group. When obeses received vitamin E, fetal deaths decreased considerably.

In both groups of obese rats, there was a significant decrease in several indicators of development and fetal growth (Table 2). Vitamin E caused a significant decrease in fetal growth and development variables, both in the controls and in the obese ones. The placental index increased with significant differences in the obese rats with vitamin E.

The control rat's offspring from group vitamin E treated showed a lower number of ossification sites in the metacarpals with respect to the untreated control group, in both sites, the caudal vertebrae and in the sternum. The offspring of the obese rats treated with Vitamin E showed lower number of ossification sites with respect to the offspring of obese rats without treatment. In summary, a slower ossification rate was observed in different groups treated with vitamin E, which is exacerbated in the obese rat's descendants (OE). When we compare the obese rat's offspring with fetuses of control rats, we find that ossification process happened in a similar way (Table 2). No Lower and Upper phalanges ossifications marks were observed.

Table 2: Indicators of fetal growth and development.

Variables	CV n =161	OV n =136	CE n =192	OE n =118
Fetal weight (g)	3,5 ± 0.3	3,4 ± 0,4*	3,3 ± 0,3[a]	3,0 ± 0,4**[b]
Fetal height (mm)	34,4 ± 1,7	34,2 ± 1,8	34,7 ± 1,7	32,5 ± 2,2** [b]
DBP (mm)	7,8 ± 1,0	7,7 ± 0,9	7,1 ± 0,7[a]	6,8 ± 1,2**
DAP (mm)	11,4 ± 1,4	10,7 ± 1,3*	11,5 ± 1,3	10,3 ± 1,6 [b]
Placental Weight (g)	0,53 ± 0,1	0,52 ± 0,07	0,51 ± 0,08[a]	0,53 ± 0,09 [b]
Placental index	0,15 ± 0,03	0,16 ± 0,03	0,15 ± 0,0	0,8 ± 0,3**[b]
Sitios de osificación				
Metacarpus	3,2 + 0,6	3,1 + 0,4	3,0 + 0,4 [a]	3,0 + 0,5
Metatarsus	4,0 + 0,3	3,9 + 0,2	3,9 + 0,3	3,7 + 0,5**[b]
Vertebrae of the tail	3,9 + 0,7	4,2 + 0,8	3,6 + 0,8 [a]	3,6 + 0,9**
Sternum	4,4 + 0,7	4,5 + 0,8	3,8 + 1 [a]	3,9 + 1**

Results are presented as mean and standard deviation

* p < 0.05 when comparing obese with vehicle (CV) with vehicle control (OV) (non-parametric U Mann-Whitney test).

** p < 0.05 when comparing obese with vehicle (OV) with obese with Vitamin E (OE) (nonparametric U Mann-Whitney test).

A p < 0.05 when comparing control with vehicle (CV) with the control with Vitamin E (CE) (non-parametric test U Mann-Whitney).

b p < 0.05 when comparing the control with Vitamin E (CE) with obese with Vitamin E (OE) (nonparametric test U Mann-Whitney).

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Lower frequency of skeletal anomalies is shown in (Figure 3). These anomalies are considered normal development variations in the fetuses of obese rats compared to the descendants of control rats with vehicle (OV = 15 % vs CV = 40 %, p <0.05). These results are reversed once the group of obese rats was treated with vitamin E (OE = 38% vs OV = 40 %). In the visceral anomalies was observed normal variation and congenital malformations in all groups without significant differences.

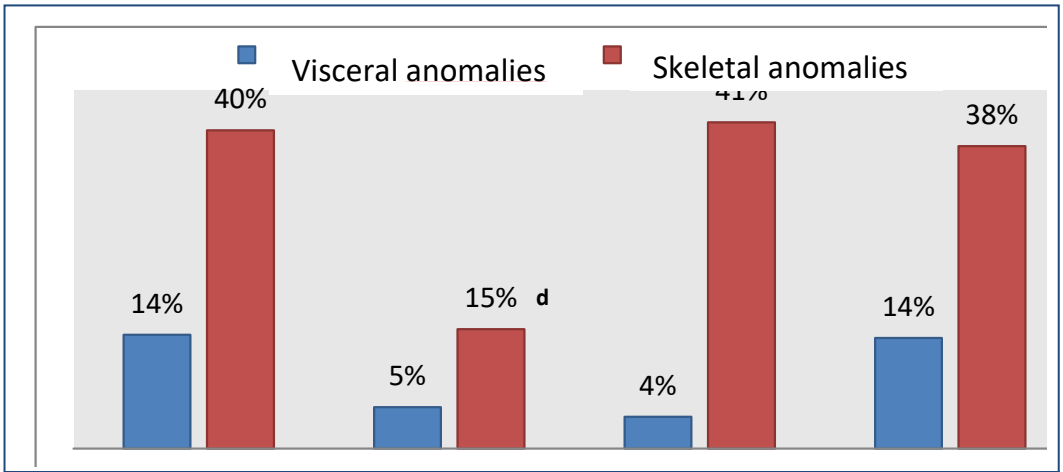

Figure 3: Visceral and skeletal anomalies in offspring of rats

d p <0.05 when comparing obese with vehicle (OV) with vehicle control (CV) (Fisher's exact test).

+ p <0.05 when comparing obese with vehicle (OV) with obese with Vitamin E (EO) (Fisher's exact test).

Incomplete ossification of the supra-occipital bone, mixed skeletal anomalies such as undulating ribs, short ribs with incomplete ossification of flat bones and elongated or elongated ureter in the form of S (not associated with another urinary tract defect) were structural alterations whose frequency showed statistical differences between the groups (Figure 4). No fetuses with neural tube defects, cardiovascular malformations or any other malformations were found (Figure 4).

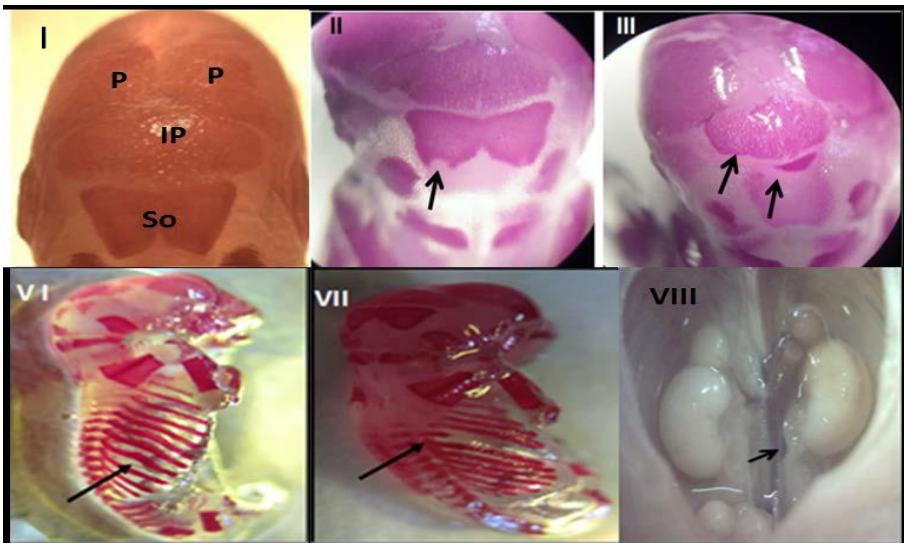

Figure 4. Skeletal and visceral alterations observed in all groups studied. I. Views of the skull of control rats stained with alizarin red showing: parietal (P) bones interparietal (IP) and superoccipital (So). II. Supraoccipital incomplete ossification III Supraoccipital and interparietal incomplete ossification. IV. Wavy rib, VI. Short rib VIII. Dilated and folded ureter. Pictures A was taken with permission of Damasceno DC, 2008 [29])

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DISCUSSION

Increase in abdominal fat was found in obese rats in relation to the control group in this study. This result is consistent with other studies where an increase in abdominal adipose tissue was observed independently of the obesity model used [36,37]. It is also proof that the model of obesity induced by MSG reproduces central obesity, which is the most adipose tissue associated with complications.

The fertility disorder that characterizes rats with obesity induced with MSG occurs at the level of the central nervous system. The neonatal injection with MSG produces ablation of neurons in the arcuate nucleus, which regulate the pulsatile secretion of Gonadotropins Releases Hormone (GnRH) at the level of the hypothalamic median eminence [38]. The loss of pulsatile activity of GnRH causes decreased synthesis and secretion of pituitary gonadotropins (LH: luteinizing hormone; FSH: follicle-stimulating hormone), especially LH. This leads to a decrease in the synthesis of estrogen and progesterone, lower production of oocytes in the ovaries and alterations in the ovulation process [37]. Other groups of researchers had also showed a decrease in litters in an obesity model induced with oral MSG [39].

In humans, it has been shown that obesity during pregnancy increases the risk of fetal death [40], as it has been reproduced in the animal model used in this study. The histological findings found in the placental biopsies of obese pregnant women with fetal death are very similar to the placentas of mothers with diabetes. These alterations are varied and include edema, immaturity and delayed villous maturation, massive deposit of perivellositic fibrinoid substances, thrombotic fetal vasculopathy and avascular terminal villi. According to current evidence, these placental findings suggest a placental dysfunction with local hypoperfusion, which may possibly be associated with the cause of fetal death [41].

Fetal deaths can also occur due to malformations of cardiovascular origin that are associated with the gestation of obese. These have their origin in the incorrect migration of the cells of the neural crests. It is known that from day 12.5 to 16.5 of gestation in rats, the embryos with severe malformations (cardiac and central nervous system) do not survive [42]. This could also explain the low presence of fetal malformations in the model used. Fetuses of obese rats showed delayed intrauterine growth. Intrauterine growth in mammals depends on the supply of nutrients from the mother to the fetus [41]. Recent research suggests that obesity alters the transport function of the placenta at the end of pregnancy through the reduction of mediators such as appelin and leptin that regulate the placental vascular tone [43-45]. In addition, maternal obesity is associated with a decrease in angiogenesis regulators and an increase in markers of inflammation and oxidative stress [46,47]. It also affects the trophoblastic invasion and the formation of vessels, which causes episodes of intermittency in the delivery of blood flow. This situation is associated with transitory states of reperfusion ischemia. High levels of leptin, cholesterol, fatty acids and insulin resistance also induce placental ischemia. This causes an increase in the response inflammatory and inflammatory cytokine synthesis in the maternal vascular bed increasing the risk of presenting pre-eclampsia and intrauterine growth retardation [48].

The model used is characterized by an excess of maternal glucocorticoids in the circulation [43,44-46], as a consequence of the loss of the inhibitory effect exerted by leptin on the secretion of glucocorticoids stimulated by the adrenocorticotropic hormone in the adrenal glands. The absence of this inhibitory effect is due to the resistance to leptin that the obese rats of this model develop, with decreased expression of the hormone receptor at the level of the adrenal gland [46,47].

The increase in maternal glucocorticoids leads to delayed intrauterine growth [48-49]. There are studies that show that the administration of natural steroids such as cortisol during pregnancy produces a decrease in fetal weight, inducing structural and functional changes in the placenta. These changes produce reduction in the density of placental vessels, inhibition of proliferation, migration and trophoblastic invasion, which causes an inadequate remodeling of the spiral arteries, stimulating a decrease in blood flow and with this the passage of nutrients favoring placental dysfunction [50-53]. From the molecular point of view, protein synthesis and stimulation of cell proliferation during pregnancy is mediated by the stimulation of the PI3K / Akt / mTOR pathway (phosphatidylinositol 3 kinase / seryl-threonyl-protein kinase / mammalian target of

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rapamycin). This leads to the phosphorylation and activation of several proteins such as p70S6K and 4EBP1 (kinase 1 of the ribosomal protein S6 and protein 1 binding to the eukaryotic initiation factor 4E) involved in the process of cell proliferation and protein synthesis. At the same time, apoptosis is inhibited by phosphorylation of Akt and inactivation of pro-apoptotic proteins such as Bad and Caspasa 9 [54].

When glucocorticoids are increased, signaling of the PI3K / Akt / mTOR pathway is suppressed due to an increase in the expression of REDD 1 (regulated in development and DNA damage responses 1) at the placental level. This cause inhibition of the synthesis or could block the translocation of transporters to the membrane, reducing the supply of amino acids and glucose to the embryo-fetus [42]. In the case of amino acid transporters, it is system A, which participates in the transport of neutral amino acids [54].

On the other hand, it has been shown that vitamin E also produces inhibition of the PI3K / Akt / mTOR pathway by blocking the phosphorylation of AKT, which contributes to the inhibition of cell proliferation and protein synthesis [55]. Some authors have also shown that it decreases the growth of smooth muscle cells linked to its effect of inhibiting the activity of protein kinase C. There are other reports that confirm this effect in other cell types such as monocytes, neutrophils, fibroblasts and mensangial cells [56]. This could explain the negative effect of the administration of vitamin E on fetal growth. It could be suggested that the excess of maternal glucocorticoids, characteristic of this obesity model, associated with the administration of vitamin E strengthened the inhibition of the PI3K / Akt / mTOR path causing the decrease in fetal growth.

The results of skeletal and visceral morphological anomalies found in the controls are within the normal range reported in other studies conducted in rats [57,58]. These skeletal and visceral anomalies are considered transient variations, that is, structural changes that occur in a population under investigation that probably do not affect the survival or health of the species. The fetal period in the rats is very short and a remodeling of the organ systems occurs postnatally, so there are anomalies found in the fetuses that disappear after birth [59]. The obese rats with vehicle had less skeletal variations, so it could be speculated that remodeling process started early from the fetal period. This effect could be due to the excess of circulating maternal glucocorticoids. It is known that one third of the glucocorticoids in fetal blood are obtained by placental transfer [60], so that overexposure to this hormone in the fetuses of obese rats could justify the fact that they possess less variation in their maturation. This result at this time of development is contradicted by that found by researchers from the University of Arkansas in other experimental model in obese mouse they found that maternal obesity produced a delay in the skeletal development of the offspring [61].

The incomplete ossification of the skull bones, specifically the supra occipital and interparietal bones, were the most frequent variation in this investigation, they are the result of delay in the ossification process and disappear after birth [59]. It has been demonstrated that the wavy ribs (Wavy rib Undulations along the length of a rib), another variation was presented in a non-isolated way, is also due to one delay in bone ossification or mineralization [59]. The force exerted by the muscles during contraction causes the alteration of the shape of the rib; this anomaly usually disappears before weaning.

In the skull bones, due to the scarce muscles that surround them, there is some delay in the bone ossification that doesn't deform it [59]. The variation of the vertebral centers also disappears later than the wavy ribs [58]. Babies or animals in neonatal periods born from obese mothers have not been systematically studied, because that, we don't known postnatal consequences [58,59]. Ossification pattern in fetuses born from obese mothers treated with vitamin E was similar to the control group. Several studies have shown that this vitamin improves bone quality, can accelerate bone formation and attenuates bone reabsorption, but is unable to restore density once decalcification has taken place [62]. This shows once again the multiple independent effects of its antioxidant activity that vitamin E has. In our case, it seems that vitamin E was able to regulate the balance in bone formation and reabsorption, which is supported by results reported in another studies [63,64].

CONCLUSIONS

Our research shows that vitamin E administration to obese dams negatively affects growth and development of the obese rat's offspring. The hidden mechanisms should be accentuating the disorder that glucocorticoids provoke in the molecular

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pathways that control the amino acids transport for protein synthesis. However, the administration of such vitamin improved offspring survival and allowed an adequate balance in fetal bone development. We think it is due to the control that this molecule can exert on signaling pathways that control survival or death at vulnerable moments of intrauterine development, even including bone maturation. Results suggest the need of experimental designs that they enable studying the functional placental capacity in this model of obesity.

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