ENDOCRINE REVIEWS

Animal Models in Diabetes and Pregnancy

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The worldwide increase in the incidence of diabetes, the increase in type 2 diabetes in women at reproductive ages, and the cross-generation of the intrauterine programming of type 2 diabetes are the bases for the growing interest in the use of experimental diabetic models in order to gain insight into the mechanisms of induction of developmental alterations in maternal diabetes.

In this scenario, experimental models that present the most common features of diabetes in pregnancy are highly required. Several important aspects of human diabetic pregnancies such as the increased rates of spontaneous abortions, malformations, fetoplacental impairments, and offspring diseases in later life can be approached by using the appropriate animal models. The purpose of this review is to give a practical and critical guide into the most frequently used experimental models in diabetes and pregnancy, discuss their advantages and limitations, and describe the aspects of diabetes and pregnancy for which these models are thought to be adequate. This review provides a comprehensive view and an extensive analysis of the different models and phenotypes addressed in diabetic animals throughout pregnancy. The review includes an analysis of the surgical, chemical-induced, and genetic experimental models of diabetes and an evaluation of their use to analyze early pregnancy defects, induction of congenital malformations, placental and fetal alterations, and the intrauterine programming of metabolic diseases in the offspring's later life. (***Endocrine Reviews*** **31: 680–701, 2010)**

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I. Introduction

Maternal diabetes constitutes an unfavorable environment for embryonic and fetoplacental development. Despite current treatments, pregnant women with either type 1 or type 2 diabetes are at increased risk of miscarriage, stillbirth, congenital malformations, placental abnormalities, and intrauterine malprogramming (1–7). Because gestational diabetes is induced after the organogenic period, there are no risks for early embryo defects or congenital malformations unless the woman presents undiagnosed pregestational diabetes; however, the fetoplacental impairments and intrauterine programming

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Abbreviations: BB, Bio-breeding; GK, Goto Kakizaki (rat); Glut, glucose transporter; NOD, nonobese diabetic; PGE_2, prostaglandin E_2.

TABLE 1. Experimental models in diabetes and pregnancy

Type of experimental model	Animal species	Phenotype	Refs.
Surgical method			
Partial pancreatectomy	Rats, sheep	Mild diabetes	14, 15, 243
Chemical methods			
Streptozotocin administration	Rats, mice, rabbits, sheep	Mild/severe diabetes	22, 52, 128, 131, 239, 266, 267
Alloxan administration	Rats, mice, rabbits, sheep, swine	Mild/severe diabetes	20, 21, 83, 176, 197, 268
Genetic models			
NOD	Mice	Mild/severe diabetes	38, 84, 162
BB	Rats	Severe diabetes	149, 249
GK	Rats	Mild diabetes	44, 184
Cohen	Rats	Mild diabetes	269
Akita	Mice	Mild diabetes	46
Db/+	Mice	Mild diabetes	224

Listed references are examples of the literature findings and not a complete list.

of diseases in the offspring's later life induced by gestational diabetes are similar to those induced by type 1 and type 2 diabetes (8).

A classification of the existing diabetic experimental models is difficult because there are obvious differences between the etiology of the human disease and that of each experimental diabetic model, as detailed in the following sections. Nevertheless, in both diabetic patients and diabetic experimental models, the degree of pancreatic β-cell dysfunction and insulin resistance determines the degree of maternal metabolic disbalance, and thus the severity of the complications in diabetes and pregnancy (9, 10).

A similar picture of the complexity of the human diabetic disease is present in the experimental models of diabetes. Indeed, the diabetes and pregnancy experimental models can present a broad range of hyperglycemia, can either lead or not lead to alterations at the earliest stages of pregnancy, can show different rates of embryo resorption and malformations, can present microsomic or macrosomic fetuses, and can either affect or not affect the offspring's health later in life.

Although several review articles have analyzed the different animal models of diabetes available (9, 11, 12), different aspects arise and should be taken into account when the pregnant state is evaluated in diabetic animals. This comprehensive review details the maternal diabetes-induced alterations in different diabetic animal models throughout pregnancy.

The purpose of this review is to give a practical and critical approach to the most frequently studied animal models of diabetes, with emphasis on the aspects of diabetes and pregnancy for which these models are thought to be appropriate.

II. Methods in Experimental Diabetes and Pregnancy

When the matter of interest requires the use of an experimental model of diabetes in pregnancy, the first decision is to choose a useful model. This section will describe the basic characteristics of the most frequently used models in diabetes and pregnancy, whereas the following sections will guide the choice of adequate experimental diabetes and pregnancy models and will detail how these models have been used to address anomalies during early gestation, congenital malformations, placental and fetal alterations, and intrauterine programming of diseases in the offspring's later life.

Experimental models of diabetes and pregnancy can be obtained by surgical procedures, chemical induction, or the use of spontaneous or genetically derived animal strains (Table 1).

A. Surgical models in diabetes and pregnancy

Although pancreatectomy in dogs led to the discovery of insulin (13), most experimental models are precluded to rodents for ethical, economic, and practical reasons. Partial pancreatectomy in rats and mice leads to a diabetic model compatible with the pregnant state (14, 15). This model has been used to study uterine dysfunction and embryonic and fetoplacental alterations in mild maternal diabetes (Table 2). The basis of this model is the removal of most pancreatic tissue (95% of weight), except for that located between the bile duct and the duodenum. Because this surgery is performed in animals after puberty, this is a model for pregestational type 1 diabetes.

The advantages of this technique are that a mild diabetic model is generated (glycemias range from 150 to 200 mg/dl), no insulin administration is needed, and pregnancy rates are good (16). The main limitations of this methodology are the expertise required to proceed with this surgery, the elevated postsurgical mortality rate (about 20%), the nonspecific reduction of the β-cell mass, and the time required between the surgical procedure and the diabetic symptoms (up to 2–3 months). Altogether these limitations lead to a diabetic model that is not frequently used nowadays because many other options are available.

B. Chemical-induced models in diabetes and pregnancy

Nonsurgical methods of inducing damage to the pancreatic β-cells are obtained through the administration of

TABLE 2. Complications analyzed in animal models in diabetes and pregnancy

Diabetes and pregnancy complications	Experimental models	Refs.
Preimplantation defects	Chemical-induced	
	Streptozotocin and alloxan in mice and rats	76, 81, 83
	Genetic models	
	NOD rats, BB rats, Akita mice	79, 80, 84
Congenital malformations	Chemical-induced	
	Streptozotocin and alloxan in mice and rats	52, 103, 119, 130, 132, 197
	Genetic models	
	NOD mice, BB rats, Cohen rats	106, 149, 185
Uterine-placental defects	Surgical methods	
	Rat partial pancreatectomy	14, 270
	Chemical-induced	
	Streptozotocin and alloxan in mice and rats	150, 155, 157–159, 166, 174, 176
	Genetic models	
	NOD mice and BB rats	78, 149
Fetal alterations	Surgical methods	
	Partial pancreatectomy in rats and sheep	14, 243
	Chemical-induced	
	Streptozotocin and alloxan in mice and rats	140, 163, 167, 181, 183, 197, 232
	Genetic models	
	NOD mice, BB rats, Akita mice, Cohen rats, db/+ mice, GK rats	46, 149, 184, 224, 249, 254, 260, 262
Offspring neonatal/later life defects	Chemical-induced	
	Streptozotocin in mice and rats	27, 61, 195, 209, 211, 215, 216, 233, 236, 238
	Genetic models	
	NOD mice, BB rats, db/+ mice, Sand rats, GK rats	37, 38, 42, 44, 110, 222, 224, 250

Listed references are examples of the literature findings and not a complete list.

drugs such as streptozotocin, a nitrosurea derivative isolated from *Streptomyces achromogenes*, and alloxan, a uric acid derivative (17, 18). At the appropriate doses, these drugs act by selectively destroying the pancreatic β-cells, even though streptozotocin is more selective than alloxan (19). These treatments lead to insulin deficiency and hyperglycemia in different animals (17, 20–22). Much of the research on diabetes and pregnancy has relied on the use of rodents rendered diabetic through the administration of these chemicals. Indeed, these models of diabetes have been widely used to address early embryo developmental defects, the induction of malformations, placental abnormalities, fetal maldevelopment, and intrauterine transmission of metabolic diseases (Table 2).

Many different approaches have been used as regards the mode of drug administration, the doses, and the animal age and stage (pregestational or gestational). Nevertheless, because the loss of pancreatic β-cell mass is a characteristic of all the different chemical-induced diabetic approaches, these models have low maternal insulin circulating levels. Despite the partial regeneration of pancreatic β-cells observed in the neonatal streptozotocin-induced diabetic models (23) and the reduced damage of the β-cells in mice injected with various low doses of streptozotocin (24), these models are considered type 1 diabetic models because their origin is due to the destruction of β-cells rather to insulin resistance. In addition, the administration of these drugs to pregnant animals leads to the destruction of β-cells, thus generating a maternal diabetic state related to type 1 diabetes during pregnancy. Differently, gestational diabetes is mostly characterized by the lack of adaptation of pancreatic β-cells to the metabolic changes that take place after midpregnancy and/or by an enhanced insulin-resistant state (8, 25, 26).

To simplify, the different chemical approaches can be classified into those that in rodent strains lead to severe hyperglycemia (glycemia levels greater than 250 mg/dl) and those that lead to mild hyperglycemia (glycemia levels lower than 250 mg/dl). Several different approaches that lead to mild and severe diabetes in rats and mice through chemical induction are shown in Table 3.

An important point is that the possibilities to obtain pregnancies in diabetic animals and obtain specific diabetes-induced alterations during early, mid, or late gestation have great variation between rodent strains and substrains, even when the same amount of streptozotocin or alloxan is given at the same period (neonatal, pregestational, or gestational). In addition, although dose dependency is achieved in rats treated with low streptozotocin doses leading to mild diabetic and pregnancy experimental models, dose dependency is difficult to achieve at higher concentrations (27). This may be due to the interaction of polygenic and nutritional factors that lead to different responses to the damaging agent in the β-cells and to the

TABLE 3. Various technical approaches to generate experimental models of diabetes and pregnancy by the use of chemicals

Chemical used and type of administration	Phenotype	Refs.
Streptozotocin (1 dose, 40–45 mg/kg iv or 50–75 mg/kg ip) given to adult rats several days before mating	Severe diabetes	119, 135, 155
Streptozotocin (1 dose, 200–240 mg/kg ip) given to adult mice several days before mating	Severe diabetes	130, 157
Streptozotocin (1 dose, 45 mg/kg iv to rats, or 100 mg/kg to mice) given several days before mating and insulin administration until d 1 of gestation	Severe diabetes	52, 131, 271
Streptozotocin (1 dose, 90–100 mg/kg sc) given to rats in the neonatal period	Mild diabetes	170, 239
Streptozotocin (1 dose, 15–65 mg/kg iv or ip) given to rats during pregnancy	Mild/severe diabetes	61, 124, 236, 272, 273
Streptozotocin (3 consecutive doses, 75–90 mg/kg iv or ip) given to mice prior to mating	Mild/severe diabetes	54, 142, 144
Alloxan given to mice (300 mg/kg) prior to induced superovulation and mating or to rats (40 mg/kg ip) during pregnancy	Mild/severe diabetes	83, 176

Listed references are examples of the literature findings and not a complete list.

triggering of different repairing and compensatory responses, although further research on this subject is needed (12, 28–30).

Therefore, as a starting point to using chemical-induced models in diabetes and pregnancy, researchers should first carry out a pilot study addressing whether or not a specific rodent substrain bred in a determinate animal facility and fed with a determinate food leads to the phenotype to be evaluated. Indeed, depending on the election of the diabetic animal model chosen, glycemia levels can be either similar to those frequently observed in diabetic patients or very elevated, such as those found in humans in severe diabetic conditions (Table 3). On the other hand, although severe diabetic models have glycemia levels higher than those usually found in patients, this is due to the patient's insulin treatment. Thus, insulin-treated chemical-induced diabetic animals, in which glycemia is not completely corrected, are also diabetes and pregnancy animal models that deserve further studies.

Some of the limitations that arise depending on the dose and rodent strains used are the difficulties in achieving reasonable pregnant rates. Indeed, although pregnancy in the mild hyperglycemic rodent models is not impeded, animal models with severe hyperglycemia often stop cycling 2 or 3 wk after streptozotocin/alloxan administration (31, 32).

In those cases, some of the approaches to obtain pregnant diabetic animals are: the use of a different strain, the mating of the animals in the two or three reproductive cycles that follow the drug administration, the preconceptional insulin administration, the induction of ovulation, or the drug administration during pregnancy (Table 3). The limitations of some of these approaches are described in *Sections III and IV*.

One of the main advantages of the chemical-induced diabetic models is the vast literature that supports their use as models to address the impact of the metabolic alterations induced by maternal diabetes and the mechanisms of induction of the most common complications in diabetes and pregnancy (Table 2). Another advantage is that obtaining the diabetic animals through this methodology is relatively easy.

Together with the possible criticisms of the use of a toxic agent to induce the β-cell damage and thus the diabetic pathology, another disadvantage of chemical-induced diabetic models is that the genetic and immune components of the diabetic disease are not present. Nevertheless, although the causes of β-cell death in human diabetes and in chemical-induced diabetic models are clearly different, the chemical destruction of β-cells induces a series of proinflammatory reactions similar to those occurring in the autoimmune destruction of the β-cells in human diabetes and, indeed, even pancreatic macrophage infiltration occurs in models such as those that involve multiple low-dose streptozotocin administration (33, 34).

C. Genetic models in diabetes and pregnancy

Both type 1 and type 2 genetic models of diabetes have been successfully used to address the complications induced by diabetes and pregnancy. Inbreeding to select for hyperglycemia and insulin resistance leads to several diabetic models with different degrees of β-cell failure and/or insulin resistance (9).

The nonobese diabetic (NOD) mice and bio-breeding (BB) rats are the most commonly used animals that spontaneously develop type 1 diabetes. In common with the human disease, the pancreatic islets are subjected to an immune attack with T cells, B cells, macrophages and natural killer cells being recruited to the insulitis (35). Genetic

studies have localized multiple susceptibility genes, and, as in human type 1 diabetes, the major histocompatibility complex regions play a key role (36). These models have been used to address causes of subfertility and embryo loss, embryo malformations, fetoplacental abnormalities, and offspring's macrosomia and later life diseases (Table 2).

Advantages of these models are the similarity of their immunological origin with that of type 1 human diabetes. It should be taken into account that the genetic background that leads to the diabetic phenotype will depend on the breeding strategy and can thus be present or not in the fetuses of genetic models in diabetes and pregnancy. Due to the polygenic origin of these models, breeding strategies and embryo transfer techniques are useful to analyze the maternal effects independently of the fetal genotype (37). It should also be noted that insulin-dependent diabetes develops spontaneously in 9% of NOD mice by 12 wk and in 80% of them by 30 wk of age (38). Therefore, a disadvantage of this model is that studies are sometimes performed in females older than 30 wk of age, whereas other models are usually used at much younger female ages. On the other hand, whereas NOD mice constitute a model of mild diabetes, BB rats develop severe diabetes, and ketoacidosis may be fatal unless exogenous insulin is administered (39).

The Akita mouse has a single autosomal dominant mutation in the insulin II gene (ins2) that disrupts normal insulin processing and causes a failure in the secretion of mature insulin and a reduction of β-cell mass, a phenotype similar to maturity-onset diabetes of the young diabetic patients (40). This model has been used for the analysis of preimplantation and fetoplacental defects (Table 2).

There are many animal models of type 2 diabetes that are as heterogeneous as the human condition (41). However, not many of them have been used to study the diabetic pregnancy condition. The db/db mouse diabetic model results from a point autosomal recessive mutation in the leptin receptor gene, and although these mice are infertile, db/+ mice are glucose intolerant and develop diabetes during gestation, therefore providing a gestational diabetic experimental model (42). On the other hand, most type 2 diabetic models used to analyze diabetic and pregnancy complications have a polygenic origin, such as the Cohen diabetic rat, the Sand rat (*Psammomys obesus*), and the Goto Kakizaki (GK) rat (43). Their several genetic mutations are transmitted from generation to generation, and an excess of maternal transmission of the diabetic disease has been reported in some studies (44, 45). These type 2 diabetic models have been used to address maternal diabetes-induced developmental defects, the induction of congenital malformations, fetal alterations, and the programming of diseases in the offspring's later life (Table 2).

An advantage of these genetic type 2 diabetic models is the wide range of phenotypes regarding the degree of obesity, hyperglycemia, and insulin resistance. It should be noted that the evaluation of these models requires the analysis of the influence of maternal diabetes separately from the fetal genotype. This can be performed through breeding or embryo transfer strategies (45). Genotypic typification of the fetuses when their mothers are mated with wild-type males is also useful mostly in those models arising from a single mutation (46).

III. Choosing an Adequate Experimental Model in Diabetes and Pregnancy

A. Ethical, economic, and practical issues

Animal models in diabetes and pregnancy, used after the approval from the appropriate ethical committees, are useful when studies addressing a particular investigative purpose cannot be conducted in humans due to ethical concerns and cannot be addressed by alternative methods that do not imply live animals (47). These situations are common when addressing embryo and fetal development in maternal diabetes. Nevertheless, because there is no animal model equal to the human situation, caution should be taken to extrapolate the results obtained to the human disease, and validation of the results obtained is always required.

In diabetes and pregnancy animal models, water and food consumption is usually increased, and care should be taken to provide adequate housing considering their increased urination. Although the diabetic state is not painful, ketoacidosis or severe alterations in organs such as the kidneys and the eyes may occur in animals with severe hyperglycemia (48).

Ethically, in any animal model selected, the number of animals used should be as low as possible to lead to the expected result (49, 50). Thus, regarding the selection of the animal model, when the purpose of the investigation allows the use of rodents, this choice is recommended. This is because of the lower number of animals required as a result of their multiparity, their human-like hemochorial placentation, the short duration of their pregnancies, and their easy maintenance. Nevertheless, it should be taken into account that multiparity is not common in humans and that differences arise in placental development and structure when compared with humans (51).

When comparing rats and mice, the latter offer a broader range of possibilities of genetic manipulation and are thus recommended in case genetic strategies are pursued. Care should be taken in the selection of the mice

genetic background when diabetes is planned to be induced by chemicals because there are important differences in the doses of the chemicals required under different genetic backgrounds (52–54). On the other hand, the small size of the mice may challenge the surgical models in diabetes and pregnancy. Finally, mice constitute excellent models to study the early embryo and organogenesis stages, although their smaller size may challenge the studies at the fetal stage.

Rats have been mostly studied in chemical-induced type 1 diabetes and pregnancy experimental models. Although surgical procedures have been used to obtain type 1 diabetes and pregnancy models in rats (14, 15), they should not be chosen unless specifically needed because this major surgery has a high mortality rate and requires high expertise, and there are several other chemical-induced and genetic options to obtain experimental models of diabetes with less ethical concerns.

There are several worthy genetic models of both type 1 and type 2 diabetes in rodents (9, 55). However, the need to purchase most of these animals from specialized companies, the lack of commercial availability of some of these strains, and the resulting increased costs are the main reasons for the reduced number of studies in these strains when compared with the chemical-induced models in diabetes and pregnancy.

The chemicals to induce diabetes and pregnancy experimental models can be used in a wide range of animals such as ewes, pigs, and monkeys (56–58). Due to the increased cost of maintenance of many of these animal species, their use is recommended when the aims of the study require them. For example, monkeys are much better models than rodents when addressing the cognitive consequences of maternal diabetes, and big animals like sheep are very useful in the study of fetoplacental circulation (56).

B. A critical comparison between the expected and available experimental models in diabetes and pregnancy

The available experimental models in diabetes and pregnancy have limitations when compared with an ideal diabetes and pregnancy experimental model.

Indeed, an ideal experimental model of type 1 diabetes should have an autoimmune destruction of the β-cells during its early life. In surgical models in diabetes and pregnancy, the lack of β-cells is the product of the removal of the pancreas (16), whereas in the chemical-induced models in diabetes and pregnancy, β-cells are destroyed due to a specific β-cell-induced death (17, 23). Thus, although the resulting metabolic impairments in these experimental models can be compared with those found in type 1 diabetic patients, the causes that lead to the β-cell damage differ from the human situation. In both NOD and BB rats, the destruction of β-cells is the product of an autoimmune reaction, but in the NOD mice this occurs in aged animals rather than in young ones (38).

An ideal experimental model of type 2 diabetes should have insulin resistance and impairments in the pancreatic response secondary to the insulin resistance. This is not the case with the chemical-induced diabetic models, in which the main insult is the destruction of the β-cells. Dietary treatments such as those containing increased sucrose, fructose, and fat lead to an insulin-resistant state that, combined with the chemical destruction of the β-cells, could constitute type 2 diabetes experimental models (59), although not yet evaluated during pregnancy. On the other hand, although there is a wide range of genetic type 2 diabetic models, many of them have not been addressed during pregnancy (55).

An ideal experimental model of gestational diabetes should have normal glycemia levels before gestation but glucose intolerance and impaired insulin secretion and/or function after midpregnancy, which leads to alterations in both glucose and lipid metabolism in the mother and consequently in the fetus. In the insulin-resistant db/+ mice, diabetes develops during pregnancy, and therefore, this model can be used as a gestational diabetic model, although the deficiency in the leptin receptor that causes this phenotype differs from the etiology of human gestational diabetes (42). On the other hand, the chemical destruction of the β-cells during pregnancy leads to a diabetic experimental model during the pregnant state. Due to the direct damaging effect on the β-cells, there are low circulating maternal insulin levels, whereas failures in the adaptation of β-cells to pregnancy and/or an exaggerated insulin resistance are main features in gestational diabetes (25, 26). Nevertheless, the elevated glucose and other metabolic substrates in the maternal compartment reach the fetuses and are involved in the induction of macrosomia, placentomegaly, and/or the related programming of metabolic diseases, thus allowing the use of this experimental model to analyze these typical gestational diabetes features (60, 61).

It should be noted that possible *per se* effects of the streptozotocin administered during pregnancy cannot be ruled out. Nevertheless, although studies performed in monkeys show that streptozotocin can cross the placenta, due to its short half-life (5–15 min), the streptozotocin concentrations that reach the fetus when the mother is rendered diabetic are very low and do not induce damage in the fetal pancreas (62, 63).

C. Future perspectives

Future studies will be needed to provide models in diabetes and pregnancy that better represent gestational di-

abetes as well as to analyze the pregnant state in the available type 2 diabetic models.

Many studies in which nutritional challenges (low protein or increased fat diets) lead to glucose intolerance and diabetes in the offspring's adult life are in progress. Further studies addressing the pregestational or gestational diabetic state in these animals will be valuable.

Transgenic or knockout diabetic animals have not yet been used as models in diabetes and pregnancy, although future research is likely to make such models available (64). Indeed, transgenic approaches have already been proved to be useful as tools to obtain animals with malformations similar to those induced by maternal diabetes and to address the mechanisms of induction of congenital malformations in streptozotocin-induced diabetes (65–67). In recent studies, the fetal outcome has been analyzed in the normoglycemic and insulin-signaling defective Insr (−/−) and Insr (−/+) mice (68). Moreover, the $H19^{\Delta 13}$ disruption of the H19 gene (a gene that regulates IGF-II imprinting and expression, and is reciprocally imprinted with respect to IGF-II) leads, when inherited from their mothers, to an increased fetal growth (69) as well as to maternal hyperglycemia on d 16 of gestation, thus constituting a fetal-induced gestational diabetes experimental model in mice (70).

IV. Animal Models to Study Early Embryo Development and Embryo Loss in Maternal Diabetes

A. Overview

Ovulation is the first step greatly altered by the abnormal ovarian microvasculature, loss of connectivity in the developing follicle, and the proinflammatory environment in both diabetic patients and experimental diabetic models (71–73). Ovulation failure may be due to other relevant factors such as failures in sexual hormone secretion/function (31, 74), as well as to the fat loss and consequent insufficient leptin signaling to the central nervous system (75).

In the severe diabetic experimental models, insulin may be required to bypass the ovulation defects and obtain pregnancies. Therefore, defects in early gestation have been studied mostly in mild diabetic experimental models and in rodent strains in which estrous cycles are maintained despite hyperglycemia, and oocyte quality and fertilization are not severely affected (76–79). Other approaches include the use of animals superovulated with pregnant mare serum gonadotropin and human chorionic gonadotropin before mating (80, 81). It should be noted that superovulation may compromise the development of the embryos, leading to confounding effects that should be addressed with the appropriate controls, and these treatments will be effective when oocyte quality is sufficient and uterine receptivity is not impeded.

Indeed, as reviewed elsewhere (73, 82), the oocytes from chemical and genetic experimental models of diabetes show important alterations in their quality, the levels/function of signaling molecules, and mitochondrial dysfunction, alterations that can lead to the induction of defects after fertilization.

B. The preimplantation embryo

Both chemical-induced and genetic diabetic experimental models have identified delayed early embryo development (76, 79, 83–85). Besides, streptozotocin-induced diabetic mice and some transgenic approaches have served to identify hyperglycemia-induced metabolic abnormalities in preimplantatory embryos (86, 87).

Progress in the field suggests that viable alterations occurring during the first stages of embryo development have impact on the periimplantatory and postimplantatory developmental stages. Indeed, a recent study has shown that the malformation rate is increased when either one-cell embryos or blastocysts obtained from superovulated streptozotocin-induced diabetic mice are transferred to control recipients (88).

Regarding the immunological aspects of subfertility and embryo loss, NOD mice have been used to address this issue, and recent studies in the pregnant uterus of NOD mice have identified an insufficiency of natural killer cells probably due to a decreased expression of adhesion molecules (89, 90). On the other hand, a proinflammatory environment and altered remodeling processes characterize the uterus of streptozotocin-induced diabetic rats and NOD mice during the periimplantation period (77, 78, 91, 92).

This abnormal environment is probably involved in the pathways that lead to increased embryo loss and in apoptotic events. Indeed, as a key feature of the early embryo developmental defects induced by maternal diabetes, apoptosis is increased in preimplantation embryos obtained from NOD mice and alloxan/streptozotocin-induced diabetic experimental models. Indeed, these models have served to identify several signaling pathways leading to the embryonic cell apoptotic events (93, 94).

Therefore, different experimental models of type 1 diabetes have been useful to address mechanisms of induction of early embryo defects (Table 2), and can be used to gain further insights into the possible inducers causing early embryo damage and their later effects.

For future studies in the preimplantation stage, mild chemical-induced models and genetic models such as NOD mice can be recommended because no insulin administration or superovulation strategies are required. Besides, considering that most diabetic patients are insulin-

treated, studies in diabetes and pregnancy models in which insulin is administered to control severe hyperglycemia are also encouraged. On the other hand, although early embryo loss is increased in type 2 diabetic women, early embryo defects have not yet been studied in experimental models of type 2 diabetes and thus deserve to be evaluated.

V. Animal Models to Study the Induction of Congenital Anomalies in Maternal Diabetes

A. Overview

A higher incidence of congenital malformations as a result of an impaired maternal metabolic control is a feature in both human type 1 and type 2 diabetes and in most experimental models of diabetes evaluated (6, 95, 96). Although clearly dependent on the degree of maternal metabolic control, it is very difficult to reduce the malformation rate to control values even in well-controlled diabetic patients (97–100). Accordingly, the malformation rate may be elevated even in mild diabetic experimental models (73).

B. The postimplantation embryo

As in human diabetic pregnancies, malformations in streptozotocin/alloxan-induced experimental models of diabetes occur mainly in the neural system, heart, and skeleton (1, 101–103).

Morphological, functional, and developmental mitochondrial defects are also found in organogenetic embryos from streptozotocin-induced experimental diabetic models (104, 105).

Diabetic NOD mice also show an increased malformation rate (mostly neural tube defects and skeleton alterations) when compared with controls (106). The cause for the induction of congenital malformations in NOD mice is highly related to the maternal environment because malformations are increased in control embryos transferred into NOD mice. Besides, malformations are also increased in NOD embryos transferred into control recipients, thus highlighting the relevance of the embryo genetic background and/or the programming during oocyte development and preimplantation stages in the induction of malformations (88, 106). A higher incidence of chromosomal anomalies, associations in nucleolar organizing regions, and an increased genomic DNA mutation frequency have been found in embryos from NOD mice and streptozotocin-induced diabetic rodents (107, 108).

The Cohen diabetic rat is the type 2 diabetic model most studied during early organogenesis (109), although fetal alterations have also been found in the GK and Sand rat (110, 111).

Therefore, both chemical-induced and genetic diabetic models can lead to the induction of congenital malformations (Table 2). Indeed, both experimental diabetic models and *in vitro* culture of embryos during early organogenesis have been very helpful in the understanding of the multifactorial aspects that can lead to malformations due to maternal diabetes in this very susceptible developmental period (1, 112, 113).

Whole rat embryo cultures during the organogenetic period have clearly served to establish the increased concentrations of glucose, triglycerides, and β-hydroxybutyrate as teratogens; indeed, these metabolites are elevated in streptozotocin-induced diabetic rats during early organogenesis (114, 115).

Several signaling pathways are impaired within the embryo due to the abnormal maternal metabolic environment and are related to the induction of malformations. The first one studied, described in streptozotocin-induced diabetic rodents and further corroborated in *in vitro* studies, consists of a disturbed arachidonic-prostaglandin pathway that leads to decreased prostaglandin E_2 (PGE_2) concentrations, an alteration involved in the induction of neural tube defects (73, 116, 117). Moreover, PGE_2 concentrations are also reduced in yolk sacs of pregnant diabetic women (118). In addition, the concentrations of two other prostaglandins, PGI_2 and 15-deoxydelta$^{12,14}PGJ_2$, are also decreased in embryos from streptozotocin-induced diabetic rats during early organogenesis and are regulators of PGE_2 and nitric oxide concentrations, respectively (119, 120). Dietary supplementation with safflower and olive oils is capable of both increasing PGE_2 and reducing nitric oxide embryonic concentrations and is also able to reduce both malformation and resorption rates in streptozotocin-induced diabetic rats (121).

On the other hand, chemical-induced diabetic experimental models and *in vitro* studies have also been useful to discover embryonic disturbances in inositol uptake that lead to low intracellular inositol concentrations that impair proper embryo morphogenesis (122–124).

Both increased oxidative stress and nitrosative stress are crucial features in diabetes-induced embryopathy and have been characterized in chemical-induced and genetic models of diabetes and even in mild diabetic experimental models (95, 125–127). Impairment of the oxidative and nitrosative stress balance can dysregulate multiple signaling pathways and cause massive cell damage, apoptotic events, and defective embryonic development (52, 128–132). Indeed, apoptosis is increased in embryos and their yolk sacs in streptozotocin-induced diabetic rats and mice (54, 133–135).

It is interesting to state the relevance of the genetic background in determining the malformation rate. The streptozotocin-induced diabetic rats of the U substrain, derived

from Sprague-Dawley rats, and the Cohen diabetes-sensitive rat substrain, derived from Wistar rats, have increased risks for congenital malformations (109, 136). As stated by comparing different genetic backgrounds in these chemical-induced and genetic diabetic experimental models, the capacity to deal with oxidative stress is an important feature in determining the degree of induction of congenital malformations, and changes in catalase and superoxide dismutase expression are clearly relevant in this context (127, 137).

Folic acid has antioxidant properties, and its deficiency is involved in the induction of congenital malformations in the general population and also in streptozotocin-induced diabetic rats and mice (138–140).

Congenital anomalies in maternal diabetes can also be the result of an impaired expression of the genes that control essential developmental processes. In particular, a decreased expression of the transcription factor Pax-3 has been clearly involved in the induction of neural tube and cardiac defects in streptozotocin-induced diabetic mice (52, 141, 142). In addition, recent works have identified an altered expression of several other neural tube and cardiac defect-related genes in embryos from streptozotocin-induced diabetic mice (53, 142). Moreover, microarray analysis in embryos from streptozotocin-induced diabetic mice has shown that hundreds of genes exhibit changes in their expression levels in whole embryos (143) and in the developing neural tube (144), thus suggesting that much experimental research is still needed to fully understand the etiology of congenital malformations in maternal diabetes.

For future studies in the postimplantation embryo, both chemical-induced and type 1 and type 2 genetic models of diabetes can be recommended. Addressing the induction of congenital malformations may require the use of severe diabetic animals (glycemia higher than 250 mg/dl) or the use of rodent strains prone to malformations to allow sufficient malformed embryos for the analysis. Besides, because the malformation rate in women is clearly correlated with the increased glucose concentrations, but responses to insulin are very variable in patients, addressing congenital malformations induced in experimental models in diabetes and pregnancy that present variable responses to insulin would be valuable.

VI. Animal Models to Study the Placenta in Maternal Diabetes

A. Overview

Despite the existence of several developmental and morphological differences in the placenta from rodents and women, there are many similarities in the alterations induced by maternal diabetes in the placenta from diabetic patients and diabetic experimental models (51, 125, 145–148).

B. The placenta

Placentomegalia is observed in various mild and severe chemical-induced diabetic experimental models and in some genetic models of diabetes such as the BB rat (149–153). Structural, functional, and developmental abnormalities are found in the placenta of streptozotocin-induced diabetic rodents (152, 154–156). Moreover, array studies have shown an aberrant gene expression pattern in placentas from streptozotocin-induced diabetic mice (157).

Increased amounts of lipids, glycogen, and DNA characterize the placentas from streptozotocin-induced diabetic rodents (150, 158). Glucose transfer through the placenta increases linearly with the maternal glucose in streptozotocin-induced diabetic rats (159). The placentas from these animals, as well as from NOD mice, also have alterations in glucose transfer, transporters, and metabolism (159–162).

On the other hand, lipid transfer is also enhanced in streptozotocin-induced diabetic rats. Several impairments, including increased maternal lipid concentrations, altered expression of lipid transporters, and impaired lipid metabolic pathways, contribute to the increased placental accumulation and transfer of lipids (163–166).

The nuclear peroxisome proliferator-activated receptors and their endogenous ligands, involved in both lipid metabolism and antiinflammatory processes, are also differently expressed in placentas from streptozotocin-induced diabetic rodents throughout gestation (151, 153, 167–169).

Aberrant concentrations of several prostaglandins that regulate the balance between proinflammatory/antiinflammatory pathways and between vascular relaxation/dilation are found in placentas from streptozotocin-induced diabetic rats and ewes (56, 151, 170). On the other hand, enhanced vascularization and increased angiogenic factors like vascular endothelial growth factor and placental growth factor are found in placentas from streptozotocin-induced diabetic rodents (153, 171, 172).

Oxidative and nitrosative stress in the placenta is enhanced even in mild chemical-induced diabetic models (170, 173). These alterations have been related to an overactivity of matrix metalloproteinases (168, 174, 175), proteases capable of degrading all components of the extracellular matrix. Indeed, several alterations in the components of the extracellular matrix have been found in streptozotocin- and alloxan-induced diabetic rodents (176, 177).

Altogether, the many changes present in both chemical-induced and genetic diabetic models (Table 2), which have

served to study and gain insights into the development of the many features common to the placentas from both human and rodent diabetic experimental models, suggest that the placenta is a compromised target that largely suffers the impact of maternal diabetes.

For future studies, the use of chemical-induced and genetic diabetes and pregnancy experimental models can be recommended because many alterations similar to those found in the human diabetic placenta have been observed in these experimental models.

VII. Animal Models to Study the Fetuses and the Intrauterine Programming of Diseases in Maternal Diabetes

A. Overview

Maternal diabetes-induced impairments in fetal and neonatal development have both short- and long-term adverse effects. Short-term outcomes are characterized by increased neonatal morbidity and mortality, in part due to the increased rate of congenital malformations, premature delivery, macrosomia, shoulder dystocia, growth retardation, fetal hypoxia, neonatal hypoglycemia, and respiratory distress syndrome (2, 5, 8, 98, 178). Long-term effects in the newborns are increased risks for development of overweight, obesity, impaired glucose tolerance, type 2 diabetes mellitus, metabolic syndrome, and minor neurological deficits (4, 10, 179, 180).

Many of these short- and long-term effects have been addressed in diabetes and pregnancy experimental models (Table 2). The results of these studies clearly show that the abnormal intrauterine environment causes many of these derangements during fetal development, and that the fetuses present several impairments in different experimental diabetic models, as described in *Sections VII. B, C, and D*. Thus, the experimental models in diabetes and pregnancy provide the possibility to study the fetus, to gain insights into the mechanisms of induction of fetal and neonatal impairments, and to test different approaches to prevent fetal alterations and their long-term effects.

When evaluating the fetus and the fetal outcome, the chemical-induced diabetic models are those that have been most used (Table 2). Besides, the use of inbred genetic models of diabetes to address the consequences of fetal exposure to maternal diabetes in the offspring should take into account that the genetic background that leads to the diabetic phenotype can be present or not depending on the breeding strategy. Therefore, breeding and embryo transfer strategies are very useful approaches to address the relevance of environmental *vs.* genetic factors in inducing diabetes in the offspring.

B. The fetus

Although the origin of congenital malformations has already been discussed because it occurs at earlier developmental stages, skeletal, facial, heart, and visceral malformations are clearly evident in the fetuses at late gestation in streptozotocin-induced diabetic rats (103, 181–183). Congenital malformations are also found in fetuses from genetic models of diabetes such as the GK and Cohen rats (184, 185).

Congenital heart malformations are mostly evident on d 17–18 of rat gestation because some of them are lethal and lead to fetal death and resorption at late gestation (186). Nonspecific immune stimulation with both interferon γ and Freund's complete adjuvant has been recently shown to reduce the heart malformation rate in streptozotocin-induced diabetic mice, although the mechanisms of these beneficial effects remain unclear (187).

Several diabetes and pregnancy experimental models have served to address the role of hyperglycemia in fetal development and outcome, and to state that besides hyperglycemia, the multifactorial metabolic derangement resulting from the impaired maternal insulin action seems to play an important role in these fetal disorders and in its consequences (115, 125, 164, 188–191). Indeed, diabetes induced by streptozotocin previous to or during rat gestations clearly impairs fetal lipid metabolism, alterations closely related to fetal impairments (163, 164, 167, 192, 193).

As a consequence of the maternal metabolic derangements, increased oxidative stress and impaired antioxidant enzymes have been found in fetuses and neonates from mild and severe streptozotocin-induced diabetic rodents and alloxan-induced diabetic experimental models (173, 194–197). Nitric oxide production is increased in the fetuses from mild diabetic rats induced by streptozotocin administration, an alteration related to the overexpression of matrix metalloproteinase-2 during fetal development (174, 175). In streptozotocin-induced diabetic rats, changes in uterine perfusion have been found related to changes in the expression of genes that regulate antioxidant defenses and angiogenesis and to the fetal outcome (198). Dietary supplementation with n-3 polyunsaturated fatty acids can suppress abnormal antioxidant status in macrosomic fetuses from streptozotocin-induced diabetic rats (199). Reduction of congenital malformations by folic acid and antioxidants has been reported in term fetuses from streptozotocin-induced mice and rats (103, 135, 140). Thus, several approaches are in progress aiming to prevent maternal diabetes-induced fetal defects.

C. Fetal organs

Alterations in the development of several fetal organs can be detected in most diabetes and pregnancy experi-

mental models (Table 2). These alterations, induced by the abnormal intrauterine environment, can be detected during the fetal stage and the neonatal period and can also be involved in the programming of diseases in the newborn's later life. Indeed, the disruption of multiple organ systems in ways that permanently impair their function and predispose the offspring to chronic diseases that emerge in later life has been considered the basis of intrauterine programming (200, 201).

Besides the already described induction of congenital heart defects, the impact of maternal diabetes-induced derangements during heart development can be detected in fetuses that do not present heart malformations (202). Maternal diabetes-induced increases in heart apoptosis have been related to malformations and heart lesions in fetuses from streptozotocin-induced diabetic rodents (203, 204) and have also been found in the offspring of diabetic animals rendered diabetic with streptozotocin during pregnancy (205). In Akita hypoinsulinemic mice, fetal myocardial hypertrophy and triglyceride accumulation do not occur, but reduction in the expression of several lipid metabolizing genes such as fatty acid transporter protein and fatty acid translocase is observed, suggesting that the changes that control fatty acid uptake prevent cardiac lipid overaccumulation (46).

Fetuses from streptozotocin-induced diabetic rats can present cardiac hypertrophy, which has been related to increases in atrial natriuretic peptide (206). The fetal myocardium in streptozotocin-induced diabetic rats shows a reduced expression of glucose transporter isoforms 1 and 4 (Glut 1 and Glut 4) changes probably related to compensatory effects to fetal hyperglycemia, which may be involved in the programming of insulin resistance (207). Indeed, in the offspring from streptozotocin-induced diabetic rats, the programming of insulin resistance is related to changes in translocation of Glut 4 in adipose and skeletal muscle and to alterations in the concentrations of neuropeptide Y in the hypothalamus (61, 208). Interestingly, in offspring from streptozotocin-induced diabetic rats, the availability of nutrients during weaning can induce gender-dependent changes in Glut 4 translocation and neuropeptide Y concentrations, as well as in the programming of obesity and glucose intolerance in the offspring's adult life (61). In addition, impaired cardiovascular function has been reported in 2-month-old offspring from streptozotocin-induced diabetic rats (209).

Regarding fetal kidney development, nephrogenesis has been found impaired in fetuses obtained from streptozotocin-induced diabetic rats (210). In streptozotocin-induced diabetic rodents in which fetal microsomia is observed, both a reduced kidney weight and a reduced nephron number are observed, alterations probably related to an increase in proinflammatory and apoptotic pathways and to alterations in the remodeling of the extracellular matrix during development (211, 212). Developmental changes in IGF-I expression during nephrogenesis and alterations in tubular reabsorption of calcium and magnesium in the neonates have also been reported in streptozotocin-induced diabetic rats (213, 214). In addition, streptozotocin-induced diabetes both before and during gestation leads to impaired vascular and renal function in the adult rat offspring, thus showing that hypertension and renal dysfunction can be determined *in utero* in chemical-induced diabetic models (215–217).

The lung is also a fetal organ affected by maternal diabetes, and reduced surfactant phospholipids, surfactant proteins, and the number of type II pneumocytes are found in term fetuses from streptozotocin-induced diabetic rats (218–221). Delayed lung maturation has been found both in fetuses from streptozotocin-induced diabetic rats and in offspring from diabetic db/+ mice (222, 223). Defects in fetal lung production of PGE_2 have been found in alloxan-induced diabetic rabbits, an alteration probably related to lung immaturity (20).

Liver lipid accumulation occurs in fetuses and neonates from streptozotocin-induced diabetic rats and in db/+ mice (27, 224). Alterations in arachidonic acid and docosahexaenoic acid ratio have been found in fetal and neonatal livers from streptozotocin-induced diabetic mothers either fed or not fed with high-fat diets (225). Supplementation with arachidonic acid during pregnancy and lactation ameliorates neurodevelopmental parameters in offspring from streptozotocin-induced diabetic rats (226). In rats rendered diabetic through alloxane administration at early gestation, the offspring's intestine is affected, showing decreased weight and length, elevated brush boarder enzymes, and increased absorption of glucose and glycine (227).

The fetal pancreas is extremely sensitive to maternal diabetes. Different changes in the fetal β-cell mass and function, related to the macrosomic and the microsomic phenotypes, can be observed in diabetes and pregnancy experimental models (10). In streptozotocin-induced diabetic rats, a positive correlation between maternal glycemia and fetal weight has been found in mild diabetic rats, whereas a negative correlation between these variables has been found in severe diabetic rats. These alterations are related to the impact of maternal diabetes on the fetal β-cell mass (228). Indeed, in mild hyperglycemic mothers, fetuses present islet hyperplasia and increased pancreatic and plasma insulin concentrations (229, 230). Possibly dependent on the rat substrain, the nutritional/metabolic factors, and the animal facility environment, which will lead to different rates of both β-cell death and

adaptive responses (12, 28, 29), the fetal pancreatic β-cell mass can be found either reduced or increased in streptozotocin-induced severely hyperglycemic rats. The reduced pancreatic β-cell mass leads to a reduced capacity of insulin secretion (231). On the other hand, the increased pancreatic β-cell mass leads to an increased insulin secretion, an alteration related to hyperplasia and degranulation of fetal pancreatic β-cells, which in turn leads to neonatal exhaustion of the insulin secretory capacity at term (232, 233). This alteration is restored in the neonatal period, but the β-cell mass is increased and insulin action is decreased in the adult state (233). Through this way, streptozotocin-induced females can transmit the glucose intolerant state to the next generation (234). Moreover, in the second generation, the offspring of both severe and mild hyperglycemic females develop gestational diabetes, and their offspring (third generation) also present the same disorders as the offspring of mildly hyperglycemic rats (232, 235, 236). These results show that streptozotocin-induced diabetic rodents can be useful animal models to analyze the involvement of the development of the fetal pancreatic β-cell mass in the induction of glucose intolerance and diabetes in the offspring's later life.

D. Fetal growth

Depending on the maternal metabolic and proinflammatory derangements, macrosomia can arise in fetuses from experimental diabetic models due to the excessive availability of nutrients and an increase in fetal insulin release, a phenotype related to the programming of glucose intolerance (27, 193).

Neonatal macrosomia and increased circulating lipids and liver triglycerides are found in the offspring from streptozotocin-induced diabetic rats (27, 237). Neonatal macrosomia and an aberrant lipid metabolism are also observed when streptozotocin is administered during gestation, alterations that have been found to be suppressed by the supplementation of n-3 polyunsaturated fatty acids (60, 238).

Dietary supplementation with safflower and olive oils, enriched in linoleic and oleic acids, respectively, and both capable of activating the ligand-activated peroxisome proliferator-activated receptors, is also able to prevent the aberrant lipid metabolism induced during fetal development in streptozotocin-induced mildly diabetic rats (239).

The degree of fetal damage and placental dysfunction and the availability and utilization of fetal substrates, among others, can lead to the induction of macrosomia or microsomia in some or all fetuses within a litter. Indeed, similar to that found in clinical studies, a U-shaped relationship between offspring weight and metabolic impairments is observed in streptozotocin-induced experimental models of diabetes (10, 240, 241). Nevertheless, further research is needed to fully understand the mechanisms that govern fetal overgrowth in maternal diabetes.

It is interesting to note that both macrosomia and microsomia are related to the induction of diabetes in the offspring's later life. Indeed, in diabetic pregnant animals in which the fetuses have normal weight, compensatory effects are usually functional enough, and thus, fewer alterations are induced. Because insulin is a hormone related to fetal growth, both macrosomia and microsomia are phenotypes that reflect the abnormal concentrations of insulin and other fetal growth factors in the fetus. Indeed, together with insulin, other growth-related factors such as fetal leptin and IGFs can be found reduced, enhanced, or unchanged in diabetes and pregnancy experimental models (21, 242–244).

In fetuses from streptozotocin-induced diabetic mice, impaired methylation and expression of the imprinted genes H19 and IGF-II have been found related to the microsomic phenotype (245). Levels of angiogenic factors like vascular endothelial growth factor-A and placental growth factor-2 are reduced in fetuses smaller in weight from streptozotocin-induced diabetic rats (171).

In macrosomic offspring from streptozotocin-induced diabetic animals, fetal hyperinsulinism has been shown to be a critical feature involved in the *in utero* programming of obesity and glucose intolerance. Indeed, hyperinsulinemia and an increase in the insulin concentrations within the hypothalamus have been observed in the perinatal period in offspring from streptozotocin-induced diabetic rats (246). The hyperinsulinemia persists throughout life, leads to spontaneous gestational diabetes in the F1 females, and is nongenetically transmitted to the next generations (F2 and F3) (247, 248). Studies performed in offspring of diabetic mothers and in offspring treated with insulin administration in the hypothalamus have led to the conclusion that these alterations are the result of a neuroendocrine malprogramming, which contributes to the occurrence of hyperphagia, overweight, and hyperinsulinemia throughout life, which may be passed on to the succeeding generations (3).

On the other hand, the genetic models of diabetes can lead to fetal microsomia or macrosomia, phenotypes that also depend on the degree of damage to the fetal organs. In the BB type 1 diabetic rats, the fetuses are small and present skeleton malformations, large hearts, reduced pancreatic and plasma insulin content, and small kidneys and lungs, fetal alterations probably associated with the classical genetic heredity (149, 249). Induction of diabetes in the BB rat offspring has been shown to be delayed and reduced through the administration of diabetes-promoting food antigens and immune modulators administered in the neonatal period (250).

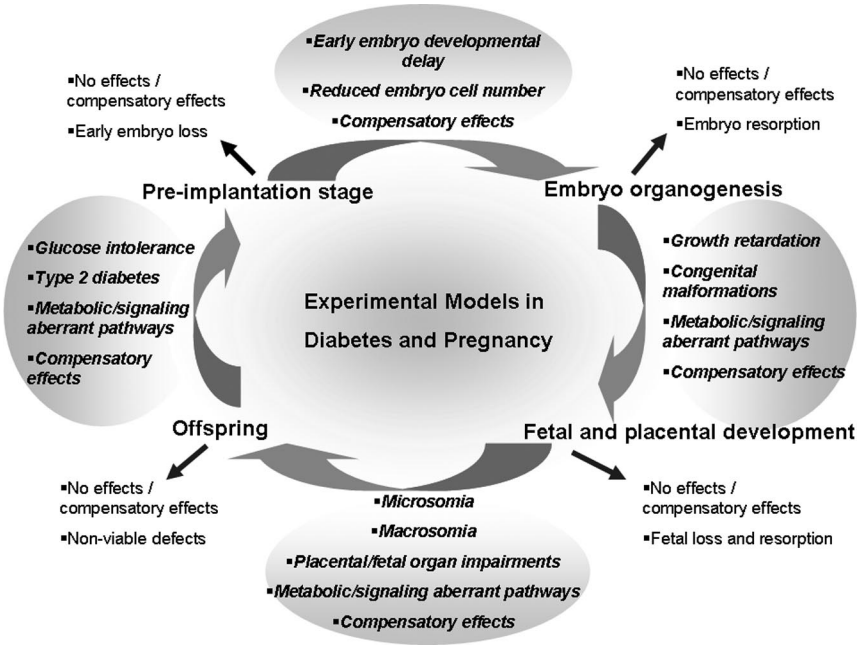

FIG. 1. Schematic representation of possible outcomes in experimental models of diabetes and pregnancy. The diagram indicates the different kinds and degrees of damage that can be induced during embryo and fetoplacental development and in the offspring of diabetic animals, which will vary according to the degree of maternal metabolic impairment and to several genetic and environmental factors. The *gray arrows* indicate the effects that can have consequences at later developmental stages and in the next generation, and the *black arrows* indicate the effects that do not lead to later developmental defects.

Macrosomia develops in the offspring of heterozygous CSTBLKS/J-Lepr (db/+) mice, and studies indicate that both genetic transmission and the abnormal environment are involved in the programming of aberrant adipose tissue development (42, 251).

In NOD mice, it has been found that glycosylated hemoglobin levels lower than 2.5% are not related to neonatal weight, those ranging from 2.6 to 4% are positively correlated with fetal growth, and those higher that 4% were negatively related to fetal growth (252). In NOD mice mated between 26 and 52 wk of age, the macrosomic offspring present organomegaly, elevated pancreatic insulin content, and smaller litter size (38). Indeed, NOD mice show several impairments in the pancreas in the neonatal period (253). Also, the glucose metabolic enzyme hexokinase is overexpressed in the fetal brain from NOD mice (254).

Both the administration of insulin in the neonatal period and the administration of diets, either reduced in proteins or in energy or enriched in zinc, during gestation have been shown to suppress the induction of diabetes in NOD mice (255–258). Taurine supplementation throughout pregnancy and weaning prevents pancreatic insulitis and delays the onset of diabetes in NOD mice (259). Moreover, the elimination of maternally transmitted autoantibodies by the use of B cell-deficient NOD mothers and by transferring NOD embryos to nonautoimmune strains protects the susceptible offspring from the induction of diabetes, thus suggesting a maternal effect rather than effects of the genetic background (37).

In the GK rat, the neonatal β-cell mass is severely reduced, an alteration that is due to a reduction in cell proliferation, defective IGF signaling pathways, and an increase in apoptosis in the fetal pancreas (111, 260). Nutrient restriction during the last week of gestation in GK rats improves pancreatic IGF-II and increases β-cell mass in their fetuses (261). Other studies performed in this genetic model of type 2 diabetes have shown that diabetes during pregnancy predisposes offspring to develop obesity and abnormal glucose tolerance later in life, at least in part independently of classic genetic transmission (44). In addition, offspring developed from Wistar rat 1-d embryos transferred to GK mothers show increased risks of hyperglycemia at adult ages, highlighting the intrauterine transmission of diabetes in this diabetic experimental model (45).

In the Cohen diabetic rat, fetal growth restriction is evident at term, and impaired oxidative stress is observed in different fetal organs (262). In the Sand rat fed with a high-energy diet, which is another type 2 diabetic model, maternal diabetes leads to low-weight offspring with impaired neurodevelopmental parameters that become overweight and diabetic in the third and fourth weeks of life (110).

Although all these data indicate that intrauterine exposure in diabetes and pregnancy experimental models is associated with the programming of glucose intolerance and type 2 diabetes, further research on this subject and on the molecular mechanisms responsible for these alterations would be valuable.

For future research, both chemical-induced and genetic experimental models in diabetes and pregnancy can be useful to address both the mechanisms of induction of fetal anomalies and the possible long-term effects of these alterations. Importantly, it should be noted that the alterations detected at the fetal stage may have been induced at the earliest stages of development, an issue that deserves further study.

Finally, it should be stated that beyond the scope of this review, but thoroughly reviewed elsewhere, there is vast information regarding the nutritional aspects that lead to the programming of type 2 diabetes, obesity, and cardio-

vascular disease in the offspring of nondiabetic pregnant animals (190, 263–265).

VIII. Concluding Remarks

Several important aspects of human diabetic pregnancies such as the increases in the rates of early embryo loss, spontaneous abortions, malformations, fetoplacental impairments and offspring's diseases in later life can be studied using the appropriate animal models. The scheme in Fig. 1 illustrates the broad putative uses of experimental models of diabetes and pregnancy, indicating that there are multiple experimental possibilities to approach the evaluation of the numerous possible phenotypes that comprise the human development in maternal diabetes.

In maternal diabetes, both the maternal environment and the genetic background are important in the complex and multifactorial processes that induce damage to the embryo, the placenta, the fetus, and the offspring. Thus, the use of experimental models of diabetes is crucial in the determination of these damaging pathways. Although there is no doubt that several diabetic models present similar patterns of the most characteristic features of human diabetic pregnancies, the mechanisms involved in these alterations and those mechanisms developed to prevent these anomalies require corroboration because the same mechanisms do not always explain the diabetic phenotype in diabetic patients and animals. However, there are obvious limitations in the study of diabetic pregnant women together with an important need of new strategies to improve and help in the difficult issue of managing these patients to prevent developmental impairments. Therefore, animal studies are critical for understanding the pathophysiology of diabetes-induced defects throughout pregnancy, and the use of experimental models of diabetes is justified and highly encouraged as a first stage for the evaluation of possible approaches to prevent diabetes-induced developmental defects.

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References

1. **Eriksson UJ** 2009 Congenital anomalies in diabetic pregnancy. Semin Fetal Neonatal Med 14:85–93
2. **Melamed N, Hod M** 2009 Perinatal mortality in pregestational diabetes. Int J Gynaecol Obstet 104(Suppl 1):S20–S24
3. **Plagemann A, Harder T, Dudenhausen JW** 2008 The diabetic pregnancy, macrosomia, and perinatal nutritional programming. Nestle Nutr Workshop Ser Pediatr Program 61:91–102
4. **Simeoni U, Barker DJ** 2009 Offspring of diabetic pregnancy: long-term outcomes. Semin Fetal Neonatal Med 14: 119–124
5. **Michael Weindling A** 2009 Offspring of diabetic pregnancy: short-term outcomes. Semin Fetal Neonatal Med 14: 111–118
6. **Balsells M, García-Patterson A, Gich I, Corcoy R** 2009 Maternal and fetal outcome in women with type 2 versus type 1 diabetes mellitus: a systematic review and meta-analysis. J Clin Endocrinol Metab 94:4284–4291
7. **Sheffield JS, Butler-Koster EL, Casey BM, McIntire DD, Leveno KJ** 2002 Maternal diabetes mellitus and infant malformations. Obstet Gynecol 100:925–930
8. **Reece EA, Leguizamón G, Wiznitzer A** 2009 Gestational diabetes: the need for a common ground. Lancet 373: 1789–1797
9. **Rees DA, Alcolado JC** 2005 Animal models of diabetes mellitus. Diabet Med 22:359–370
10. **Aerts L, Van Assche FA** 2003 Intra-uterine transmission of disease. Placenta 24:905–911
11. **Shafrir E** 1992 Animal models of non-insulin-dependent diabetes. Diabetes Metab Rev 8:179–208
12. **Kargar C, Ktorza A** 2008 Anatomical versus functional β-cell mass in experimental diabetes. Diabetes Obes Metab 10(Suppl 4):43–53
13. **Bliss M** 1993 The history of insulin. Diabetes Care 16(Suppl 3):4–7
14. **Foglia VG, Cattáneo de Peralta R, Ibarra R, Rivera Cortés L** 1967 [Fetal and placental characteristics of pregnancy in pancreatectomized rats]. Rev Soc Argent Biol 43:187–198
15. **Jawerbaum A, Catafau JR, Gonzales ET, Rodriguez RR, Gelpi E, Gomez G, Gimeno AL, Gimeno MA** 1993 Eicosanoid production by uterine strips and by embryos obtained from diabetic pregnant rats. Prostaglandins 45: 487–495
16. **Foglia VG** 1970 Fetuses and newborns of 95 per cent pancreatectomized female rats. Adv Metab Disord 1(Suppl 1):221–227
17. **Junod A, Lambert AE, Stauffacher W, Renold AE** 1969 Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J Clin Invest 48:2129–2139
18. **Lenzen S, Panten U** 1988 Alloxan: history and mechanism of action. Diabetologia 31:337–342
19. **Junod A, Lambert AE, Orci L, Pictet R, Gonet AE, Renold AE** 1967 Studies of the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med 126:201–205
20. **Tsai MY, Schallinger LE, Josephson MW, Brown DM** 1982 Disturbance of pulmonary prostaglandin metabolism in fetuses of alloxan-diabetic rabbits. Biochim Biophys Acta 712:395–399
21. **Ramsay TG, Wolverton CK, Steele NC** 1994 Alteration in

IGF-I mRNA content of fetal swine tissues in response to maternal diabetes. Am J Physiol 267:R1391–R1396
22. **Dickinson JE, Meyer BA, Chmielowiec S, Palmer SM** 1991 Streptozocin-induced diabetes mellitus in the pregnant ewe. Am J Obstet Gynecol 165:1673–1677
23. **Portha B, Picon L, Rosselin G** 1979 Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. Diabetologia 17:371–377
24. **Müller A, Schott-Ohly P, Dohle C, Gleichmann H** 2002 Differential regulation of Th1-type and Th2-type cytokine profiles in pancreatic islets of C57BL/6 and BALB/c mice by multiple low doses of streptozotocin. Immunobiology 205:35–50
25. **Catalano PM, Kirwan JP, Haugel-de Mouzon S, King J** 2003 Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. J Nutr 133:1674S–1683S
26. **Buchanan TA** 2001 Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. J Clin Endocrinol Metab 86:989–993
27. **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 Diabesity Res 4:107–118
28. **Wilson GL, Patton NJ, McCord JM, Mullins DW, Mossman BT** 1984 Mechanisms of streptozotocin- and alloxan-induced damage in rat B cells. Diabetologia 27:587–591
29. **Cardinal JW, Allan DJ, Cameron DP** 1998 Differential metabolite accumulation may be the cause of strain differences in sensitivity to streptozotocin-induced β cell death in inbred mice. Endocrinology 139:2885–2891
30. **Lefebvre DE, Powell KL, Strom A, Scott FW** 2006 Dietary proteins as environmental modifiers of type 1 diabetes mellitus. Annu Rev Nutr 26:175–202
31. **Tesone M, Ladenheim RG, Oliveira-Filho RM, Chiauzzi VA, Foglia VG, Charreau EH** 1983 Ovarian dysfunction in streptozotocin-induced diabetic rats. Proc Soc Exp Biol Med 174:123–130
32. **Kim NN, Stankovic M, Cushman TT, Goldstein I, Munarriz R, Traish AM** 2006 Streptozotocin-induced diabetes in the rat is associated with changes in vaginal hemodynamics, morphology and biochemical markers. BMC Physiol 6:4
33. **Kolb H** 1987 Mouse models of insulin dependent diabetes: low-dose streptozocin-induced diabetes and nonobese diabetic (NOD) mice. Diabetes Metab Rev 3:751–778
34. **González E, Roselló-Catafau J, Jawerbaum A, Vela J, Sinner D, Pustovrh C, White V, Xaus C, Peralta C, Gimeno MA** 2001 Involvement of inducible isoforms of COX and NOS in streptozotocin-pancreatic damage in the rat: interactions between nitridergic and prostanoid pathway. Prostaglandins Leukot Essent Fatty Acids 64:311–316
35. **Kay TW, Thomas HE, Harrison LC, Allison J** 2000 The β cell in autoimmune diabetes: many mechanisms and pathways of loss. Trends Endocrinol Metab 11:11–15
36. **Hashimoto L, Habita C, Beressi JP, Delepine M, Besse C, Cambon-Thomsen A, Deschamps I, Rotter JI, Djoulah S, James MR, et al** 1994 Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371:161–164
37. **Greeley SA, Katsumata M, Yu L, Eisenbarth GS, Moore DJ, Goodarzi H, Barker CF, Naji A, Noorchashm H** 2002 Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. Nat Med 8:399–402
38. **Formby B, Schmid-Formby F, Jovanovic L, Peterson CM** 1987 The offspring of the female diabetic "nonobese diabetic" (NOD) mouse are large for gestational age and have elevated pancreatic insulin content: a new animal model of human diabetic pregnancy. Proc Soc Exp Biol Med 184: 291–294
39. **Nakhooda AF, Like AA, Chappel CI, Murray FT, Marliss EB** 1977 The spontaneously diabetic Wistar rat. Metabolic and morphologic studies. Diabetes 26:100–112
40. **Yoshioka M, Kayo T, Ikeda T, Koizumi A** 1997 A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes 46:887–894
41. **Ktorza A, Bernard C, Parent V, Penicaud L, Froguel P, Lathrop M, Gauguier D** 1997 Are animal models of diabetes relevant to the study of the genetics of non-insulin-dependent diabetes in humans? Diabetes Metab 23(Suppl 2):38–46
42. **Lambin S, van Bree R, Caluwaerts S, Vercruysse L, Vergote I, Verhaeghe J** 2007 Adipose tissue in offspring of Lepr(db/+) mice: early-life environment vs. genotype. Am J Physiol Endocrinol Metab 292:E262–E271
43. **Goto Y, Kakizaki M, Masaki N** 1976 Production of spontaneous diabetic rats by repetition of selective breeding. Tohoku J Exp Med 119:85–90
44. **Gauguier D, Nelson I, Bernard C, Parent V, Marsac C, Cohen D, Froguel P** 1994 Higher maternal than paternal inheritance of diabetes in GK rats. Diabetes 43:220–224
45. **Gill-Randall R, Adams D, Ollerton RL, Lewis M, Alcolado JC** 2004 Type 2 diabetes mellitus—genes or intrauterine environment? An embryo transfer paradigm in rats. Diabetologia 47:1354–1359
46. **Lindegaard ML, Nielsen LB** 2008 Maternal diabetes causes coordinated down-regulation of genes involved with lipid metabolism in the murine fetal heart. Metabolism 57:766–773
47. **Farnaud S** 2009 The evolution of the Three Rs. Altern Lab Anim 37:249–254
48. **Sieher FE, Traystman RJ** 1993 Ethical issues involved in the development of animal models for type I diabetes. ILAR J 35:1
49. **Johnson PD, Besselsen DG** 2002 Practical aspects of experimental design in animal research. ILAR J 43:202–206
50. **Festing MF** 2006 Design and statistical methods in studies using animal models of development. ILAR J 47:5–14
51. **Georgiades P, Ferguson-Smith AC, Burton GJ** 2002 Comparative developmental anatomy of the murine and human definitive placentae. Placenta 23:3–19
52. **Morgan SC, Relaix F, Sandell LL, Loeken MR** 2008 Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. Birth Defects Res A Clin Mol Teratol 82:453–463
53. **Sato N, Sugimura Y, Hayashi Y, Murase T, Kanou Y, Kikkawa F, Murata Y** 2008 Identification of genes differentially expressed in mouse fetuses from streptozotocin-induced diabetic pregnancy by cDNA subtraction. Endocr J 55:317–323
54. **Yang P, Zhao Z, Reece EA** 2008 Activation of oxidative stress

signaling that is implicated in apoptosis with a mouse model of diabetic embryopathy. Am J Obstet Gynecol 198:130.e1–e7
55. **Srinivasan K, Ramarao P** 2007 Animal models in type 2 diabetes research: an overview. Indian J Med Res 125: 451–472
56. **Dickinson JE, Meyer BA, Brath PC, Chmielowiec S, Walsh SW, Parisi VM, Palmer SM** 1990 Placental thromboxane and prostacyclin production in an ovine diabetic model. Am J Obstet Gynecol 163:1831–1835
57. **Kemnitz JW, Eisele SG, Lindsay KA, Engle MJ, Perelman RH, Farrell PM** 1984 Changes in food intake during menstrual cycles and pregnancy of normal and diabetic rhesus monkeys. Diabetologia 26:60–64
58. **Ezekwe MO, Ezekwe EI, Sen DK, Ogolla F** 1984 Effects of maternal streptozotocin-diabetes on fetal growth, energy reserves and body composition of newborn pigs. J Anim Sci 59:974–980
59. **Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P** 2005 Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 52:313–320
60. **Soulimane-Mokhtari NA, Guermouche B, Yessoufou A, Saker M, Moutairou K, Hichami A, Merzouk H, Khan NA** 2005 Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. Clin Sci (Lond) 109:287–295
61. **Thamotharan M, McKnight RA, Thamotharan S, Kao DJ, Devaskar SU** 2003 Aberrant insulin-induced GLUT4 translocation predicts glucose intolerance in the offspring of a diabetic mother. Am J Physiol Endocrinol Metab 284: E901–E914
62. **Reynolds WA, Chez RA, Bhuyan BK, Neil GL** 1974 Placental transfer of streptozotocin in the rhesus monkey. Diabetes 23:777–782
63. **Schein PS, Loftus S** 1968 Streptozotocin: depression of mouse liver pyridine nucleotides. Cancer Res 28:1501–1506
64. **Karnik SK, Chen H, McLean GW, Heit JJ, Gu X, Zhang AY, Fontaine M, Yen MH, Kim SK** 2007 Menin controls growth of pancreatic β-cells in pregnant mice and promotes gestational diabetes mellitus. Science 318:806–809
65. **Heilig CW, Saunders T, Brosius 3rd FC, Moley K, Heilig K, Baggs R, Guo L, Conner D** 2003 Glucose transporter-1-deficient mice exhibit impaired development and deformities that are similar to diabetic embryopathy. Proc Natl Acad Sci USA 100:15613–15618
66. **Hagay ZJ, Weiss Y, Zusman I, Peled-Kamar M, Reece EA, Eriksson UJ, Groner Y** 1995 Prevention of diabetes-associated embryopathy by overexpression of the free radical scavenger copper zinc superoxide dismutase in transgenic mouse embryos. Am J Obstet Gynecol 173:1036–1041
67. **Pavlinkova G, Salbaum JM, Kappen C** 2008 Wnt signaling in caudal dysgenesis and diabetic embryopathy. Birth Defects Res A Clin Mol Teratol 82:710–719
68. **Nandi A, Wang X, Accili D, Wolgemuth DJ** 2010 The effect of insulin signaling on female reproductive function independent of adiposity and hyperglycemia. Endocrinology 151:1863–1871
69. **Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A, Tilghman SM** 1995 Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature 375:34–39
70. **Petry CJ, Evans ML, Wingate DL, Ong KK, Reik W, Constância M, Dunger DB** 2010 Raised late pregnancy glucose concentrations in mice carrying pups with targeted disruption of H19δ13. Diabetes 59:282–286
71. **Jungheim ES, Moley KH** 2008 The impact of type 1 and type 2 diabetes mellitus on the oocyte and the preimplantation embryo. Semin Reprod Med 26:186–195
72. **Powers RW, Chambers C, Larsen WJ** 1996 Diabetes-mediated decreases in ovarian superoxide dismutase activity are related to blood-follicle barrier and ovulation defects. Endocrinology 137:3101–3110
73. **Jawerbaum A, Gonzalez E** 2005 The role of alterations in arachidonic acid metabolism and nitric oxide homeostasis in rat models of diabetes during early pregnancy. Curr Pharm Des 11:1327–1342
74. **Codner E** 2008 Estrogen and type 1 diabetes mellitus. Pediatr Endocrinol Rev 6:228–234
75. **Aubert ML, Pierroz DD, Gruaz NM, d'Allèves V, Vuagnat BA, Pralong FP, Blum WF, Sizonenko PC** 1998 Metabolic control of sexual function and growth: role of neuropeptide Y and leptin. Mol Cell Endocrinol 140:107–113
76. **Vercheval M, De Hertogh R, Pampfer S, Vanderheyden I, Michiels B, De Bernardi P, De Meyer R** 1990 Experimental diabetes impairs rat embryo development during the preimplantation period. Diabetologia 33:187–191
77. **Novaro V, Jawerbaum A, Faletti A, Gimeno MA, González ET** 1998 Uterine nitric oxide and prostaglandin E during embryonic implantation in non-insulin-dependent diabetic rats. Reprod Fertil Dev 10:217–223
78. **Burke SD, Dong H, Hazan AD, Croy BA** 2007 Aberrant endometrial features of pregnancy in diabetic NOD mice. Diabetes 56:2919–2926
79. **Lea RG, McCracken JE, McIntyre SS, Smith W, Baird JD** 1996 Disturbed development of the preimplantation embryo in the insulin-dependent diabetic BB/E rat. Diabetes 45:1463–1470
80. **Chang AS, Dale AN, Moley KH** 2005 Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. Endocrinology 146: 2445–2453
81. **Beebe LF, Kaye PL** 1990 Preimplantation development in the streptozotocin-induced diabetic mouse. Reprod Fertil Dev 2:407–412
82. **Wang Q, Moley KH** 2010 Maternal diabetes and oocyte quality. Mitochondrion DOI: 10.1016/j.mito.2010.03.002
83. **Diamond MP, Moley KH, Pellicer A, Vaughn WK, DeCherney AH** 1989 Effects of streptozotocin- and alloxan-induced diabetes mellitus on mouse follicular and early embryo development. J Reprod Fertil 86:1–10
84. **Moley KH, Vaughn WK, DeCherney AH, Diamond MP** 1991 Effect of diabetes mellitus on mouse pre-implantation embryo development. J Reprod Fertil 93:325–332
85. **Beebe LF, Kaye PL** 1991 Maternal diabetes and retarded preimplantation development of mice. Diabetes 40:457–461
86. **Moley KH, Chi MM, Mueckler MM** 1998 Maternal hyperglycemia alters glucose transport and utilization in mouse preimplantation embryos. Am J Physiol 275: E38–E47
87. **Doblado M, Moley KH** 2007 Glucose metabolism in pregnancy and embryogenesis. Curr Opin Endocrinol Diabetes Obes 14:488–493
88. **Wyman A, Pinto AB, Sheridan R, Moley KH** 2008 One-cell

zygote transfer from diabetic to nondiabetic mouse results in congenital malformations and growth retardation in offspring. Endocrinology 149:466–469
89. **Dong H, Burke SD, Croy BA** 2008 Vascular addressins in the uterus and pancreas of type 1 diabetic mice in early pregnancy. Placenta 29:201–209
90. **Wang W, Lin Y, Zeng S, Li DJ** 2009 Improvement of fertility with adoptive CD25+ natural killer cell transfer in subfertile non-obese diabetic mice. Reprod Biomed Online 18:95–103
91. **Pampfer S, Vanderheyden I, De Hertogh R** 1997 Increased synthesis of tumor necrosis factor-α in uterine explants from pregnant diabetic rats and in primary cultures of uterine cells in high glucose. Diabetes 46:1214–1224
92. **Pustovrh C, Jawerbaum A, Sinner D, White V, Capobianco E, González E** 2002 Metalloproteinase 2 activity and modulation in uterus from neonatal streptozotocin-induced diabetic rats during embryo implantation. Reprod Fertil Dev 14:479–485
93. **Pampfer S** 2000 Apoptosis in rodent peri-implantation embryos: differential susceptibility of inner cell mass and trophectoderm cell lineages–a review. Placenta 21 Suppl A:S3–S10
94. **Moley KH** 2001 Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women. Trends Endocrinol Metab 12:78–82
95. **Eriksson UJ, Cederberg J, Wentzel P** 2003 Congenital malformations in offspring of diabetic mothers—animal and human studies. Rev Endocr Metab Disord 4:79–93
96. **Jensen DM, Korsholm L, Ovesen P, Beck-Nielsen H, Moelsted-Pedersen L, Westergaard JG, Moeller M, Damm P** 2009 Peri-conceptional A1C and risk of serious adverse pregnancy outcome in 933 women with type 1 diabetes. Diabetes Care 32:1046–1048
97. **Evers IM, de Valk HW, Visser GH** 2004 Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in The Netherlands. BMJ 328:915
98. **Langer O, Conway DL** 2000 Level of glycemia and perinatal outcome in pregestational diabetes. J Matern Fetal Med 9:35–41
99. **García-Patterson A, Corcoy R, Rigla M, Caballero A, Adelantado JM, Altirriba O, de Leiva A** 1997 Does preconceptional counselling in diabetic women influence perinatal outcome? Ann Ist Super Sanita 33:333–336
100. **Dheen ST, Tay SS, Boran J, Ting LW, Kumar SD, Fu J, Ling EA** 2009 Recent studies on neural tube defects in embryos of diabetic pregnancy: an overview. Curr Med Chem 16: 2345–2354
101. **Martínez-Frías ML** 1994 Epidemiological analysis of outcomes of pregnancy in diabetic mothers: identification of the most characteristic and most frequent congenital anomalies. Am J Med Genet 51:108–113
102. **Schaefer-Graf UM, Buchanan TA, Xiang A, Songster G, Montoro M, Kjos SL** 2000 Patterns of congenital anomalies and relationship to initial maternal fasting glucose levels in pregnancies complicated by type 2 and gestational diabetes. Am J Obstet Gynecol 182:313–320
103. **Simán CM, Gittenberger-De Groot AC, Wisse B, Eriksson UJ** 2000 Malformations in offspring of diabetic rats: morphometric analysis of neural crest-derived organs and effects of maternal vitamin E treatment. Teratology 61:355–367
104. **Alcolea MP, Lladó I, García-Palmer FJ, Gianotti M** 2007 Responses of mitochondrial biogenesis and function to maternal diabetes in rat embryo during the placentation period. Am J Physiol Endocrinol Metab 293:E636–E644
105. **Yang X, Borg LA, Eriksson UJ** 1995 Altered mitochondrial morphology of rat embryos in diabetic pregnancy. Anat Rec 241:255–267
106. **Otani H, Tanaka O, Tatewaki R, Naora H, Yoneyama T** 1991 Diabetic environment and genetic predisposition as causes of congenital malformations in NOD mouse embryos. Diabetes 40:1245–1250
107. **Tatewaki R, Hashimoto R, Tanigawa K, Furuse K, Tanaka O** 1995 Relationship between associations of NOR and chromosomal anomalies in the abnormal embryos of nonobese diabetic and STZ-diabetic mouse. Biol Neonate 67:132–139
108. **Lee AT, Reis D, Eriksson UJ** 1999 Hyperglycemia-induced embryonic dysmorphogenesis correlates with genomic DNA mutation frequency in vitro and in vivo. Diabetes 48:371–376
109. **Zangen SW, Yaffe P, Shechtman S, Zangen DH, Ornoy A** 2002 The role of reactive oxygen species in diabetes-induced anomalies in embryos of Cohen diabetic rats. Int J Exp Diabetes Res 3:247–255
110. **Patlas N, Avgil M, Ziv E, Ornoy A, Shafrir E** 2006 Pregnancy outcome in the *Psammomys obesus* gerbil on low- and high-energy diets. Biol Neonate 90:58–65
111. **Calderari S, Gangnerau MN, Thibault M, Meile MJ, Kassis N, Alvarez C, Portha B, Serradas P** 2007 Defective IGF2 and IGF1R protein production in embryonic pancreas precedes β cell mass anomaly in the Goto-Kakizaki rat model of type 2 diabetes. Diabetologia 50:1463–1471
112. **Wentzel P** 2009 Can we prevent diabetic birth defects with micronutrients? Diabetes Obes Metab 11:770–778
113. **Loeken MR** 2005 Current perspectives on the causes of neural tube defects resulting from diabetic pregnancy. Am J Med Genet C Semin Med Genet 135:77–87
114. **Eriksson UJ, Borg LA, Forsberg H, Styrud J** 1991 Diabetic embryopathy. Studies with animal and in vitro models. Diabetes 40(Suppl 2):94–98
115. **Styrud J, Thunberg L, Nybacka O, Eriksson UJ** 1995 Correlations between maternal metabolism and deranged development in the offspring of normal and diabetic rats. Pediatr Res 37:343–353
116. **Reece EA, Eriksson UJ** 1996 The pathogenesis of diabetes-associated congenital malformations. Obstet Gynecol Clin North Am 23:29–45
117. **Goldman AS, Baker L, Piddington R, Marx B, Herold R, Egler J** 1985 Hyperglycemia-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. Proc Natl Acad Sci USA 82:8227–8231
118. **Schoenfeld A, Erman A, Warchaizer S, Ovadia J, Bonner G, Hod M, Bonner J** 1995 Yolk sac concentration of prostaglandin E2 in diabetic pregnancy: further clues to the etiology of diabetic embryopathy. Prostaglandins 50:121–126
119. **Higa R, González E, Pustovrh MC, White V, Capobianco E, Martínez N, Jawerbaum A** 2007 PPARδ and its activator PGI2 are reduced in diabetic embryopathy: involvement of PPARδ activation in lipid metabolic and signalling

pathways in rat embryo early organogenesis. Mol Hum Reprod 13:103–110
120. **Jawerbaum A, Sinner D, White V, Pustovrh C, Capobianco E, Gonzalez E** 2002 Modulation of nitric oxide concentration and lipid metabolism by 15-deoxy delta12,14prostaglandin J2 in embryos from control and diabetic rats during early organogenesis. Reproduction 124:625–631
121. **Higa R, White V, Martínez N, Kurtz M, Capobianco E, Jawerbaum A** 2010 Safflower-oil and olive-oil dietary treatments rescues aberrant embryonic arachidonic acid and nitric oxide metabolism, and prevents diabetic embryopathy in rats. Mol Hum Reprod 16:286–295
122. **Weigensberg MJ, Garcia-Palmer FJ, Freinkel N** 1990 Uptake of myo-inositol by early-somite rat conceptus. Transport kinetics and effects of hyperglycemia. Diabetes 39: 575–582
123. **Hod M, Star S, Passonneau J, Unterman TG, Freinkel N** 1990 Glucose-induced dysmorphogenesis in the cultured rat conceptus: prevention by supplementation with myo-inositol. Isr J Med Sci 26:541–544
124. **Reece EA, Wu YK, Zhao Z, Dhanasekaran D** 2006 Dietary vitamin and lipid therapy rescues aberrant signaling and apoptosis and prevents hyperglycemia-induced diabetic embryopathy in rats. Am J Obstet Gynecol 194:580–585
125. **Jawerbaum A, González E** 2006 Diabetic pregnancies: the challenge of developing in a pro-inflammatory environment. Curr Med Chem 13:2127–2138
126. **King GL, Loeken MR** 2004 Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol 122:333–338
127. **Ornoy A** 2007 Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. Reprod Toxicol 24:31–41
128. **Jawerbaum A, Higa R, White V, Capobianco E, Pustovrh C, Sinner D, Martínez N, González E** 2005 Peroxynitrites and impaired modulation of nitric oxide concentrations in embryos from diabetic rats during early organogenesis. Reproduction 130:695–703
129. **Reece EA, Ma XD, Zhao Z, Wu YK, Dhanasekaran D** 2005 Aberrant patterns of cellular communication in diabetes-induced embryopathy in rats: II, apoptotic pathways. Am J Obstet Gynecol 192:967–972
130. **Sugimura Y, Murase T, Oyama K, Uchida A, Sato N, Hayasaka S, Kano Y, Takagishi Y, Hayashi Y, Oiso Y, Murata Y** 2009 Prevention of neural tube defects by loss of function of inducible nitric oxide synthase in fetuses of a mouse model of streptozotocin-induced diabetes. Diabetologia 52:962–971
131. **Viana M, Aruoma OI, Herrera E, Bonet B** 2000 Oxidative damage in pregnant diabetic rats and their embryos. Free Radic Biol Med 29:1115–1121
132. **Sivan E, Lee YC, Wu YK, Reece EA** 1997 Free radical scavenging enzymes in fetal dysmorphogenesis among offspring of diabetic rats. Teratology 56:343–349
133. **Gäreskog M, Cederberg J, Eriksson UJ, Wentzel P** 2007 Maternal diabetes in vivo and high glucose concentration in vitro increases apoptosis in rat embryos. Reprod Toxicol 23:63–74
134. **Sun F, Kawasaki E, Akazawa S, Hishikawa Y, Sugahara K, Kamihira S, Koji T, Eguchi K** 2005 Apoptosis and its pathway in early post-implantation embryos of diabetic rats. Diabetes Res Clin Pract 67:110–118
135. **Cederberg J, Eriksson UJ** 2005 Antioxidative treatment of pregnant diabetic rats diminishes embryonic dysmorphogenesis. Birth Defects Res A Clin Mol Teratol 73:498–505
136. **Eriksson UJ, Dahlström VE, Lithell HO** 1986 Diabetes in pregnancy: influence of genetic background and maternal diabetic state on the incidence of skeletal malformations in the fetal rat. Acta Endocrinol Suppl (Copenh) 277:66–73
137. **Cederberg J, Galli J, Luthman H, Eriksson UJ** 2000 Increased mRNA levels of Mn-SOD and catalase in embryos of diabetic rats from a malformation-resistant strain. Diabetes 49:101–107
138. **van der Put NM, van Straaten HW, Trijbels FJ, Blom HJ** 2001 Folate, homocysteine and neural tube defects: an overview. Exp Biol Med (Maywood) 226:243–270
139. **Wentzel P, Gäreskog M, Eriksson UJ** 2005 Folic acid supplementation diminishes diabetes- and glucose-induced dysmorphogenesis in rat embryos in vivo and in vitro. Diabetes 54:546–553
140. **Oyama K, Sugimura Y, Murase T, Uchida A, Hayasaka S, Oiso Y, Murata Y** 2009 Folic acid prevents congenital malformations in the offspring of diabetic mice. Endocr J 56:29–37
141. **Loeken MR** 2006 Advances in understanding the molecular causes of diabetes-induced birth defects. J Soc Gynecol Investig 13:2–10
142. **Kumar SD, Dheen ST, Tay SS** 2007 Maternal diabetes induces congenital heart defects in mice by altering the expression of genes involved in cardiovascular development. Cardiovasc Diabetol 6:34
143. **Pavlinkova G, Salbaum JM, Kappen C** 2009 Maternal diabetes alters transcriptional programs in the developing embryo. BMC Genomics 10:274
144. **Jiang B, Kumar SD, Loh WT, Manikandan J, Ling EA, Tay SS, Dheen ST** 2008 Global gene expression analysis of cranial neural tubes in embryos of diabetic mice. J Neurosci Res 86:3481–3493
145. **Radaelli T, Varastehpour A, Catalano P, Hauguel-de Mouzon S** 2003 Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. Diabetes 52:2951–2958
146. **Lappas M, Permezel M, Rice GE** 2004 Release of proinflammatory cytokines and 8-isoprostane from placenta, adipose tissue, and skeletal muscle from normal pregnant women and women with gestational diabetes mellitus. J Clin Endocrinol Metab 89:5627–5633
147. **Desoye G, Shafrir E** 1994 Placental metabolism and its regulation in health and diabetes. Mol Aspects Med 15: 505–682
148. **Kuhn DC, Crawford MA, Stuart MJ, Botti JJ, Demers LM** 1990 Alterations in transfer and lipid distribution of arachidonic acid in placentas of diabetic pregnancies. Diabetes 39:914–918
149. **Eriksson UJ, Bone AJ, Turnbull DM, Baird JD** 1989 Timed interruption of insulin therapy in diabetic BB/E rat pregnancy: effect on maternal metabolism and fetal outcome. Acta Endocrinol (Copenh) 120:800–810
150. **Diamant YZ, Metzger BE, Freinkel N, Shafrir E** 1982 Placental lipid and glycogen content in human and experimental diabetes mellitus. Am J Obstet Gynecol 144:5–11
151. **Capobianco E, Jawerbaum A, Romanini MC, White V, Pustovrh C, Higa R, Martinez N, Mugnaini MT, Soñez C, Gonzalez E** 2005 15-Deoxy-delta(12,14)-prostaglandin

J2 and peroxisome proliferator-activated receptor γ (PPARγ) levels in term placental tissues from control and diabetic rats: modulatory effects of a PPARγ agonist on nitridergic and lipid placental metabolism. Reprod Fertil Dev 17:423–433

152. **Acar N, Korgun ET, Cayli S, Sahin Z, Demir R, Ustunel I** 2008 Is there a relationship between PCNA expression and diabetic placental development during pregnancy? Acta Histochem 110:408–417
153. **Suwaki N, Masuyama H, Masumoto A, Takamoto N, Hiramatsu Y** 2007 Expression and potential role of peroxisome proliferator-activated receptor γ in the placenta of diabetic pregnancy. Placenta 28:315–323
154. **Padmanabhan R, al-Zuhair AG** 1990 Ultrastructural studies on the placentae of streptozotocin induced maternal diabetes in the rat. Z Mikrosk Anat Forsch 104:212–230
155. **Caluwaerts S, Pijnenborg R, Luyten C, Van Assche FA** 2000 Growth characteristics of diabetic rat ectoplacental cones in vivo and in vitro. Diabetologia 43:939–945
156. **Barash V, Gutman A, Shafrir E** 1985 Fetal diabetes in rats and its effect on placental glycogen. Diabetologia 28:244–249
157. **Yu Y, Singh U, Shi W, Konno T, Soares MJ, Geyer R, Fundele R** 2008 Influence of murine maternal diabetes on placental morphology, gene expression, and function. Arch Physiol Biochem 114:99–110
158. **Shafrir E, Barash V** 1987 Placental function in maternal-fetal fat transport in diabetes. Biol Neonate 51:102–112
159. **Herrera E, Palacin M, Martin A, Lasuncion MA** 1985 Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. Diabetes 34(Suppl 2):42–46
160. **Boileau P, Mrejen C, Girard J, Hauguel-de Mouzon S** 1995 Overexpression of GLUT3 placental glucose transporter in diabetic rats. J Clin Invest 96:309–317
161. **Jansson T, Cetin I, Powell TL, Desoye G, Radaelli T, Ericsson A, Sibley CP** 2006 Placental transport and metabolism in fetal overgrowth—a workshop report. Placenta 27(Suppl A):S109–S113
162. **Devaskar SU, Devaskar UP, Schroeder RE, deMello D, Fiedorek Jr FT, Mueckler M** 1994 Expression of genes involved in placental glucose uptake and transport in the nonobese diabetic mouse pregnancy. Am J Obstet Gynecol 171:1316–1323
163. **Shafrir E, Khassis S** 1982 Maternal-fetal fat transport versus new fat synthesis in the pregnant diabetic rat. Diabetologia 22:111–117
164. **Herrera E, Amusquivar E** 2000 Lipid metabolism in the fetus and the newborn. Diabetes Metab Res Rev 16: 202–210
165. **Herrera E, Amusquivar E, Lopez-Soldado I, Ortega H** 2006 Maternal lipid metabolism and placental lipid transfer. Horm Res 65(Suppl 3):59–64
166. **Capobianco E, White V, Higa R, Martínez N, Jawerbaum A** 2008 Effects of natural ligands of PPARγ on lipid metabolism in placental tissues from healthy and diabetic rats. Mol Hum Reprod 14:491–499
167. **Martínez N, Capobianco E, White V, Pustovrh MC, Higa R, Jawerbaum A** 2008 Peroxisome proliferator-activated receptor α activation regulates lipid metabolism in the feto-placental unit from diabetic rats. Reproduction 136:95–103
168. **Pustovrh MC, Capobianco E, Martínez N, Higa R, White V, Jawerbaum A** 2009 MMP/ TIMP balance is modulated in vitro by 15dPGJ(2) in fetuses and placentas from diabetic rats. Eur J Clin Invest 39:1082–1090
169. **Kurtz M, Capobianco E, Martínez N, Fernández J, Higa R, White V, Jawerbaum A** 2010 Carbaprostacyclin, a PPARδ agonist, ameliorates excess lipid accumulation in diabetic rat placentas. Life Sci 86:781–790
170. **White V, Jawerbaum A, Sinner D, Pustovrh C, Capobianco E, González E** 2002 Oxidative stress and altered prostanoid production in the placenta of streptozotocin-induced diabetic rats. Reprod Fertil Dev 14:117–123
171. **Salim MD, Al-Matubsi HY, El-Sharaky AS, Kamel MA, Oriquat GA, Helmy MH, El-Bassiouni EA** 2009 The levels of vascular endothelial growth factor-A and placental growth factor-2 in embryopathy associated with experimental diabetic gestation. Growth Factors 27:32–39
172. **Koh PO, Sung JH, Won CK, Cho JH, Moon JG, Park OS, Kim MO** 2007 Streptozotocin-induced diabetes decreases placenta growth factor (PlGF) levels in rat placenta. J Vet Med Sci 69:877–880
173. **Pustovrh MC, Jawerbaum A, Capobianco E, White V, Martínez N, López-Costa JJ, González E** 2005 Oxidative stress promotes the increase of matrix metalloproteinases-2 and -9 activities in the feto-placental unit of diabetic rats. Free Radic Res 39:1285–1293
174. **Pustovrh MC, Jawerbaum A, Capobianco E, White V, López-Costa JJ, González E** 2005 Increased matrix metalloproteinases 2 and 9 in placenta of diabetic rats at midgestation. Placenta 26:339–348
175. **Pustovrh MC, Jawerbaum A, White V, Capobianco E, Higa R, Martínez N, López-Costa JJ, González E** 2007 The role of nitric oxide on matrix metalloproteinase 2 (MMP2) and MMP9 in placenta and fetus from diabetic rats. Reproduction 134:605–613
176. **Giachini FR, Carriel V, Capelo LP, Tostes RC, Carvalho MH, Fortes ZB, Zorn TM, San Martin S** 2008 Maternal diabetes affects specific extracellular matrix components during placentation. J Anat 212:31–41
177. **Forsberg H, Wentzel P, Eriksson UJ** 1998 Maternal diabetes alters extracellular matrix protein levels in rat placentas. Am J Obstet Gynecol 179:772–778
178. **Schwartz R, Teramo KA** 2000 Effects of diabetic pregnancy on the fetus and newborn. Semin Perinatol 24: 120–135
179. **Schaefer-Graf UM, Pawliczak J, Passow D, Hartmann R, Rossi R, Bührer C, Harder T, Plagemann A, Vetter K, Kordonouri O** 2005 Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. Diabetes Care 28:1745–1750
180. **Catalano PM, Kirwan JP** 2001 Maternal factors that determine neonatal size and body fat. Curr Diab Rep 1:71–77
181. **Eriksson RS, Thunberg L, Eriksson UJ** 1989 Effects of interrupted insulin treatment on fetal outcome of pregnant diabetic rats. Diabetes 38:764–772
182. **Damasceno DC, Volpato GT, de Mattos Paranhos Calderon I, Cunha Rudge MV** 2002 Oxidative stress and diabetes in pregnant rats. Anim Reprod Sci 72:235–244
183. **Roest PA, Molin DG, Schalkwijk CG, van Iperen L, Wentzel P, Eriksson UJ, Gittenberger-de Groot AC** 2009 Specific local cardiovascular changes of Nε-(carboxymethyl)l-ysine, VEGF, and Smad2 in the developing embryos coin-

cide with maternal diabetes induced congenital heart defects. Diabetes 58:1222–1228
184. **Malaisse-Lagae F, Vanhoutte C, Rypens F, Louryan S, Malaisse WJ** 1997 Anomalies of fetal development in GK rats. Acta Diabetol 34:55–60
185. **Zusman I, Ornoy A** 1986 The effects of maternal diabetes and high sucrose diets on the intrauterine development of rat fetuses. Diabetes Res 3:153–159
186. **Molin DG, Roest PA, Nordstrand H, Wisse LJ, Poelmann RE, Eriksson UJ, Gittenberger-De Groot AC** 2004 Disturbed morphogenesis of cardiac outflow tract and increased rate of aortic arch anomalies in the offspring of diabetic rats. Birth Defects Res A Clin Mol Teratol 70: 927–938
187. **Claudio Gutierrez J, Prater MR, Hrubec TC, Smith BJ, Freeman LE, Holladay SD** 2009 Heart changes in 17-day-old fetuses of diabetic ICR (Institute of Cancer Research) mothers: improvement with maternal immune stimulation. Congenit Anom (Kyoto) 49:1–7
188. **Metzger BE** 1991 Biphasic effects of maternal metabolism on fetal growth. Quintessential expression of fuel-mediated teratogenesis. Diabetes 40(Suppl 2):99–105
189. **Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF** 2006 Consequences of fetal exposure to maternal diabetes in offspring. J Clin Endocrinol Metab 91:3718–3724
190. **Reusens B, Ozanne SE, Remacle C** 2007 Fetal determinants of type 2 diabetes. Curr Drug Targets 8:935–941
191. **Shafrir E, Desoye G** 2008 Pregnancy in diabetic animals. In: Hod M, Jovanovic LG, Di Renzo GC, De Leiva A, Langer O, eds. Textbook in diabetes and pregnancy. 2nd ed. London: Informa HelathCare; 86–99
192. **Knopp RH, Warth MR, Charles D, Childs M, Li JR, Mabuchi H, Van Allen MI** 1986 Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes. Biol Neonate 50:297–317
193. **Khan NA** 2007 Role of lipids and fatty acids in macrosomic offspring of diabetic pregnancy. Cell Biochem Biophys 48: 79–88
194. **Raza H, John A** 2004 Glutathione metabolism and oxidative stress in neonatal rat tissues from streptozotocin-induced diabetic mothers. Diabetes Metab Res Rev 20: 72–78
195. **Kinalski M, Sledziewski A, Telejko B, Zarzycki W, Kinalska I** 2000 Lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes. Acta Diabetol 37:179–183
196. **Gerber RT, Holemans K, O'Brien-Coker I, Mallet AI, van Bree R, Van Assche FA, Poston L** 2000 Increase of the isoprostane 8-isoprostaglandin f2α in maternal and fetal blood of rats with streptozotocin-induced diabetes: evidence of lipid peroxidation. Am J Obstet Gynecol 183: 1035–1040
197. **Zabihi S, Wentzel P, Eriksson UJ** 2008 Maternal blood glucose levels determine the severity of diabetic embryopathy in mice with different expression of copper-zinc superoxide dismutase (CuZnSOD). Toxicol Sci 105: 166–172
198. **Zabihi S, Wentzel P, Eriksson UJ** 2008 Altered uterine perfusion is involved in fetal outcome of diabetic rats. Placenta 29:413–421
199. **Yessoufou A, Soulaimann N, Merzouk SA, Moutairou K, Ahissou H, Prost J, Simonin AM, Merzouk H, Hichami A, Khan NA** 2006 N-3 fatty acids modulate antioxidant status in diabetic rats and their macrosomic offspring. Int J Obes (Lond) 30:739–750
200. **Nathanielsz PW, Poston L, Taylor PD** 2007 In utero exposure to maternal obesity and diabetes: animal models that identify and characterize implications for future health. Clin Perinatol 34:515–526, v
201. **Barker DJ** 1997 Intra-uterine programming of the adult cardiovascular system. Curr Opin Nephrol Hypertens 6:106–110
202. **Corrigan N, Brazil DP, McAuliffe F** 2009 Fetal cardiac effects of maternal hyperglycemia during pregnancy. Birth Defects Res A Clin Mol Teratol 85:523–530
203. **Chappell Jr JH, Wang XD, Loeken MR** 2009 Diabetes and apoptosis: neural crest cells and neural tube. Apoptosis 14:1472–1483
204. **Gutierrez JC, Prater MR, Smith BJ, Freeman LE, Mallela MK, Holladay SD** 2009 Late-gestation ventricular myocardial reduction in fetuses of hyperglycemic CD1 mice is associated with increased apoptosis. Birth Defects Res B Dev Reprod Toxicol 86:409–415
205. **Reinking BE, Wedemeyer EW, Weiss RM, Segar JL, Scholz TD** 2009 Cardiomyopathy in offspring of diabetic rats is associated with activation of the MAPK and apoptotic pathways. Cardiovasc Diabetol 8:43
206. **Mulay S, Conliffe PR, Varma DR** 1995 Increased natriuretic peptides in fetal hearts of diabetic rats. J Endocrinol 146:255–259
207. **Schroeder RE, Doria-Medina CL, Das UG, Sivitz WI, Devaskar SU** 1997 Effect of maternal diabetes upon fetal rat myocardial and skeletal muscle glucose transporters. Pediatr Res 41:11–19
208. **Singh BS, Westfall TC, Devaskar SU** 1997 Maternal diabetes-induced hyperglycemia and acute intracerebral hyperinsulinism suppress fetal brain neuropeptide Y concentrations. Endocrinology 138:963–969
209. **Wichi RB, Souza SB, Casarini DE, Morris M, Barreto-Chaves ML, Irigoyen MC** 2005 Increased blood pressure in the offspring of diabetic mothers. Am J Physiol Regul Integr Comp Physiol 288:R1129–R1133
210. **Amri K, Freund N, Vilar J, Merlet-Bénichou C, Lelièvre-Pégorier M** 1999 Adverse effects of hyperglycemia on kidney development in rats: in vivo and in vitro studies. Diabetes 48:2240–2245
211. **Tran S, Chen YW, Chenier I, Chan JS, Quaggin S, Hébert MJ, Ingelfinger JR, Zhang SL** 2008 Maternal diabetes modulates renal morphogenesis in offspring. J Am Soc Nephrol 19:943–952
212. **Van Huyen JP, Viltard M, Nehiri T, Freund N, Bélair MF, Martinerie C, Lelongt B, Bruneval P, Lelièvre-Pégorier M** 2007 Expression of matrix metalloproteinases MMP-2 and MMP-9 is altered during nephrogenesis in fetuses from diabetic rats. Lab Invest 87:680–689
213. **Duong Van Huyen JP, Amri K, Bélair MF, Vilar J, Merlet-Bénichou C, Bruneval P, Lelièvre-Pégorier M** 2003 Spatiotemporal distribution of insulin-like growth factor receptors during nephrogenesis in fetuses from normal and diabetic rats. Cell Tissue Res 314:367–379
214. **Bond H, Sibley CP, Balment RJ, Ashton N** 2005 Increased renal tubular reabsorption of calcium and magnesium by the offspring of diabetic rat pregnancy. Pediatr Res 57: 890–895
215. **Nehiri T, Duong Van Huyen JP, Viltard M, Fassot C,**

Heudes D, Freund N, Deschênes G, Houillier P, Bruneval P, Lelièvre-Pégorier M 2008 Exposure to maternal diabetes induces salt-sensitive hypertension and impairs renal function in adult rat offspring. Diabetes 57:2167–2175

216. **Rocha SO, Gomes GN, Forti AL, do Carmo Pinho Franco M, Fortes ZB, de Fátima Cavanal M, Gil FZ** 2005 Long-term effects of maternal diabetes on vascular reactivity and renal function in rat male offspring. Pediatr Res 58:1274–1279
217. **Segar EM, Norris AW, Yao JR, Hu S, Koppenhafer SL, Roghair RD, Segar JL, Scholz TD** 2009 Programming of growth, insulin resistance and vascular dysfunction in offspring of late gestation diabetic rats. Clin Sci (Lond) 117:129–138
218. **Rieutort M, Farrell PM, Engle MJ, Pignol B, Bourbon JR** 1986 Changes in surfactant phospholipids in fetal rat lungs from normal and diabetic pregnancies. Pediatr Res 20:650–654
219. **Singh M, Feigelson M** 1983 Effects of maternal diabetes on the levels, synthetic rates and activities of synthetic enzymes of surface-active phospholipids in perinatal rat lung. Biochim Biophys Acta 753:53–59
220. **Moglia BB, Phelps DS** 1996 Changes in surfactant protein A mRNA levels in a rat model of insulin-treated diabetic pregnancy. Pediatr Res 39:241–247
221. **Treviño-Alanís M, Ventura-Juárez J, Hernández-Piñero J, Nevárez-Garza A, Quintanar-Stephano A, González-Piña A** 2009 Delayed lung maturation of foetus of diabetic mother rats develop with a diminish, but without changes in the proportion of type I and II pneumocytes, and decreased expression of protein D-associated surfactant factor. Anat Histol Embryol 38:169–176
222. **Lawrence S, Warshaw J, Nielsen HC** 1989 Delayed lung maturation in the macrosomic offspring of genetically determined diabetic (db/+) mice. Pediatr Res 25:173–179
223. **Gewolb IH, Rooney SA, Barrett C, Ingleson LD, Light D, Wilson CM, Walker Smith GJ, Gross I, Warshaw JB** 1985 Delayed pulmonary maturation in the fetus of the streptozotocin-diabetic rat. Exp Lung Res 8:141–151
224. **Yamashita H, Shao J, Qiao L, Pagliassotti M, Friedman JE** 2003 Effect of spontaneous gestational diabetes on fetal and postnatal hepatic insulin resistance in Lepr(db/+) mice. Pediatr Res 53:411–418
225. **Ghebremeskel K, Bitsanis D, Koukkou E, Lowy C, Poston L, Crawford MA** 1999 Maternal diet high in fat reduces docosahexaenoic acid in liver lipids of newborn and sucking rat pups. Br J Nutr 81:395–404
226. **Zhao J, Del Bigio MR, Weiler HA** 2009 Maternal arachidonic acid supplementation improves neurodevelopment of offspring from healthy and diabetic rats. Prostaglandins Leukot Essent Fatty Acids 81:349–356
227. **Sharma R, Kaur J, Mahmood A** 2009 Effect of maternal diabetes on postnatal development of brush border enzymes and transport functions in rat intestine. J Pediatr Gastroenterol Nutr 49:8–15
228. **Van Assche FA, Holemans K, Aerts L** 2001 Long-term consequences for offspring of diabetes during pregnancy. Br Med Bull 60:173–182
229. **Kervran A, Guillaume M, Jost A** 1978 The endocrine pancreas of the fetus from diabetic pregnant rat. Diabetologia 15:387–393
230. **Aerts L, Van Assche FA** 1979 Is gestational diabetes an acquired condition? J Dev Physiol 1:219–225
231. **Eriksson U, Andersson A, Efendiæ S, Elde R, Hellerström C** 1980 Diabetes in pregnancy: effects on the foetal and newborn rat with particular regard to body weight, serum insulin concentration and pancreatic contents of insulin, glucagon and somatostatin. Acta Endocrinol (Copenh) 94:354–364
232. **Aerts L, van Assche FA** 1977 Rat foetal endocrine pancreas in experimental diabetes. J Endocrinol 73:339–346
233. **Aerts L, Sodoyez-Goffaux F, Sodoyez JC, Malaisse WJ, Van Assche FA** 1988 The diabetic intrauterine milieu has a long-lasting effect on insulin secretion by B cells and on insulin uptake by target tissues. Am J Obstet Gynecol 159:1287–1292
234. **Holemans K, Aerts L, Van Assche FA** 1991 Evidence for an insulin resistance in the adult offspring of pregnant streptozotocin-diabetic rats. Diabetologia 34:81–85
235. **Aerts L, Holemans K, Van Assche FA** 1990 Maternal diabetes during pregnancy: consequences for the offspring. Diabetes Metab Rev 6:147–167
236. **Oh W, Gelardi NL, Cha CJ** 1991 The cross-generation effect of neonatal macrosomia in rat pups of streptozotocin-induced diabetes. Pediatr Res 29:606–610
237. **Goldstein R, Levy E, Shafrir E** 1985 Increased maternal-fetal transport of fat in diabetes assessed by polyunsaturated fatty acid content in fetal lipids. Biol Neonate 47:343–349
238. **Soulimane-Mokhtari NA, Guermouche B, Saker M, Merzouk S, Merzouk H, Hichami A, Madani S, Khan NA, Prost J** 2008 Serum lipoprotein composition, lecithin cholesterol acyltransferase and tissue lipase activities in pregnant diabetic rats and their offspring receiving enriched n-3 PUFA diet. Gen Physiol Biophys 27:3–11
239. **Capobianco E, Martínez N, Higa R, White V, Jawerbaum A** 2008 The effects of maternal dietary treatments with natural PPAR ligands on lipid metabolism in fetuses from control and diabetic rats. Prostaglandins Leukot Essent Fatty Acids 79:191–199
240. **Nasu R, Seki K, Nara M, Murakami M, Kohama T** 2007 Effect of a high-fat diet on diabetic mother rats and their offspring through three generations. Endocr J 54:563–569
241. **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 1:21
242. **White V, González E, Pustovrh C, Capobianco E, Martínez N, Do Porto DF, Higa R, Jawerbaum A** 2007 Leptin in embryos from control and diabetic rats during organogenesis: a modulator of nitric oxide production and lipid homeostasis. Diabetes Metab Res Rev 23:580–588
243. **Gluckman PD, Butler JH, Comline R, Fowden A** 1987 The effects of pancreatectomy on the plasma concentrations of insulin-like growth factors 1 and 2 in the sheep fetus. J Dev Physiol 9:79–88
244. **Streck RD, Rajaratnam VS, Fishman RB, Webb PJ** 1995 Effects of maternal diabetes on fetal expression of insulin-like growth factor and insulin-like growth factor binding protein mRNAs in the rat. J Endocrinol 147:R5–R8
245. **Shao WJ, Tao LY, Gao C, Xie JY, Zhao RQ** 2008 Alterations in methylation and expression levels of imprinted

genes H19 and Igf2 in the fetuses of diabetic mice. Comp Med 58:341–346
246. **Plagemann A, Harder T, Rake A, Melchior K, Rittel F, Rohde W, Dörner G** 1998 Hypothalamic insulin and neuropeptide Y in the offspring of gestational diabetic mother rats. Neuroreport 9:4069–4073
247. **Dörner G, Plagemann A** 1994 Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm Metab Res 26:213–221
248. **Dörner G, Plagemann A, Rückert J, Götz F, Rohde W, Stahl F, Kürschner U, Gottschalk J, Mohnike A, Steindel E** 1988 Teratogenetic maternofoetal transmission and prevention of diabetes susceptibility. Exp Clin Endocrinol 91: 247–258
249. **Verhaeghe J, Peeters TL, Vandeputte M, Rombauts W, Bouillon R, Van Assche FA** 1989 Maternal and fetal endocrine pancreas in the spontaneously diabetic BB rat. Biol Neonate 55:298–308
250. **Scott FW, Rowsell P, Wang GS, Burghardt K, Kolb H, Flohé S** 2002 Oral exposure to diabetes-promoting food or immunomodulators in neonates alters gut cytokines and diabetes. Diabetes 51:73–78
251. **Ishizuka T, Klepcyk P, Liu S, Panko L, Liu S, Gibbs EM, Friedman JE** 1999 Effects of overexpression of human GLUT4 gene on maternal diabetes and fetal growth in spontaneous gestational diabetic C57BLKS/J Lepr(db/+) mice. Diabetes 48:1061–1069
252. **Bevier WC, Jovanovic-Peterson L, Formby B, Peterson CM** 1994 Maternal hyperglycemia is not the only cause of macrosomia: lessons learned from the nonobese diabetic mouse. Am J Perinatol 11:51–56
253. **Charré S, Rosmalen JG, Pelegri C, Alves V, Leenen PJ, Drexhage HA, Homo-Delarche F** 2002 Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period. Histol Histopathol 17:393–401
254. **Schroeder RE, Devaskar UP, Trail SE, Demello DE, Cole DP, Devaskar SU** 1993 Effect of maternal diabetes on the expression of genes regulating fetal brain glucose uptake. Diabetes 42:1487–1496
255. **Maron R, Guerau-de-Arellano M, Zhang X, Weiner HL** 2001 Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. J Autoimmun 16:21–28
256. **Oge A, Isganaitis E, Jimenez-Chillaron J, Reamer C, Faucette R, Barry K, Przybyla R, Patti ME** 2007 In utero undernutrition reduces diabetes incidence in non-obese diabetic mice. Diabetologia 50:1099–1108
257. **Chamson-Reig A, Arany EJ, Summers K, Hill DJ** 2009 A low protein diet in early life delays the onset of diabetes in the non-obese diabetic mouse. J Endocrinol 201:231–239
258. **Schott-Ohly P, Lgssiar A, Partke HJ, Hassan M, Friesen N, Gleichmann H** 2004 Prevention of spontaneous and experimentally induced diabetes in mice with zinc sulfate-enriched drinking water is associated with activation and reduction of NF-κB and AP-1 in islets, respectively. Exp Biol Med (Maywood) 229:1177–1185
259. **Arany E, Strutt B, Romanus P, Remacle C, Reusens B, Hill DJ** 2004 Taurine supplement in early life altered islet morphology, decreased insulitis and delayed the onset of diabetes in non-obese diabetic mice. Diabetologia 47:1831–1837
260. **Miralles F, Portha B** 2001 Early development of β-cells is impaired in the GK rat model of type 2 diabetes. Diabetes 50(Suppl 1):S84–S88
261. **Fernández-Millán E, Gangnerau MN, De Miguel-Santos L, Calderari S, Serradas P, Escrivá F, Portha B, Alvarez C** 2009 Undernutrition of the GK rat during gestation improves pancreatic IGF-2 and β-cell mass in the fetuses. Growth Factors 27:409–418
262. **Ornoy A, Tsadok MA, Yaffe P, Zangen SW** 2009 The Cohen diabetic rat as a model for fetal growth restriction: vitamins C and E reduce fetal oxidative stress but do not restore normal growth. Reprod Toxicol 28:521–529
263. **Waterland RA, Garza C** 1999 Potential mechanisms of metabolic imprinting that lead to chronic disease. Am J Clin Nutr 69:179–197
264. **Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L** 2004 Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol 561:355–377
265. **Devaskar SU, Thamotharan M** 2007 Metabolic programming in the pathogenesis of insulin resistance. Rev Endocr Metab Disord 8:105–113
266. **Wentzel P, Gäreskog M, Eriksson UJ** 2008 Decreased cardiac glutathione peroxidase levels and enhanced mandibular apoptosis in malformed embryos of diabetic rats. Diabetes 57:3344–3352
267. **Fletcher JM, Bassett JM** 1986 Effects of streptozotocin injection into fetal rabbits on their subsequent growth in utero. Biol Neonate 49:51–59
268. **Miodovnik M, Mimouni F, Berk M, Clark KE** 1989 Alloxan-induced diabetes mellitus in the pregnant ewe: metabolic and cardiovascular effects on the mother and her fetus. Am J Obstet Gynecol 160:1239–1244
269. **Weksler-Zangen S, Yaffe P, Ornoy A** 2003 Reduced SOD activity and increased neural tube defects in embryos of the sensitive but not of the resistant Cohen diabetic rats cultured under diabetic conditions. Birth Defects Res A Clin Mol Teratol 67:429–437
270. **Jawerbaum A, Gonzalez ET, Catafau JR, Rodriguez RR, Gomez G, Gimeno AL, Gimeno MA** 1993 Glucose, glycogen and triglyceride metabolism, as well as prostaglandin production in uterine strips and in embryos from diabetic pregnant rats. Influences of the presence of substrate in the incubation medium. Prostaglandins 46:417–431
271. **MartínA, Herrera E** 1991 Different responses to maternal diabetes during the first and second half of gestation in the streptozotocin-treated rat. Isr J Med Sci 27:442–448
272. **Franke K, Harder T, Aerts L, Melchior K, Fahrenkrog S, Rodekamp E, Ziska T, Van Assche FA, Dudenhausen JW, Plagemann A** 2005 'Programming' of orexigenic and anorexigenic hypothalamic neurons in offspring of treated and untreated diabetic mother rats. Brain Res 1031:276–283
273. **Yessoufou A, Hichami A, Besnard P, Moutairou K, Khan NA** 2006 Peroxisome proliferator-activated receptor α deficiency increases the risk of maternal abortion and neonatal mortality in murine pregnancy with or without diabetes mellitus: modulation of T cell differentiation. Endocrinology 147:4410–4418