Extracellular HSP70 levels in diabetic environment in rats

T. M. M. Santos^{1,2} · Y. K. Sinzato¹ · F. Q. Gallego¹ · I. L. Iessi¹ · G. T. Volpato^{1,2} · B. Dallaqua¹ · D. C. Damasceno^{1,2,3}

Received: 16 June 2014 / Revised: 5 March 2015 / Accepted: 8 March 2015 / Published online: 27 March 2015
© Cell Stress Society International 2015

Abstract The expression of HSP70 in embryonic cells of mammals and its role for their normal development and protection is an important aspect to be investigated in pregnancy and/or mild diabetes. In this sense, the present study evaluated the effects of mild diabetes on maternal reproductive parameters and HSP70 levels in Wistar rats at different stages of life and in their offspring. Mild diabetes was induced by a beta-cytotoxic drug (streptozotocin) at birth. Four experimental groups were evaluated: at 90 days of age: nonpregnant non-diabetic (ND90) and nonpregnant mild diabetic (D90) female rats, and at term pregnancy: pregnant female rats of both glycemic status were examined (NDP and DP, respectively). The rats were submitted to oral glucose tolerance test, and blood samples were collected for determination of HSP70 levels. In addition, the reproductive performance of pregnant rats was assessed and HSP70 levels determined in their offspring blood samples. The HSP70 levels and maternal reproductive performance presented no difference between ND and D rats, regardless of the life stage. The HSP70 levels were increased in

D90 rats and lower in offspring from D rats. Maternal HSP70 levels were positively correlated to the number of dead embryos. In conclusion, mild diabetes did not affect maternal reproductive performance, but high maternal HSP70 levels compromised embryo development. In addition, offspring from D rats exhibited lower HSP70 levels, showing that this protein can be used as an indicator of metabolic consequences of diabetes and predictor of related disorders in adulthood.

Keywords HSP70 · Rat · Pregnancy · Offspring · Diabetes

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, which is caused by dysfunctions in insulin secretion and/or action (American Diabetes Association 2014). DM is considered one of the main chronic diseases affecting all socioeconomic classes, in both developed and developing countries, lowering patient's quality of life and increasing the expenses of the healthcare system (Reece et al. 2004). In pregnancies complicated by diabetes, hyperglycemia is associated with an increase in maternal-perinatal morbidity and decrease in the reproductive performance, leading to spontaneous miscarriage, neonatal mortality, and congenital anomalies (Eriksson et al. 2003; Metzger 2002; Yang et al. 2006).

Earlier studies showed that disturbances in the intrauterine environment caused by diabetes also affect embryonic and fetal development (Maayan-Metzger et al. 2009; Higa and Jawerbaum 2013). Therefore, the investigation of the mechanisms underlying changes caused by diabetes in humans is limited by ethic reasons and by a number of uncontrollable variables that modify the intrauterine environment. These facts justify the development of reliable experimental models

✉ D. C. Damasceno
damascenofmb@gmail.com

¹ Gynecology, Obstetrics and Mastology Graduate Course, Laboratory of Experimental Research on Gynecology and Obstetrics, Botucatu Medical School, Univ. Estadual Paulista Unesp, Botucatu, São Paulo, Brazil

² Immunology and Parasitology: Basic and Applied Graduate Course, Laboratory of General Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, University Center of Araguaia, Federal University of Mato Grosso (UFMT), Barra do Garças, Mato Grosso, Brazil

³ Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (UNESP), Distrito de Rubião Júnior, s/n, CEP: 18618-970 Botucatu, São Paulo, Brazil

that reproduces human hyperglycemia using laboratory animals in order to better understand the physiopathological mechanisms enrolled (López-Soldado and Herrera 2003). Then, a number of models are used, including diabetes induction by administration of the beta-cytotoxic drug streptozotocin (Lenzen 2008).

Experimental studies on pregnant rats with mild diabetes (120–300 mg/dL glucose levels) showed a negative impact of this disease on their reproductive performance (Saito et al. 2010). In addition, diabetes causes intrauterine growth restriction (IUGR), reduces the number of corpus luteum and embryo implantation, and increases the rates of pre- and postimplantation embryo losses (Sinzato et al. 2012). Iessi et al. (2010) found intrauterine growth restriction (IUGR) in fetuses from rats with induced mild diabetes. Different biological markers are used to find out physiopathological processes enrolled in diabetes during pregnancy, including the heat shock proteins (HSP). The HSPs can amplify the immune responses to nonphysiological changes such as infections, increase in temperature, oxidative stress, nutritional deficiency, and exposure to chemical toxins (Hendrick and Hartl 1993; Pockley 2002; Campisi et al. 2003). The HSPs are also involved in reproductive processes because participate in the formation of male and female gametes as well as embryo and fetus development (Neuer et al. 2000; Anderson 1998), being expressed in embryo and maternal decidua (Grigore and Indrei 2001). The HSPs, one of the first proteins produced by mammals in the embryonic stage, are involved in embryo growth (Neuer et al. 2000). A low HSP expression can impair endometrial receptivity to embryonic implantation (Jin et al. 2009).

In a study on Sprague–Dawley rats with mild diabetes, Saito et al. (2013) showed a positive association between maternal blood HSP70 levels and congenital fetal anomalies. Considering the expression of HSP70 in embryonic cells of mammals and its role for their normal development and protection, one important aspect to be investigated is whether pregnancy and/or mild diabetes lead to changes in HSP70 levels and whether maternal hyperglycemia affects fetal HSP70 levels. In this sense, the present study evaluated the effects of mild diabetes on maternal reproductive parameters and HSP70 levels in Wistar rats at different stages of life and in their offspring.

Material and methods

Animals

The experimental protocols applied were previously approved by the Animal Research Ethics Committee of the Medical School of Botucatu, UNESP (protocol CEEA 916/2011). The Wistar rats used were obtained from the Animal Facility

of the State University of Campinas (CEMIB/UNICAMP). They were kept at the Animal Facility of the Laboratory of Experimental Research on Gynecology and Obstetrics of the Medical School of Botucatu, UNESP, under controlled conditions of temperature (22 ± 2 °C) and relative air humidity (50 ± 10 %), 12:12 h light/dark cycle. Filtered tap water and feed were available ad libitum. Normoglycemic males and females were mated to obtain the offspring tested in the study.

Experimental design

Diabetogenesis: mild diabetes induction

Diabetes was induced in the newborn female Wistar rats at first day of life, and these rats received 100 mg/kg body weight of the beta-cytotoxic drug streptozotocin (STZ; Sigma Chemical Company, St. Louis, MO, USA), diluted in citrate buffer (0.1 mol/L; pH 4.5), by subcutaneous injection in the dorsal region. The same protocol was used to inject a similar volume of citrate buffer in other newborn female rats. After 4 days of STZ administration, the glucose levels were determined, the rats given STZ presenting glucose levels ≥ 400 mg/dL were included in the diabetic group; those receiving only the vehicle and with glucose levels ≤ 120 mg/dL were included in the nondiabetic group. All the newborns were kept with their mothers until weaning (21 days). After this period, the mothers were killed with a lethal dose of sodium thiopental (Thiopentax®—50 mg/kg body weight), and the female newborns were maintained until adulthood.

Experimental groups

In the adult stage (75 days old), the animals were randomly distributed into four experimental groups ($n=10$ animals each). Two groups were constituted by nonpregnant rats that were diabetic (D) or not (ND) and were killed with 90 days old for examination (groups D90 and ND90, respectively). The other two groups were formed by D and ND pregnant rats that were killed and examined with 21 days of pregnancy (DP and NDP, respectively).

Oral glucose tolerance test

To confirm the changes in glucose metabolism, the oral glucose tolerance test (OGTT) was performed 2 days before examination of ND90 and D90 rats and in the 17th day of pregnancy of NDP and DP rats. For all the groups, OGTT was performed after 6 h fasting, by determining glucose levels in a blood drop collected from the rats by tail vein puncture (timepoint 0). A D-glucose solution (2.0 g/kg BW) was intragastrically administered by gavage. Thereafter, glucose levels were measured 30, 60, and 120 min after D-glucose overload (Mello et al. 2001; de Campos et al. 2007). When

the rats presented two or more timepoints superior to 140 mg/dL during OGTT curve, the animals were considered as mild diabetic.

Experiment on 90-day nonpregnant rats

Blood samples were collected from nonpregnant rats (groups ND90 and D90) by puncture of the tail tip to determine glucose levels (mg/dL) using a conventional glucose meter (One Touch Ultra, Johnson & Johnson®). The rats were then anesthetized with a lethal dose of sodium thiopental and decapitated for blood collection in dry tubes for measurement of glycated hemoglobin (HbA1C) rate. The blood samples were centrifuged, and serum was separated and stored under -80°C until insulin and HSP70 determinations.

Mating

At adulthood, the rats from NDP and DP groups were submitted to mating, which lasted no longer than 15 days (corresponding to three estrous cycles). Two female rats and a non-diabetic male rat were introduced in a polyethylene cage in the late afternoon. In the next morning, the male was removed from the cage and vaginal smear collected from the female rat for morphological examination. The morning on which spermatozoa were found in the smear was designated pregnancy day 0. This procedure was repeated until reaching the number of pregnant rats needed.

Pregnancy

On days 0 and 21 of pregnancy, maternal weight was determined to calculate weight gain (final weight–initial weight). On day 21, the rats were weighted, lethally anesthetized with sodium thiopental and decapitated for blood sample collection. The blood samples were processed, and the serum obtained was used for insulin and HSP70 level determinations. The uterine horns were then exposed for reproductive performance analysis, by counting of the number of corpora lutea, embryo implantation and resorption (embryo death), and live and dead fetuses. The rates of preimplantation embryo loss were calculated by the formula: (number of corpora lutea–number of implantations) $\times$ 100/number of corpora lutea. The rates of postimplantation loss were calculated by the formula: (number of implantations–number of live fetuses $\times$ 100)/number of implantations (Damasceno et al. 2008). When embryo or fetal development could not be observed, the uterus was stained by the Salewski's technique (Salewski 1964) for marking possible implantation points. Newborns from NDP and DP groups were lethally anesthetized with sodium thiopental for collection of blood samples, which were prepared as described for the mothers.

Extracellular heat shock protein (HSP70) determination

A commercial kit (Enzo Life Sciences®, code ADI-EKS-715) containing ELISA microplates was used according to manufacturer's instructions for determination of HSP70 levels.

Serum insulin determination

Serum samples were used to measure insulin levels (kit protocol number: 90060; Crystal Chemical®, USA) by ELISA kits.

Glycated hemoglobin (HbA1C) determination

Total blood samples were used to measure HbA1C rate in particular laboratory.

Statistical analysis

The results obtained are shown as mean $\pm$ standard deviation (SD). The HSP70 levels comparison between experimental groups (ND90 vs D90, NDP vs DP and NDP offspring vs DP offspring) was performed using the generalized linear model (gamma distribution) with adjustment for weights and blood glucose levels, followed by Wald's multiple comparison test. The parameters of reproductive performance were compared between groups (ND90 vs D90, NDP vs DP and NDP offspring vs DP offspring) by *t* test. Variable frequencies were evaluated by the Fisher's exact test and their association by the Pearson's correlation test. The maximum confidence limit adopted was 95 % ($p<0.05$).

Results

According to the OGTT, at 30 and 60 min after D-glucose overload D90 rats exhibited higher glucose levels and the area under curve (AUC) in relation to those of ND90 rats (Fig. 1a). In addition, the D90 rats presented no alteration in insulin levels (Fig. 1b), but these animals showed higher rate of HbA1C compared to ND90 rats (Fig. 1c). In the same timepoints (30 and 60 min) of OGTT, DP rats showed higher glucose levels and AUC compared with those of NDP rats (Fig. 2a). During pregnancy, the insulin levels were not statistically different between DP and NDP (Fig. 2b). The offspring of both groups presented no alteration in glucose levels (Fig. 2c).

No differences were observed between the reproductive performance of DP and NDP mothers (Table 1).

Correlation analysis on the relationship between extracellular serum HSP70 and blood glucose, and extracellular serum HSP70 and reproductive parameters are shown in Table 2.

Fig. 1 **a** Oral glucose tolerance test (OGTT) at day 90 of life of non-diabetic (control—ND90, $n=12$) and mild diabetic (D90, $n=12$) rats. **b** Insulin levels of nondiabetic (ND90, $n=8$) and mild diabetic (D90, $n=8$) rats. **c** HbA1C rates of nondiabetic (ND90, $n=12$) and mild diabetic (D90, $n=12$) rats. Data presented as mean $\pm$ standard deviation. * $p<0.05$, statistically significant difference compared to group ND90 (t test)

Extracellular maternal HSP70 levels were positively correlated with the prevalence of embryo death (resorption).

Figure 3 shows the mean levels of extracellular HSP70 in serum samples collected from 90-day rats (ND90 and D90), pregnant rats (NDP and DP), and

their offspring. It was observed an increase in extracellular HSP70 levels in D90 rats compared to those of NP90 rats, and the offspring from DP exhibited lower extracellular HSP70 levels than those from NDP after adjustment for body weight and glycemia level.

Fig. 2 **a** Oral glucose tolerance test (OGTT) at day 17 of pregnancy of nondiabetic (NDP, $n=12$) and mild diabetic (DP, $n=12$) rats. **b** Blood glucose levels of offspring at term from NDP (NB-NDP, $n=12$) and DP (NB-DP, $n=12$) dams. Data presented as mean $\pm$ standard deviation. $*p<0.05$, statistically significant difference compared to group NDP (t test)

Discussion

In the present study, we found that nonpregnant diabetic rats with 90 days of age exhibited alterations in mean blood glucose levels in two or more time points of the OGTT curve, confirming the presence of mild diabetes in these rats,

corroborating with other experimental study of our group (Dallaqua et al. 2012).

A number of models of mild diabetes induction are available, but their responses differ according to life stage at the time of diabetes induction, drug administration route, and animal strain (Kervran et al. 1978; Gelardi et al. 1990; Kiss et al.

Table 1 Maternal reproductive performance of nondiabetic (NDP) and mild diabetic (DP) at term pregnancy

	Nondiabetic (NDP) <i>n</i> =12	Mild diabetic (DP) <i>n</i> =12
Maternal weight gain (g) ^a	118.8±13.9	123.3±27.1
Corpora lutea ^a	12.6±1.4	12.8±1.7
Implantation ^a	11.9±1.6	10.9±3.1
Alive fetuses ^a	11.0±1.5	9.9±3.5
Resorption (%) ^b	7.6	9.2
Preimplantation embryo loss (%) ^b	5.8	14.5
Postimplantation embryo loss (%) ^b	7.6	9.2
Gravid uterus weight (g) ^a	83.1±10.2	74.1±24.9

Data presented as mean±standard deviation. $p>0.05$, nonstatistically significant difference compared to group NDP

^a *t* test

^b Fisher's exact test)

2009; Dallaqua et al. 2012; Damasceno et al. 2014). In our diabetic model, after streptozotocin administration during neonatal period, the rats present hyperglycemia in first days of life due to necrosis of beta cells (Lenzen 2008). However, the rats show beta cell regeneration from 13 to 17 days of life (Scaglia et al. 1995), leading to basal level of insulin, which is similar to level of control animals at adulthood and during pregnancy. Despite this, these animals present disturbance in the glucose metabolism, as evidenced in our findings. With respect to maternal reproductive performance, the data showed no difference between experimental groups. Nevertheless, a number of studies carried out in our laboratory found increased rates of pre- and postimplantation embryo losses and embryonic death (resorption), reduced number of live fetuses, and maternal weight gain during diabetic pregnancy. These alterations are due to metabolic changes caused by hyperglycemic peaks during

pregnancy (Kiss et al. 2009; Damasceno et al. 2011; Iessi et al. 2010; Saito et al. 2010; Dallaqua et al. 2012; Sinzato et al. 2012). The divergent results shown in our study, which does not reinforce an effect of diabetes on the reproductive performance of rats, is possibly explained by mating time. In the present study, the rats were mated with 90 days old, whereas in the other studies, they were mated with nearly 120 days of age. This fact suggests that 90 days of hyperglycemia is not long enough to affect the reproductive performance of rats significantly. Similarly, Nakhjavani et al. (2010) verified that serum level of HSP70 is significantly higher in patients with diabetes and correlates with the duration of disease. Higher HSP70 in prolonged diabetes versus newly diagnosed diabetes may be an indicator of metabolic derangement in the course of diabetes.

Extracellular HSP70 (eHSP70) can act as an intercellular stress signaling molecule, representing an ancestral danger signal of a nonphysiological condition, such as cellular stress or damage, to elicit innate and adaptive proinflammatory immune responses (Pockley 2003). In the present study, it was demonstrated that extracellular serum HSP70 levels in rats was affected by mild diabetes at day 90 of life (adult age), corroborating with Nakhjavani et al. (2010), who verified that serum level of HSP70 is significantly higher in patients with diabetes. However, at term pregnancy, the mild diabetic rats presented no alteration on eHSP70 level. This finding corroborates with Saito et al. (2013), who reported that mild diabetic rats showed no changes on eHSP70 levels but streptozotocin-induced severe diabetic rats (glucose levels ≥ 300 mg/dL) presented increased eHSP70 levels, which were positively correlated to resorption frequency and rates of postimplantation embryo losses. Based on these results and according to our findings, we suggest that the glucose levels reached with mild diabetes were not high enough to affect serum HSP70 levels at term pregnancy. Welch (1992) reports that the response to nonphysiological stress (exposure to metabolic challenges and other stressors) is characterized by a drastic increase in the expression of the HSPs, which comprise a selected group of proteins. This explains the association between HSP70 levels and hyperglycemia in cases of severe diabetes. Severe diabetes occurs in adulthood and causes sudden and abrupt changes in blood glucose levels, compromising homeostasis and increasing HSP70 levels significantly. Given that rats without diabetes or with mild diabetes showed no difference on glucose levels at term pregnancy, the lack of a stressor such as hyperglycemia did not affect HSP70 levels at euthanasia time.

Other studies indicate that, among the HSPs, HSP70 in special plays a role in embryogenesis and

Table 2 Pearson correlation analyses among HSP70 serum levels and blood glucose concentration and maternal reproductive parameter

	<i>R</i>	<i>p</i> value
Pre-pregnancy period		
HSP70 X blood glucose level	-0.26	0.35
Pregnancy period		
Maternal HSP70×blood glucose level (day 0)	0.06	0.86
Maternal HSP70×blood glucose level (day 21)	-0.13	0.72
Maternal HSP70×corpora lutea number	0.44	0.20
Maternal HSP70×implantation number	0.25	0.48
Maternal HSP70×resorption number	0.65	0.04
Maternal HSP70×offspring HSP70	0.11	0.88

Fig. 3 Serum HSP70 levels sampled of nondiabetic (ND90) and mild diabetic (D90) rats at day 90 of life, of nondiabetic (NDP) and mild diabetic (DP) rats at term pregnancy and of the offspring of these dams (NB-NDP and NB-DP). Data presented as mean $\pm$ standard deviation. * $p < 0.05$, statistically significant difference compared to respective control group (NP90 vs D90, and NB-NDP vs NB-DP—generalized linear model (gamma distribution) with adjustment for body weights and blood glucose levels, followed by Wald's multiple comparison test)

reproduction. Our findings show that maternal HSP70 is also an indicator of postimplantation embryo loss and is positively correlated to embryonic death rate. Accordingly, Tan et al. (2007) report that increase in HSP70 levels in mononuclear cell lysate from peripheral blood is associated with miscarriage, fetal death, and preterm parturition. The literature demonstrates that increased maternal HSP70 levels are a predictor of pregnancy disorders (Fukushima et al. 2005; Molvarec et al. 2011; Jaffe et al. 2013).

Some studies suggest that exposure to diabetes in the intrauterine environment increases the risk of metabolic disorders in the offspring, including processes that compromise growth (Lehnen et al. 2013). In addition, human babies born to mothers with gestational diabetes are more susceptible to the development of hypertension and other cardiovascular diseases (Vambergue and Fajardy 2011). In our study, the extracellular serum HSP70 levels were lower in the offspring of mild diabetic rats. This finding suggests that maternal mild hyperglycemia impaired fetal immune response. Our research group also verified in clinical investigation that the HSP70 levels in cord blood of diabetic pregnant woman were low (unpublished data). HSP70 may be a mobile messenger to transduce nonspecific stress information to the immune system for the initiation of innate immunity and adaptive immunity (Chen and Cao 2010). Because of its chaperone function, HSP70 is also involved in antigen processing and presentation (Cristau et al. 1994; Meyer and da Silva 1999). Additionally, Nakhjavani et al. (2011) showed that serum eHSP72 level is increased in women with long-standing diabetes compared with men, indicating the existence of some differences of gender/sex. However, the eHSP70 levels of offspring from mild diabetic rats were analyzed in

pool, preventing sex difference analysis in this investigation.

Recent studies showed that newborns from mothers with gestational diabetes mellitus present deficiency in lymphocyte subsets (Lapolla et al. 2005). Moreover, they show a great reduction in the proliferative capacity and activity of T cells and delay in their activation, even in the presence of superantigens (Badr et al. 2011, 2013). Phagocytes are considered the first line of the immune defense, and decrease in their functional activity makes the body more vulnerable to infections (Alba-Loureiro et al. 2007). Phagocytic disorders are in fact the major cause of infectious diseases, especially bacterial infections (Larijani et al. 2007). The phagocytic and bacterial activity of cord blood mononuclear phagocytes against enteropathogenic *Escherichia coli* (EPEC) is low in pregnant women with hyperglycemia (França et al. 2012). These evidences reinforce our findings on the decrease in HSP70 levels in offspring from rats with mild diabetes, which can be associated with decrease in the immune response observed in offspring from hyperglycemic dams. The reduction in HSP levels in offspring of mild diabetic rats impairs antigen presentation, thereby decreasing the protection conferred by these proteins. The inability in recognizing and responding to antigens increases the vulnerability to infections and the risk for developing a number of disorders throughout the life.

In conclusion, mild diabetes does not affect maternal reproductive performance, but there is a relationship between extracellular maternal HSP70 levels and embryonic death. In addition, offspring from mild diabetic rats show lower extracellular HSP70 levels, suggesting that this protein can be used as an indicator of metabolic consequences of diabetes and predictor of related disorders in adulthood.

Acknowledgments We are grateful to Talisia Moreto, technician in the Laboratory of Experimental Research on Gynecology and Obstetrics,

Botucatu Medical School Unesp, and to the financial support of CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Brazil.

References

- Alba-Loureiro TC, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiva P (2007) Neutrophil function and metabolism in individuals with diabetes mellitus. *Braz J Med Biol Res* 40(8):1037–1044
- American Diabetes Association (2014) Diagnosis and classification of diabetes mellitus. *Diabetes Care* 37(1):S81–S90
- Anderson RL (1998) Stress proteins and apoptosis in prenatal development, cancer and medicine. *Cell Stress Chaperones* 3(4):209–212
- Badr G, Alwasel S, Ebaid H, Mohany M, Alhazza I (2011) Perinatal supplementation with thymoquinone improves diabetic complications and T cell immune responses in rat offspring. *Cell Immunol* 267(2):133–140
- Badr G, Mahmoud MH, Farhat K, Waly H, Al-Abdin OZ, Rabah DM (2013) Maternal supplementation of diabetic mice with thymoquinone protects their offspring from abnormal obesity and diabetes by modulating their lipid profile and free radical production and restoring lymphocyte proliferation via PI3/AKT signaling. *Lipids Health Dis* 12:37
- Campisi J, Leem TH, Fleshner M (2003) Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. *Cell Stress Chaperones* 8(3):272–286
- Chen T, Cao X (2010) Stress for maintaining memory: HSP70 as a mobile messenger for innate and adaptive immunity. *Eur J Immunol* 40:1541–1544
- Cristau B, Schafer PH, Perce SK (1994) Heat shock enhances antigen processing and accelerates the formation of compact class II dimers. *J Immunol* 152(4):1546–1556
- Dallaqua B, Saito FH, Rodrigues T, Calderon IM, Rudge MV, Herrera E, Damasceno DC (2012) Treatment with Azadirachta indica in diabetic pregnant rats: negative effects on maternal outcome. *J Ethnopharmacol* 143(3):805–811
- Damasceno DC, Kempinas WG, Volpato GT, Consoni M, Rudge MVC, Paumgarten FJR (2008) Anomalias Congênitas: Estudos Experimentais, first ed. Coopmed, Belo Horizonte
- Damasceno DC, Kiss AC, Sinzato YK, de Campos KE, Rudge MV, Calderon IM, Volpato GT (2011) Maternal-fetal outcome, lipid profile and oxidative stress of diabetic rats neonatally exposed to streptozotocin. *Exp Clin Endocrinol Diabetes* 119(7):408–13
- Damasceno DC, Netto AO, Iessi IL, Gallego FQ, Corvino SB, Dallaqua B, Sinzato YK, Bueno A, Calderon IM, Rudge MV (2014) Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes. *Biomed Res Int* 2014:819065
- de Campos KE, Sinzato YK, Pimenta Wde P, Rudge MV, Damasceno DC (2007) Effect of maternal obesity on diabetes development in adult rat offspring. *Life Sci* 81(19–20):1473–1478
- Eriksson UJ, Cederberg J, Wentzel P (2003) Congenital malformations in offspring of diabetic mothers—animal and human studies. *Rev Endocr Metab Disord* 4(1):79–93
- França EL, Calderon IM, Vieira EL, Morceli G, Honório-França AC (2012) Transfer of maternal immunity to newborns of diabetic mothers. *Clin Dev Immunol* 2012:928187
- Fukushima A, Kawahara H, Isurugi C, Syoji T, Oyama R, Sugiyama T, Horiuchi S (2005) Changes in serum levels of heat shock protein 70 in preterm delivery and preeclampsia. *J Obstet Gynaecol Res* 31(1):72–77
- Gelardi NL, Cha CJM, Oh W (1990) Glucose metabolism in adipocytes of obese offspring of mild hyperglycemic rats. *Pediatr Res* 28(6):641–645
- Grigore M, Indrei A (2001) The role of heat shock proteins in reproduction. *Rev Med Chir Soc Med Nat Iasi* 105(4):674–676
- Hendrick JP, Hartl FU (1993) Molecular chaperone functions of heat shock proteins. *Annu Rev Biochem* 62:349–384
- Higa R, Jawerbaum A (2013) Intrauterine effects of impaired lipid homeostasis in pregnancy diseases. *Curr Med Chem* 20(18):2338–2350
- Iessi IL, Bueno A, Sinzato KY, Taylor KN, Rudge MV, Damasceno DC (2010) Evaluation of neonatally-induced mild diabetes in rats: maternal and fetal repercussions. *Diabetol Metab Syndr* 2(1):37
- Jaffe S, Doulaveris G, Orfanelli T, Arantes M, Damasceno D, Calderon I, Rudge MV, Witkin SS (2013) Induction of the 72 kDa heat shock protein by glucose ingestion in black pregnant women. *Cell Stress Chaperones* 18(4):527–530
- Jin XY, Li JX, Cui LL, Xue SZ, Tao L, Min C, Zhao YH, Fei G, Yi XL (2009) Increased expression of heat shock protein 105 in rat uterus of early pregnancy and its significance in embryo implantation. *Reprod Biol Endocrinol* 7:23
- Kervran A, Guillaume M, Jost A (1978) The endocrine pancreas of the fetus from diabetic pregnant rat. *Diabetologia* 15(5):387–393
- Kiss AC, Lima PH, Sinzato YK, Takaku M, Takeno MA, Rudge MV, Damasceno DC (2009) Animal models for clinical and gestational diabetes: maternal and fetal outcomes. *Diabetol Metab Syndr* 1(1):21
- Lapolla A, Dalfrà MG, Sanzari M, Fedele D, Betterle C, Masin M, Zanchetta R, Faggian D, Masotti M, Nucera V, Plebani M (2005) Lymphocyte subsets and cytokines in women with gestational diabetes mellitus and their newborn. *Cytokine* 31:280–287
- Larijani B, Shooshtarizadeh P, Mosaffa N, Heshmat R (2007) Polymorphonuclear leucocyte respiratory burst activity correlates with serum zinc level in type 2 diabetic patients with foot ulcers. *Br J Biomed Sci* 64(1):13–17
- Lehnen H, Zechner U, Haaf T (2013) Epigenetics of gestational diabetes mellitus and offspring health: the time for action is in early stages of life. *Mol Hum Reprod* 19(7):415–422
- Lenzen S (2008) The mechanisms of alloxan and streptozotocin-induced diabetes. *Diabetologia* 51(2):216–226
- López-Soldado I, Herrera E (2003) Different diabetogenic response to moderate doses of streptozotocin in pregnant rats, and its long-term consequences in the offspring. *Exp Diabetes Res* 4(2):107–118
- Maayan-Metzger A, Lubin D, Kuint J (2009) Hypoglycemia rates in the first days of life among term infants born to diabetic mothers. *Neonatology* 96(2):80–85
- Mello MAR, Souza T, Braga LR, Santos W, Ribeiro IA, Gobatto CA (2001) Glucose tolerance and insulin action in monosodium glutamate (MSG) obese exercise-trained rats. *Physiol Chem Phys Med* 33(1):63–71
- Metzger BE (2002) The hyperglycemia and adverse pregnancy outcome (HAPO) study. *Int Gynecol Obst* 78:69–77
- Meyer TN, da Silva AL (1999) Resposta celular ao estresse. *Rev Ass Med Bras* 45(2):181–188
- Molvarec A, Szarka A, Walentin S, Beko G, Karádi I, Prohászka Z, Rigó J Jr (2011) Serum heat shock protein 70 levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in women with preeclampsia. *Clin Chim Acta* 412(21–22):1957–1962
- Nakhjavani M, Morteza A, Khajeali L, Esteghamati A, Khalilzadeh O, Asgarani F, Outeiro TF (2010) Increased serum HSP70 levels are associated with the duration of diabetes. *Cell Stress Chaperones* 15(6):959–964
- Nakhjavani M, Morteza A, Meysamie A, Esteghamati A, Khalilzadeh O, Esfahanian F, Khajeali L, Feiz F (2011) Serum heat shock protein 70 and oxidized LDL in patients with type 2 diabetes: does sex matter? *Cell Stress Chaperones* 16(2):195–201
- Neuer A, Spandorfer SD, Giraldo P, Dieterle S, Rosenwalks Z, Witkin SS (2000) The role of heat shock proteins in reproduction. *Hum Reprod Update* 6(2):149–159
- Pockley AG (2002) Heat shock proteins, inflammation, and cardiovascular disease. *Circulation* 105(8):1012–1017

- Pockley AG (2003) Heat shock proteins as regulators of the immune response. *Lancet* 362:469–476
- Reece EA, Couston DR, Gabbe SG (2004) *Diabetes in women: adolescence, pregnancy and menopause*, 3rd edn. Lippincott Williams & Wilkins, New York (NY)
- Saito FH, Damasceno DC, Kempinas WG, Morceli G, Sinzato YK, Taylor KN, Rudge MVC (2010) Repercussions of mild diabetes on pregnancy in Wistar rats and on the fetal development. *Diabetol Metab Syndr* 2(1):26
- Saito SH, Damasceno DC, Dallaqua B, Linhares IM, Rudge MV, Calderon IMP, Witkin SS (2013) Heat shock protein production and immunity and altered fetal development in diabetic pregnant rats. *Cell Stress Chaperones* 18(1):25–33
- Salewski E (1964) Farbemethode zum makroskopischen nachweis von implantations-stellen am uterus der ratte. *Arch Exp Pathol Pharmacol* 247(4):367
- Scaglia L, Smith FS, Bonner-Weir S (1995) Apoptosis contributes to the involution of beta-cell mass in the post-partum rat pancreas. *Endocrinology* 136:5461–5468
- Sinzato YK, Volpato GT, Iessi LI, Bueno A, Calderon Ide M, Rudge MV, Damasceno DC. (2012) Neonatally induced mild diabetes in rats and its effect on maternal, placental, and fetal parameters. *Exp Diabetes Res* 2012:108163
- Tan H, Xu Y, Xu J, Wang F, Nie S, Yang M, Tanguay RM, Wu T (2007) Association of increased heat shock protein 70 levels in the lymphocyte with high risk of adverse pregnancy outcomes in early pregnancy: a nested case-control study. *Cell Stress Chaperones* 12(3):230–236
- Vambergue A, Fajardy I (2011) Consequences of gestational and pregestational diabetes on placental function and birth weight. *World J Diabetes* 2(11):196–203
- Welch WJ (1992) Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* 72(4):1063–1081
- Yang J, Cummings EA, O'Connell C, Jangaard K (2006) Fetal and neonatal outcomes of diabetic pregnancies. *Obstet Gynecol* 108(3 Pt1):644–650