HHS Public Access

Author manuscript

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2016 October 19.

Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2010 October ; 88(10): 779–790. doi:10.1002/bdra.20704.

Understanding Diabetic Teratogenesis: Where Are We Now and Where Are We Going?

Sheller Zabihi and **Mary R. Loeken***

Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215

Abstract

Maternal pregestational diabetes (type 1 or type 2) poses an increased risk for a broad spectrum of birth defects. To our knowledge, this problem first came to the attention of the Teratology Society at the 14th Annual Meeting in Vancouver, B.C. in 1974, with a presentation by Lewis Holmes, “Etiologic heterogeneity of neural tube defects”. Although advances in the control of diabetes in the decades since the discovery of insulin in the 1920’s have reduced the risk for birth defects during diabetic pregnancy, the increasing incidence of diabetes among women of childbearing years indicates that this cause of birth defects is a growing public health concern. Major advances in understanding how a disease of maternal fuel metabolism can interfere with embryogenesis of multiple organ systems have been made in recent years. In this review, we trace the history of the study of diabetic teratogenesis and discuss a model in which tissue-specific developmental control genes are regulated at specific times in embryonic development by glucose metabolism. The major function of such genes is to suppress apoptosis, perhaps to preserve proliferative capability, and inhibit premature senescence.

Keywords

diabetic pregnancy; diabetic embryopathy; neural tube defect; cardiac outflow tract defect; hyperglycemia; oxidative stress; *Pax3*; p53

INTRODUCTION

The focus of this review is to describe the molecular causes of birth defects in diabetic pregnancy as revealed by experimentation using animal models. The motivation behind this research derives from recognition of the clinical problems and the potential for basic experimentation to reduce these problems. As this issue celebrates 50 years of the Teratology Society, we begin with a brief summary of the past 90+ years of clinical observations of the teratogenic effects of maternal diabetes.

*Correspondence to: Mary R. Loeken, Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. mary.loeken@joslin.harvard.edu.

DIABETIC PREGNANCY IN THE EARLY YEARS AFTER THE DISCOVERY OF INSULIN

Before the availability of insulin in the early 1920s, most type 1 diabetic subjects died within a few months of onset of the disease. Indeed, pregnancy increased the risk for fatal diabetic ketoacidosis, so maternal death was a common occurrence in diabetic pregnancy (Joslin, 1915; Gabbe, 1993). The association of birth defects with diabetic pregnancy was not recognized until the mid-1930s, when use of insulin allowed the focus of clinical care to shift from maternal survival to neonatal outcome. In 1936 Pricilla White wrote, “Congenital defects [including heart defects, gastrointestinal atresia, microcephaly, and achondroplasia] are beyond our therapeutic control and are, we believe, related to a disease which is genetic in origin” (White, 1937).

White appeared to be explaining what she thought was an unfortunate, but unimprovable, consequence of survivability of women with diabetes. Yet she could not have appreciated how remarkably prescient, and, at the same time, inaccurate, she was. First of all, advances in treatment of diabetes have demonstrated that congenital defects are not beyond our therapeutic control. Nevertheless, even today, diabetic embryopathy, like diabetic complications in general, has not been eliminated. Second, congenital malformations are not genetically linked to diabetes, because embryos of women with type 2 diabetes are also at risk for malformations, and paternal diabetes does not increase risk for malformations. Moreover, the same kinds of malformations that occur in human offspring of diabetic mothers can be induced in experimental animals with chemically induced diabetes. On the other hand, although congenital defects are not genetically linked to diabetes, susceptibility to malformations induced by diabetic pregnancy is genetically determined in rats and mice (Eriksson, 1988; Pani et al., 2002a) and probably humans, as well.

DIABETIC TERATOGENESIS: TYPES OF MALFORMATIONS AND RELATIONSHIP TO GLYCEMIC CONTROL

In the approximately 60 years following the discovery of insulin, two major advances drastically improved the survival rate of the offspring of women with diabetes. One was the recognition that improved control of maternal diabetes reduced morbidity and mortality of both the mother and the fetus/neonate. Indeed, White’s Classification Index, in which poor fetal outcome was found to be related to early age of onset or long duration of diabetes as well as severity of vascular and renal complications, served as a guide for care of diabetic pregnancies (White, 1949). The other was the discovery and availability of surfactant to significantly reduce perinatal mortality from respiratory distress syndrome (RDS). The infants of diabetic mothers were at increased risk for RDS because maternal diabetes increased the incidence of pre-term birth (both spontaneous and iatrogenic), and fetal insulin, stimulated by maternal hyperglycemia, inhibits lung maturation (Robert et al., 1976; Freinkel et al., 1990). Thus, by the 1980s, with RDS largely treatable, congenital malformations became the major cause of neonatal mortality. On the other hand, spontaneous abortions continued to be increased in poorly controlled diabetes (Miodovnik et al., 1986; Mills et al., 1988; Miodovnik et al., 1990). At least some spontaneous abortions

could result from malformation of structures required for fetal viability, such as the cardiovascular system or the placenta, but could also be attributable to maternal effects, such as endocrinopathies or vascular complications affecting uterine perfusion.

The congenital malformations associated with diabetes can affect several different organ systems, but neural tube defects (NTDs), including spina bifida, anencephaly, encephalocele, and holoprosencephaly, and cardiovascular defects, are among the most commonly observed (Kucera, 1971; Kitzmiller et al., 1978; Mills, 1982; Barr et al., 1983; Cousins, 1983; Fuhrmann et al., 1984; Becerra et al., 1990; Kalter, 1993; Cohen and Shiota, 2002; Correa et al., 2008). Caudal regression syndrome, in which there is underdevelopment of the lower spine and sometimes the bowel and bladder, is an extremely rare defect that was observed in increased frequency in diabetic pregnancies (Mills, 1982; Kalter, 1993). The birth defects associated with diabetic pregnancy are induced before the seventh gestational week (Mills et al., 1979). Different organ systems become established in an ordered developmental sequence; thus, the time(s) during gestation at which maternal glycemic control is disturbed probably determines which organ system(s) will be affected. Malformations are not caused by maternal insulin (which is not transported to the embryo) but are related to poor control of maternal hyperglycemia (Miller et al., 1981; Widness et al., 1983; Fuhrmann et al., 1984; Ylinen et al., 1984; Greene et al., 1989). However, whether hyperglycemia is directly responsible for the malformations, or whether other metabolic disturbances secondary to poor glycemic control play a role, could not be determined from these studies.

2010: IMPROVED PREGNANCY OUTCOME, BUT A GROWING PUBLIC HEALTH PROBLEM

Within the last approximately 20 years, there has been a significant reduction in risk for birth defects resulting from diabetic pregnancy. This can be attributed to more rigorous glycemic control to reduce all diabetic complications as recommended by the Diabetes Complications Control Trial (DCCT) and the St. Vincent Declaration (WHO/IDF Europe, 1990; DCCT Research Group, 1996). Of note, there has been a reduction in congenital malformations in nondiabetic pregnancies during this time as well, which is largely due to widespread measures to increase folic acid consumption by women of childbearing years (MRC Vitamin Study Research Group, 1991; Hall and Solehdin, 1998). Thus, some diabetic pregnancies may have benefited from increased folic acid intake just as in the general population. Nevertheless, birth defects in diabetic pregnancies are still higher than in the nondiabetic population in several studies (Evers et al., 2004; Hawthorne, 2005; Jonasson et al., 2007).

In recent years, pre-pregnancy obesity has been shown to increase risk for neural tube and other defects (Shaw et al., 1996; Werler et al., 1996; Moore et al., 2000; Watkins et al., 2003; Gilboa et al., 2010). These malformations may be the result of undiagnosed pregestational type 2 diabetes (Buchanan, 1995; Mokdad et al., 2001; Biggio et al., 2010). The incidence of diabetes existing prior to pregnancy more than doubled in a racially/ethnically diverse population in southern California between 1999 and 2005 (Lawrence et al., 2008). Thus, the

incidence of birth defects caused by pregestational diabetic pregnancy will likely increase in coming years, due to the increasing prevalence of type 2 diabetes.

In summary, during the past century, the discovery of insulin, improvements in monitoring and control of diabetes, and advances in prenatal and neonatal medicine have shifted the clinical focus of diabetic pregnancy from solely maternal survival to reducing the incidence and severity of neonatal morbidity and mortality. Nevertheless, adequate control of diabetes that will reduce the incidence of birth defects to that of nondiabetic pregnancy remains challenging. Experimental research may reveal how these malformations occur and may direct the design of novel strategies to prevent them.

RESEARCH TO STUDY THE MOLECULAR MECHANISMS RESPONSIBLE FOR DIABETIC TERATOGENESIS

Basic science approaches to study diabetic teratogenesis began approximately 50 years ago. Here we provide a historical perspective, summarize the current understanding resulting from our research, and consider whether current and future research will determine general mechanisms that can explain the variety of defects that can occur during diabetic pregnancy.

DEVELOPMENT OF ANIMAL MODELS TO STUDY DIABETIC TERATOGENESIS

The discoveries that the toxic glucose analogs alloxan and streptozotocin (STZ) could be used to chemically destroy pancreatic β cells in laboratory animals allowed the development of animal models to study diabetic pregnancy. The first reports using rats or mice showed that many of the malformations that occur in human pregnancy, such as open NTDs and malformation of heart chambers, were also induced in rat or mouse embryos (Angervall, 1959). Incomplete sacral ossification, resembling caudal regression syndrome, was also sometimes observed (Deuchar, 1977). As in humans, the malformation rate was reduced by insulin treatment (Horii et al., 1966). Notably, the timing of alloxan or STZ administration affected the kinds of malformations that were observed, and whether or not fetal outcome was affected. Induction of diabetes before pregnancy or before implantation led to NTDs, heart chamber defects, and skeletal defects. Induction of diabetes early during organogenesis (day 8.5–10.5 in the mouse) did not cause neural tube or cardiac malformations but caused cleft palate and some skeletal defects (deformed ribs, club foot). However, induction of diabetes after organogenesis rarely caused structural malformations (Watanabe and Ingalls, 1963; Horii et al., 1966; Deuchar, 1977). Administration of alloxan during organogenesis also increased maternal deaths and resorptions (Watanabe and Ingalls, 1963).

A shortcoming of these experiments was that it was difficult to distinguish between potential teratogenic or toxic effects of alloxan or STZ when it was administered after conception, hypoglycemia (caused by insulin release to maternal circulation following drug administration), hyperglycemia (caused by β cell depletion), and other consequences of diabetes (such as ketoacidosis). The development of postimplantation embryo culture techniques (New et al., 1973) allowed the study of the effects of diabetic serum or high

glucose directly on the embryo. In fact, the first of these studies were published in *Teratology* in 1977 and 1980 (Cockroft and Coppola, 1977; Sadler, 1980a; Sadler, 1980b). Cockroft and Coppola found that culture of head-fold stage rat embryos in rat serum containing 6–15 mg/ml glucose (compared to approximately 1.3 mg/ml control serum) for 48 hr induced malformations, especially NTDs, and growth retardation in up to 100% of embryos (Cockroft and Coppola, 1977). Three years later, Sadler showed that culture of day 8.5 mouse embryos in serum from rats that had previously been made diabetic with STZ, or in serum containing up to 9.2 mg/ml glucose, increased NTDs compared to culture in control serum (Sadler, 1980a; Sadler, 1980b). The incidence of NTDs was dose-related to glucose concentrations. Notably, diabetic serum and high glucose-induced malformations were more frequent in younger (0–3 somites) than in older (4–6 somites) embryos. Sadler interpreted this observation that younger embryos were more susceptible to perturbations. However, these results could be reinterpreted in light of our current understanding of the molecular regulation of embryogenesis, that the temporal susceptibility of embryos to a diabetic milieu depends upon the particular embryonic genes that are induced during the teratogenic exposure. Also of note, a transient increase in pyknotic neuroepithelial and neural crest cells was observed during the first 4–8 hr after exposure to high glucose or diabetic serum (Sadler, 1980a; Sadler, 1980b). These pyknotic nuclei were interpreted as necrotic, but they might have been apoptotic. These were the first observations of cell death associated with diabetic embryopathy. Nonetheless, because the cell death was not extensive and was transient, Sadler was unsure whether it could be responsible for the malformations.

There are also several limitations of the embryo culture model: (a) the concentrations of glucose that induced malformations were far in excess of those normally experienced in human diabetes, (b) at least some of the effects could be explained by hyperosmolarity, (c) embryos cannot be cultured beyond approximately 3 days, so that effects of high glucose exposure on later fetal development or viability cannot be studied, and (d) during incubation of postimplantation embryos, culture bottles are equilibrated with gas mixtures that are significantly hyperoxic compared to the normal intrauterine environment. Hyperoxia-induced oxidative stress may confound effects of high glucose or diabetic serum.

Although all of these early experiments were descriptive and did not determine the biochemical or cellular mechanisms responsible for diabetic teratogenesis, they laid the foundation for subsequent research. Furthermore, they demonstrated that birth defects caused by diabetic pregnancy were not genetically linked to diabetes as originally proposed by Pricilla White.

FUEL-MEDIATED TERATOGENESIS

Norbert Freinkel coined the term, “fuel-mediated teratogenesis”, which proposed that increased delivery of fuels, including glucose and ketones, to the embryo during diabetic pregnancy is responsible for defective organogenesis, as well as other perturbations of fetal development later during gestation (Freinkel et al., 1986; Freinkel et al., 1990).

Glucose is transferred from maternal circulation and reaches equilibrium within embryo tissues (Sussman and Matschinsky, 1988). While the frequency and severity of

malformations during embryo culture were correlated with glucose concentrations in rat serum, it was not clear whether glucose was solely responsible. As noted above, adding only glucose to rat serum increased malformation of postimplantation cultured embryos (Cockroft and Coppola, 1977; Sadler, 1980a), but at concentrations well in excess of those that normally occur in humans, even with poor glycemic control. β-hydroxybutyrate, a ketone produced during diabetic hypoglycemia, itself is teratogenic when added to rat embryo cultures (Horton and Sadler, 1983). Two groups investigated this further by testing the teratogenic effects of serum from STZ-diabetic rats that had been insulin treated. Insulin infusion for 2 hr, or injection once a day for 1 week, normalized serum glucose concentrations in STZ-diabetic rats and significantly reduced, but did not prevent, malformations in cultured rat or mouse embryos (Buchanan et al., 1994; Wentzel and Eriksson, 1996). Addition of glucose or β-hydroxybutyrate to serum from nondiabetic rats had a synergistic teratogenic effect, but both together were not as teratogenic as serum from untreated STZ-diabetic rats (Buchanan et al., 1994). This suggested that excess glucose and ketones each contributed to diabetic teratogenesis, but that other (unidentified) factors in diabetic serum were also important. On the other hand, administration of insulin during pregnancy to diabetic animals prevented malformations (Eriksson et al., 1982; Eriksson et al., 1989). This suggests either that glucose is responsible for diabetic teratogenesis, or that there are other metabolic disturbances secondary to hypoinsulinemia that are responsible. It should be noted, however, that excess ketone production is not a general feature of type 2 diabetes. Therefore, if hyperglycemia alone is not sufficient to induce malformations, there must be additional metabolic disturbances, instead of ketosis, that contribute to diabetic teratogenesis in pregnancies affected by type 2 diabetes.

Just as maternal hyperglycemia will increase glucose delivery to the embryo, maternal hypoglycemia, which can occur during untreated diabetes or following excessive insulin administration, could limit glucose availability to the embryo. Reduced availability of fuel to generate ATP for the highly metabolically active embryo might interfere with organogenesis. Hypoglycemic exposure of cultured rat embryos increases malformations (Hunter and Sadler, 1989; Smoak and Sadler, 1990).

BIOCHEMICAL DISTURBANCES IN EMBRYOS OF DIABETIC RODENTS

Several studies using both in vivo (i.e., pregnancy in diabetic animals) and in vitro (i.e., postimplantation embryo culture) attempted to identify biochemical disturbances that were associated with malformations in animal models of diabetic pregnancy. One hypothesis was that arachidonic acid release from plasma membranes by phospholipase A_2 is reduced (Goldman et al., 1985; Goldman and Goto, 1991). Formation of several embryonic structures, such as the palate, the neural tube, the heart, and external genitalia, involve folding and fusion of opposing layers and require phosphatidylinositol turnover and arachidonic acid signaling (Goldman and Goto, 1991). In support of this hypothesis, administration of arachidonic acid suppressed malformations (Goldman et al., 1985; Goldman and Goto, 1991). Eriksson and colleagues also considered that insufficient arachidonic, as well as palmitic, acid in embryo cells was involved, but that this was due to decreased transport to the embryo, rather than due to decreased plasma membrane release (Engstrom et al., 1991). For a time, it was considered that the polyol pathway, in which

excess glucose is reduced to sorbitol by aldose reductase and that had been implicated in other diabetic complications, could be involved. However, failure of aldose reductase inhibitors to prevent diabetic malformations suggested that the polyol pathway was not involved (Eriksson et al., 1986; Hod et al., 1986). However, decreased myoinositol, which is derived from glucose-6-phosphate, might be involved (Hod et al., 1986; Sussman and Matschinsky, 1988). Abnormal synthesis of basement membrane has been implicated in diabetic complications, and altered levels of *laminin B1* and *fibronectin* mRNA were found in embryos of diabetic rats (Cagliero et al., 1993). As noted above, there was evidence that β-hydroxybutyrate is teratogenic on its own and that it synergized with glucose. β-hydroxybutyrate inhibits the pentose shunt pathway, and it was thought that inhibition of the pentose shunt pathway is teratogenic because it is necessary for production of ribose sugars for nucleotide synthesis and de novo pyrimidine synthesis (Hunter et al., 1987). The ability of β-hydroxybutyrate to inhibit the pentose shunt pathway, and whether ribose or pyrimidine synthesis was inhibited, depended on the developmental age of the embryos (2–3 somites vs. 5–6 somites) (Shum and Sadler, 1990).

INVOLVEMENT OF OXIDATIVE STRESS

Increased glucose metabolism enhances production of reactive oxygen species (ROS) (Jain et al., 1989; Brownlee, 2001). Activity of the free radical scavenging enzyme, superoxide dismutase (SOD), was found to be increased in rat embryos cultured in high glucose, providing evidence for enhanced ROS production (Eriksson and Borg, 1993). This was associated with increased expression of the genes encoding Cu/Zn-SOD, Mn-SOD, and glutathione peroxidase, but decreased expression of catalase (Forsberg et al., 1996). Others did not observe altered activities of SOD or glutathione peroxidase in rat embryos cultured in high glucose, but the important cellular antioxidant glutathione (GSH) and expression of the enzyme encoding the rate-limiting GSH-synthesizing enzyme gamma-glutamylcysteine synthetase were reduced (Trocino et al., 1995; Sakamaki et al., 1999). In addition, vitamin E content of tissues of diabetic humans and experimental animals is reduced, suggesting that it is depleted by enhanced free radical production (Jain et al., 1991). Embryos appear to be particularly vulnerable to increased production of ROS because free radical enzymes are not highly expressed until shortly before birth (el-Hage and Singh, 1990; Ishibashi et al., 1997).

Several approaches to increase free radical scavenging provided evidence that oxidative stress is mechanistically involved in diabetic teratogenesis. First, addition of SOD, catalase, and glutathione peroxidase to embryo culture inhibited malformations induced by high glucose (Eriksson and Borg, 1991). Second, transgenic overexpression of human Cu/Zn-SOD reduced malformations in embryos of STZ-diabetic mice (Hagay et al., 1995). Third, administration of supplemental antioxidants, including vitamin E, vitamin C, butylated hydroxytoluene, and GSH ester to STZ-diabetic animals or high glucose culture inhibited malformations (Trocino et al., 1995; Viana et al., 1996; Siman and Eriksson, 1997a; Siman and Eriksson, 1997b; Sivan et al., 1997; Sakamaki et al., 1999). N-acetylcysteine, which is converted to GSH, also inhibited malformation of rat embryos in high glucose culture (Wentzel et al., 1997). The aforementioned decrease in arachidonic acid release or transport leads to decreased prostaglandin synthesis (Goldman and Goto, 1991), and addition of N-

acetylcysteine restored prostaglandin concentrations in embryos cultured in high glucose (Wentzel et al., 1999).

Thus, several biochemical disturbances were associated with malformations in embryos during diabetic pregnancy or high glucose culture. Increased production of ROS, as well as insufficient free radical scavenging activities, generated oxidative stress, which appeared to mediate the effects of a diabetic/hyperglycemic milieu. Nevertheless, while these processes might be expected to disrupt normal cellular function, how any of these pathways might interfere with embryogenesis and lead to specific structural malformations, rather than malformation of all embryonic structures, was not clear.

ALTERED EXPRESSION OF ESSENTIAL DEVELOPMENTAL CONTROL GENES

By the early 1990s, much was known about many of the regulatory pathways that controlled pattern formation during early embryogenesis (Gurdon, 1992). Numerous developmental control genes had been identified, and the mechanisms by which many of them regulate morphogenesis had been studied in mouse embryos using transgenic and targeted mutagenesis (i.e., “knockout”) technology. In some cases, diversity of functions of genes whose homologs had previously been studied in *Drosophila* or *Xenopus* had been deciphered in mouse embryos (Kessel and Gruss, 1990). Some genes, in particular, the homeotic or *Hox* genes and the Paired box or *Pax* genes, belonged to gene families whose members had amplified during evolution of Metazoans (Dressler and Gruss, 1988; Kessel and Gruss, 1990; Chalepakis et al., 1992).

Our laboratory hypothesized that maternal diabetes interferes with the expression of embryonic genes that control essential developmental processes. The timing and severity of poor control of maternal diabetes, relative to times during embryonic development that such genes are induced, would determine which genes would be affected and the magnitude of altered gene expression. If expression of essential genes is reduced below a critical threshold, then this would lead to a malformation.

In our mouse model of STZ-induced diabetic pregnancy, NTDs (primarily exencephaly) were significantly increased in embryos of diabetic mice on days 10.5–11.5 (Phelan et al., 1997). The NTDs resembled those that occur in homozygous *Splotch* (*Pax3*$^{Sp/Sp}$) embryos that carry a loss-of-function *Pax3* mutation (Auerbach, 1954; Goulding et al., 1991; Epstein et al., 1993; Goulding et al., 1993). *Pax3* encodes a transcription factor that is expressed in neuroepithelium and neural crest beginning on day 8.5 (Goulding et al., 1991). Homozygous *Splotch* (i.e., *Pax3*-mutant) embryos develop NTDs with 100% penetrance (Auerbach, 1954). Therefore, if *Pax3* expression were reduced below a critical threshold in embryos of diabetic mothers, this could cause NTDs.

By in situ hybridization and reverse transcription-polymerase chain reaction (RT-PCR), we found that *Pax3* mRNA was significantly reduced in embryos of diabetic mice on day 8.5 (when *Pax3* expression is normally induced and the neural tube starts to form; Phelan et al., 1997). There was no growth or developmental delay that would account for reduced *Pax3*

expression, and embryos were morphologically normal on day 8.5. Because *Pax3*$^{Sp/Sp}$ embryos develop NTDs with 100% penetrance, this indicates that there are no redundant genes to compensate for loss of *Pax3* function. While there may be other genes that participate in neural tube development whose expression might be altered in embryos of diabetic mice, simply reducing *Pax3* expression below a critical threshold would be sufficient to induce a NTD. Therefore, most of our efforts to understand diabetic teratogenesis have focused on how *Pax3* is regulated during diabetic pregnancy, and how insufficient *Pax3* expression leads to NTDs.

By simply inducing transient hyperglycemia on day 7.5 of pregnancy of nondiabetic mice, or by only normalizing blood glucose concentrations in pregnant diabetic mice with phlorizin (to stimulate renal glucose excretion) on day 7.5, we demonstrated that maternal hyperglycemia is necessary and sufficient to inhibit *Pax3* expression and to induce malformations in diabetic pregnancy (Fine et al., 1999). In light of these in vivo findings, some of the previously reported in vitro observations bear reevaluation. For example, it is not necessary for (β-hydroxybutyrate, or other serum factors that are altered during type 1 diabetes, to contribute to malformations. In addition, significant inhibition of *Pax3* expression and induction of NTDs were observed beginning when maternal blood glucose concentrations were >250 mg/dl (13.75 mM), which is much lower than teratogenic glucose concentrations in vitro. Hyperglycemic excursions around 250 mg/dl might commonly occur in diabetes, even with good glycemic control. It is possible that much higher glucose concentrations are required to induce malformations in vitro because culture conditions do not accurately replicate the nutrient and gas exchange of the embryo and yolk sac that occur during perfusion by maternal circulation in utero. Most significantly, hyperglycemia disturbed *Pax3* expression and neural tube closure only when it occurred on day 7.5, when the embryo is at the primitive streak stage. There was no effect of maternal hyperglycemia, which returned after discontinuation of phlorizin treatment, on somite-stage embryos. This is consistent with Sadler's early observation that younger embryos were more susceptible to malformations induced by culture in diabetic serum or high glucose (Sadler, 1980a; Sadler, 1980b) and suggests that the molecular processes that lead to the initiation of *Pax3* expression on day 8.5 occur approximately 24 hr before *Pax3* expression begins, and that these processes are susceptible to interference by excess glucose metabolism.

It had previously been noted that early mouse embryos express the high K_M Glut2 glucose transporter (Hogan et al., 1991), as well as the low K_M Glut1 and Glut3 transporters (Takao et al., 1993; Trocino et al., 1994). While glucose transport by low K_M transporters might saturate during maternal hyperglycemia, glucose transport by a high K_M transporter would be optimal when glucose concentrations approximate the transporter's K_M. Indeed, the K_M of Glut2 (~15 mM) is equivalent to the threshold maternal glucose concentration at which we observed significant inhibition of *Pax3* expression and induction of NTDs (Thorens, 1996; Fine et al., 1999). Glut2 may be expressed by the early embryo, not because it is needed to transport glucose (Glut1 and Glut3 can do that, and more efficiently than Glut2 at physiologic glucose concentrations) but to serve as a glucosamine transporter (Uldry et al., 2002). And yet, Glut2 expression would enhance glucose uptake by the embryo during episodes of maternal hyperglycemia and could be responsible for the susceptibility of the embryo to maternal hyperglycemia. To test this, we crossed heterozygous Glut2 knockout

mice (*Glut2*$^{+/-}$) and found that homozygous knockout embryos (*Glut2*$^{-/-}$) failed to develop NTDs in response to maternal hyperglycemia; moreover, NTDs were less frequent in heterozygous embryos (*Glut2*$^{+/-}$) than in wild-type (*Glut2*$^{+/+}$) embryos (Li et al., 2007). This indicated that Glut2 is responsible for glucose transport and subsequent teratogenesis during maternal hyperglycemia. This also suggests that maternal blood glucose concentration must reach the K_M of Glut2 to induce NTDs.

OXIDATIVE STRESS INDUCED BY EXCESS GLUCOSE METABOLISM IS RESPONSIBLE FOR ALTERED EMBRYO GENE EXPRESSION

Considering the prior evidence that oxidative stress mediated the effects of high glucose to cause embryonic malformations, we hypothesized that oxidative stress inhibited expression of essential developmental control genes, such as *Pax3*. Consistent with the hypothesis, we found that supplementary vitamin E, or GSH-ethyl ester, prevented the inhibition of *Pax3* and increase in NTDs in embryos of diabetic mice (Chang et al., 2003). Moreover, a single injection of antimycin A (AA), a complex III electron transport inhibitor that stimulates superoxide production, on day 7.5 inhibited *Pax3* expression and induced NTDs (Chang et al., 2003). Concentrations of AA that were sufficient to inhibit *Pax3* expression were not sufficient to induce DNA strand breaks, suggesting that inhibition of gene expression was not due to DNA damage or cell death, but may be due to altered redox status of signaling molecules that regulate *Pax3* induction.

The mechanisms by which oxidative stress occurs in embryos in response to excess glucose metabolism are complex, involving not only increased mitochondrial respiration, but also decreased availability of GSH and hypoxic stress. In embryos of hyperglycemic mice, we observed that increased glycolysis stimulated the hexosamine synthetic pathway, in which fructose-6-phospate is converted to glucosamine-6-phosphate (Horal et al., 2004). Glucosamine-6-phosphate inhibits activity of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose shunt pathway; since G6PD activity is coupled to reduction of $NADP^+$ to NADPH, which is coupled to reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), increased hexosamine flux lowers cellular GSH levels (Horal et al., 2004). Furthermore, administering glucosamine to pregnant mice on day 7.5 increased markers of oxidative stress in embryos, decreased GSH, inhibited *Pax3* expression, and induced NTDs, and all of these effects were blocked by GSH-ethyl ester (Horal et al., 2004). It is possible that the previously noted inhibition of the pentose shunt pathway by β-hydroxybutyrate (Hunter et al., 1987) caused malformations, not due to inhibition of ribose or pyrimidine synthesis, but due to decreased reduction of GSSG to GSH.

We also observed that production of diacylglycerol (DAG) and activity of protein kinase C (PKC) were increased in embryos and decidua of diabetic mice, especially in embryos with NTDs (Hiramatsu et al., 2002). DAG-sensitive PKC can stimulate NADPH oxidase (Hua et al., 2003), which would further increase oxidative stress. However, because DAG and PKC were assayed in embryos after NTDs occurred, we do not know whether DAG production and PKC activity were increased prior to the onset of *Pax3* expression, and whether

increased PKC activity could have contributed to the NTDs. Others have also observed increased PKC activity in embryos of diabetic rats, although they also assayed PKC activity after the embryopathy-susceptible period (Gareskog and Wentzel, 2004).

Energy metabolism of the early embryo is predominantly anaerobic (Shepard et al., 1970; Akazawa et al., 1994). A high anaerobic:aerobic metabolism ratio even when O_2 is not limiting (the Warburg effect) is characteristic of embryonic and cancer cells and provides anabolic substrates for rapidly growing cells (Warburg, 1956a; Warburg, 1956b). The embryo is not yet vascularized during the diabetic embryopathy susceptible period; this creates a physiologic hypoxia (Rodesch et al., 1992; Fischer and Bavister, 1993). However, excess fuel substrate drives increased glucose oxidation, as well as glycolysis, and this has the potential to deplete oxygen within the embryo faster than it can be replenished. Genetic manipulations that block formation of the circulatory system produce excess embryo hypoxia and cause NTDs (Carmeliet et al., 1996; Maltepe et al., 1997; Tian et al., 1998; Yao et al., 1998; Kotch et al., 1999). Hypoxia can increase superoxide production by inhibiting mitochondrial complexes I, II, and III (Paddenberg et al., 2003). Thus, we hypothesized that increased glucose metabolism might cause excess hypoxia, and that hypoxic stress might contribute to oxidative stress in the embryo. In support of this hypothesis, we found that oxygen flux, an indicator of oxygen availability, was reduced in the head folds of embryos after 3 hr of maternal hyperglycemia (Li et al., 2005). Moreover, inducing hypoxia by housing pregnant mice at 12% O_2 on day 7.5 increased markers of oxidative stress (H_2O_2 production, decreased GSH, and lipid peroxidation), inhibited *Pax3* expression, and increased NTDs; the magnitude of the effects of maternal hypoxia on markers of oxidative stress, *Pax3* expression, and NTDs were the same as those induced by maternal hyperglycemia (>250 mg/dl) on day 7.5. Conversely, 30% O_2 on day 7.5 suppressed superoxide production, inhibition of *Pax3* expression, and NTDs that occurred in embryos of diabetic mice (Li et al., 2005). Notably, the effects of inducing hypoxia on day 7.5 to inhibit *Pax3* expression and induce NTDs were suppressed by vitamin E and GSH-ethyl ester (Li et al., 2005).

Thus, oxidative stress induced by excess embryo glucose metabolism causes NTDs because it inhibits expression of a gene, *Pax3*, which is essential for neural tube closure. *Pax3* expression has a limited tissue-specific distribution, which explains how selective structural malformations can result from oxidative stress. The oxidative stress results from increased superoxide production resulting from hypoxic stress and increased glucose oxidation, as well as diminished production of GSH.

INSUFFICIENT APOPTOSIS INHIBITION CAUSES MALFORMATIONS

In trying to understand a cellular mechanism to explain how NTDs arise as a result of inadequate *Pax3* expression, we considered that apoptosis of the migrating neural folds might abort the process of neural tube closure. Using a whole mount TUNEL assay to detect apoptotic nuclei, we observed that the epithelial ridges of the neural folds of embryos of diabetic mice were undergoing apoptosis prior to neural tube fusion; however, no apoptosis was seen in embryos of nondiabetic mice (Phelan et al., 1997). The apoptotic cells were limited to *Pax3*-expressing cells and were also seen at sites of NTDs in $Pax3^{Sp/Sp}$ embryos

(Phelan et al., 1997). This suggested that apoptosis as a result of *Pax3* insufficiency leads to NTDs in embryos of diabetic mice. Sadler was uncertain whether the neuroepithelial cell death observed in rat embryos cultured in high glucose or diabetic serum could be responsible for NTDs because it was transient (Sadler, 1980a; Sadler, 1980b). However, nuclear pyknosis, which he detected, is an early process during apoptosis and would have been completed before NTDs would be apparent.

To further investigate the regulation of apoptosis by *Pax3*, and to ask if the p53 tumor suppressor protein mediates *Pax3*-inhibited apoptosis, $Pax3^{Sp/+}$ mice were crossed with p53 knockout mice ($p53^{+/-}$). Double heterozygous offspring were crossed to generate $Pax3^{Sp/Sp}$ embryos with or without knockout *p53* alleles. As observed previously (Phelan et al., 1997), all $Pax3^{Sp/Sp}$ embryos that were wild type for both *p53* alleles developed NTDs and displayed apoptotic nuclei at sites of NTDs (Pani et al., 2002b). However, none of the $Pax3^{Sp/Sp}$ $p53^{-/-}$ embryos displayed apoptosis or NTDs. That neural tube closure was normal in embryos that lacked any functional *Pax3* demonstrated that *Pax3* is not necessary to regulate genes that direct neural tube closure, as was previously thought, but it is necessary to inhibit p53-dependent apoptosis. There was no difference in *p53* mRNA between wild-type and $Pax3^{Sp/Sp}$ embryos, but there was significantly less p53 protein in wild-type compared to $Pax3^{Sp/Sp}$ embryos (Pani et al., 2002b). This suggested that *Pax3* blocks p53-mediated apoptosis by inhibiting synthesis or stability of p53 protein.

As indicated previously, expression of many embryonic genes may be affected during diabetic pregnancy, but simply inhibiting *Pax3* expression below a critical threshold will lead to derepression of p53-mediated apoptosis and cause a NTD. However, diabetic teratogenesis can also occur in structures that do not express *Pax3*. Whether similar or distinct mechanisms can explain malformation of structures other than the neural tube is addressed later in this review.

SUSCEPTIBILITY TO DIABETIC TERATOGENESIS IS MODIFIED BY GENETIC BACKGROUND

Although Priscilla White was mistaken in concluding that congenital malformations are genetically linked to diabetes, there is evidence from animal models that susceptibility to malformations induced by diabetic pregnancy is genetically determined. In the rat, susceptibility appears to be due to free radical scavenging capacity, as mRNA levels encoding Mn-SOD and catalase are higher in embryos of diabetic rats of a malformation-resistant strain than in a susceptible strain (Cederberg and Eriksson, 1997; Cederberg et al., 2000). In the mouse, however, there is no difference in expression of any of six genes encoding free radical scavenging enzymes between malformation-resistant (C57Bl/6J) and malformation-susceptible (FVB) strains (Pani et al., 2002a). Resistance to diabetic pregnancy-induced malformations is due to the genotype of the embryo, not the teratogenicity of the mother's diabetes, and is dominant over susceptibility (Pani et al., 2002a). Susceptibility to congenital malformations in the general population appears to be complex, involving interaction of environmental factors and multiple genetic loci (Melvin et al., 2000). Thus, susceptibility to diabetic teratogenesis might not involve individual genes.

Rather, like other diabetic complications such as nephropathy (Doria et al., 1995), the metabolic disturbances caused by diabetic pregnancy may unmask the activities of genes that predispose the embryo to structural malformation.

CAUSES OF MALFORMATIONS OTHER THAN NEURAL TUBE DEFECTS

The recognition that reduced expression of *Pax3* during diabetic pregnancy could cause NTDs, and the availability of mouse lines with mutant *Pax3* alleles, allowed our laboratory to delineate a pathway from excess glucose metabolism to increased NTDs. However, detailed molecular pathways that can explain most of the malformations affecting structures other than the neural tube, with the exception of some defects of neural crest-derived structures, have been less well elucidated.

The neural crest is a transient population of mesenchymal cells that arise from the neural tube and contribute to a wide range of structures throughout the body (Sauka-Spengler and Bronner-Fraser, 2006). Cranial neural crest cells give rise to cartilage, bone, and connective tissue of the skull and jaw. Micrognathia (a reduction in size of the lower jaw) is often observed in embryos of diabetic rats (Eriksson et al., 1982). In vitro migration of explanted cranial neural crest from embryos of diabetic rats was reduced compared to that of control embryos (Suzuki et al., 1996), suggesting that reduced population of the mandible by cranial neural crest leads to micrognathia in embryos of diabetic rats. Although micrognathia is not a distinctive malformation associated with human diabetic pregnancy, some of the craniofacial defects that are observed in human offspring of diabetic mothers may result from cranial neural crest deficiencies.

Cardiac neural crest cells induce septation of the single cardiac outflow tract into the aorta and pulmonary arteries. Cardiac outflow tract defects (COTDs), in which outflow tract septation into the aorta and pulmonary arteries fails, occur in rat embryos of diabetic mothers and chicken embryos whose neural tubes were glucose injected (Siman et al., 2000; Roest et al., 2007). Administration of antioxidants blocks the effects of maternal diabetes or high glucose (Siman et al., 2000; Roest et al., 2007). COTDs are a common malformation in human diabetic pregnancy (Loffredo et al., 2001), suggesting that defective cardiac neural crest development contributes to these malformations.

Recognizing that cardiac neural crest cells express *Pax3*, and that cardiac neural crest cell migration and outflow tract septation are defective in $Pax3^{Sp/Sp}$ embryos, we tested whether COTDs in embryos of diabetic mothers might result from failure of *Pax3* to inhibit p53-mediated apoptosis. We found that maternal hyperglycemia induced on day 7.5, which inhibits *Pax3* expression in the neural crest as well as the neuroepithelium, led to apoptosis of migrating cardiac neural crest cells on day 9.5. However, suppressing oxidative stress with antioxidants on day 7.5 prevented cardiac neural crest apoptosis and resulting COTDs (Morgan et al., 2008b). Moreover, apoptosis of migrating cardiac neural crest cells and COTDs in *Pax3*-null embryos was prevented by p53 loss of function (Morgan et al., 2008a).

These results indicate that COTDs in diabetic pregnancy result from the same molecular processes as NTDs: oxidative stress during the stage of development in which *Pax3*

expression is induced leads to insufficient expression of *Pax3*, and insufficient production of *Pax3* derepresses p53-mediated apoptosis; consequently, processes depending on neuroepithelium or cardiac neural crest aborts. This pathway may also explain micrognathia in rat embryos of diabetic mothers. Increased mandibular apoptosis has been observed in embryos of diabetic rats (Wentzel et al., 2008), and micrognathia, which occurs in $Pax3^{Sp/Sp}$ embryos, is inhibited by p53 loss of function (Pani and Loeken, unpublished results).

Whereas both NTDs and COTDs (as well as defects of other neural crest-dependent processes) always develop in *Pax3*-null embryos (and often in embryos of diabetic mice, Morgan and Loeken, unpublished results), they are rarely coincident in humans. This is probably because maternal hyperglycemia is episodic, and the specific times at which *Pax3* expression is susceptible to inhibition by oxidative stress at different sites of the embryo may not be the same.

There have been no single gene pathways that explain malformations affecting the heart, skeleton, or gastrointestinal or genitourinary tracts, during diabetic teratogenesis. Several groups have employed microarray analyses and have observed differences in expression of multiple genes in rat or mouse embryos of diabetic mothers; however, since these have been performed using embryos after malformations were induced, it cannot be determined whether any of these genes could be mechanistically involved in malformation (Reece et al., 2006; Kumar et al., 2007; Pavlinkova et al., 2009). Similarly, altered expression of genes involved in free radical scavenging and embryonic development (glutathione peroxidase-1, vascular endothelial growth factor-A, Cu/Zn-SOD, Mn-SOD, sonic hedgehog, and bone morphogenetic protein-4) has been detected in embryos of diabetic rats (Wentzel et al., 2008), but after defects had formed. cDNA subtraction has identified several genes that are differentially expressed during embryogenesis in embryos of diabetic mice (Sato et al., 2008), but whether altered expression of any of these genes is responsible for any malformations has not been tested. However, one of the genes identified by cDNA subtraction and whose altered expression was confirmed by RT-PCR encodes the folic acid transporter Folbp1 (Sato et al., 2008). Reduced folate transport could explain the effect of folic acid supplementation to decrease NTDs in embryos of diabetic mice and rats (Wentzel and Eriksson, 2005; Zabihi et al., 2007; Oyama et al., 2008).

The skeletal defects that can occur in human diabetic pregnancy, particularly caudal regression syndrome, are rarely observed in animal models, making it difficult to study their molecular etiology. However, hyperglycemia potentiates the effects of retinoic acid to cause caudal regression syndrome in mouse embryos, and this is associated with decreased *Wnt-3a* expression and increased apoptosis (Chan et al., 2002; Leung et al., 2004). Thus, enhanced activation of a retinoic acid signaling pathway, or under-expression of *Wnt-3a*, may modify risk for caudal regression in embryos of diabetic mothers. There is decreased growth and decreased incorporation of thymidine and sulfate by pre-chondrocytes from embryos of diabetic rats, or by pre-chondrocytes from normal embryos cultured with high glucose or diabetic serum (Styrud and Eriksson, 1990; Styrud and Eriksson, 1991). The decreased growth and differentiation appears to be due to decreased growth factor signaling (Styrud and Eriksson, 1991), but whether there is decreased production of locally acting cytokines,

or decreased expression of growth factor receptors on specific populations of prechondrocytes, that can explain specific skeletal defects has not been determined.

Transgenic over-expression of the pancreas transcription factor *Isl-1* induces a caudal regression phenotype (Muller et al., 2003), although it has not been demonstrated that *Isl-1* is expressed in the sacral region of the embryo, and if expression is enhanced during diabetic pregnancy. Interestingly, *Isl-1*–positive cells located in the embryonic heart field have been found to be the progenitor cell for multiple cardiac cell lineages (Moretti et al., 2006). This raises the possibility that *Isl-1* expression might be regulated by glucose (or oxidative stress) in some embryonic progenitor cells, and abnormal expression of *Isl-1* could lead to defective cell lineage development.

FUEL-MEDIATED TERATOGENESIS: A 2010 PERSPECTIVE

As already mentioned, fuel metabolism in early embryo cells is highly anaerobic (Shepard et al., 1970; Akazawa et al., 1994). There is emerging evidence that p53 promotes aerobic glucose metabolism (Ma et al., 2007). p53 also inhibits activity of the muscle isoform of the glycolytic enzyme, phosphoglycerate mutase (PGM-m), that is expressed in undifferentiated cells (Kondoh et al., 2005). A high glycolytic flux and low mitochondrial oxygen consumption promote proliferation and inhibit senescence (Kondoh et al., 2007). Therefore, in early differentiating neuroepithelium and neural crest, production of *Pax3* is regulated by fuel metabolism. But, by titrating the activity of p53 to promote aerobic metabolism, *Pax3* may protect neuroepithelial and neural crest cells from premature senescence and loss of proliferative capability. This is shown schematically in Figure 1.

It is likely that there are genes, in addition to *Pax3*, that encode proteins that play a similar role to regulate p53 or related proteins such as p63 and p73, in the progenitor cells that lead to formation of other embryonic structures. Thus, insufficient expression of these genes in response to excess glucose metabolism may provide a unifying explanation for diabetic teratogenesis. A flow diagram showing how increased glucose metabolism can lead to malformation is shown in Figure 2.

This model may also explain the stronger association of pregestational maternal diabetes with multiple, than with isolated, malformations (Correa et al., 2008). In diabetic subjects, blood glucose levels fluctuate. Thus, the exposure of the embryo to glucose concentrations above the Glut2 K_M is likely to be episodic, not chronic. Genes that perform functions like *Pax3*, that are activated early during the development of different organs, are turned on at different times during embryogenesis; therefore, the timing at which these genes are susceptible to excess glucose metabolism will depend on the tissue in which they are expressed. If there are multiple episodes of hyperglycemia that are sufficient to impair critical embryo gene expression, then multiple malformations will occur. However, the timing at which sufficiently hyperglycemic episodes occur will vary between pregnancies, which can explain why the specific combinations of multiple malformations are unpredictable.

Additional research is necessary to identify such genes, as well as to investigate how fuel metabolism regulates self-renewal versus differentiation during embryogenesis. Furthermore, it is necessary to understand how redox signaling regulates expression of such genes at critical stages during embryogenesis in order to consider whether strategies to reduce oxidative or hypoxic stress during diabetic pregnancy might be clinically useful.

Acknowledgments

Grant support for this paper was RO1 DK52865, RO1 DK58300 (to M.R.L.).

REFERENCES

Akazawa S, Unterman T, Metzger BE, et al. Glucose metabolism in separated embryos and investing membranes during organogenesis in the rat. Metabolism. 1994; 43:830–835. [PubMed: 8028505]

Angervall L. Alloxan diabetes and pregnancy in the rat: effects of offspring. Acta Endocrinol Suppl (Cph). 1959; 31(Supp 44):1–86.

Auerbach R. Analysis of the developmental effects of a lethal mutation in the house mouse. J Exp Zool. 1954; 127:305–329.

Barr M Jr, Hanson JW, Currey K, et al. Holoprosencephaly in infants of diabetic mothers. J Pediatr. 1983; 102(4):565–568. [PubMed: 6834191]

Becerra JE, Khoury MJ, Cordero JF, et al. Diabetes mellitus during pregnancy and the risks for the specific birth defects: a population-based case-control study. Pediatrics. 1990; 85:1–9. [PubMed: 2404255]

Biggio JR Jr, Chapman V, Neely C, et al. Fetal anomalies in obese women: the contribution of diabetes. Obstet Gynecol. 2010; 115(2 Pt 1):290–296. [PubMed: 20093901]

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414(6865):813–820. [PubMed: 11742414]

Buchanan, TA. Pregnancy in prexisting diabetes. In: Harris, MI.; Cowie, CC.; Stern, MP.; Boyko, EJ.; Reiber, GE.; Bennett, PH., editors. Diabetes in America. 2nd. Bethesda, MD: National Institutes of Health; 1995. p. 719-733.

Buchanan TA, Denno KM, Sipos GF, et al. Diabetic teratogenesis: in vitro evidence for a multifactorial etiology with little contribution from glucose per se. Diabetes. 1994; 43:656–660. [PubMed: 8168642]

Cagliero E, Forsberg H, Sala R, et al. Maternal diabetes induces increased expression of extracellular matrix components in rat embryos. Diabetes. 1993; 42:975–980. [PubMed: 7685721]

Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996; 380(6573):435–439. [PubMed: 8602241]

Cederberg J, Eriksson UJ. Decreased catalase activity in malformation-prone embryos of diabetic rats. Teratology. 1997; 56(6):350–357. [PubMed: 9485544]

Cederberg J, Galli J, Luthman H, et al. Increased mRNA levels of Mn-SOD and catalase in embryos of diabetic rats from a malformation-resistant strain. Diabetes. 2000; 49(1):101–107. [PubMed: 10615956]

Chalepakis G, Tremblay P, Gruss P, et al. Pax genes, mutants and molecular function. J Cell Sci Suppl. 1992; 16:61–67. [PubMed: 1363663]

Chan BW, Chan KS, Koide T, et al. Maternal diabetes increases the risk of caudal regression caused by retinoic acid. Diabetes. 2002; 51(9):2811–2816. [PubMed: 12196475]

Chang TI, Horal M, Jain S, et al. Oxidant regulation of gene expression and neural tube development: insights gained from diabetic pregnancy on molecular causes of neural tube defects. Diabetologia. 2003; 46:538–545. [PubMed: 12739027]

Cockroft DL, Coppola PT. Teratogenic effects of excess glucose on head-fold rat embryos in culture. Teratology. 1977; 16:141–146. [PubMed: 412267]

Cohen MM Jr, Shiota K. Teratogenesis of holoprosencephaly. Am J Med Genet. 2002; 109(1):1–15. [PubMed: 11932986]

Correa A, Gilboa SM, Besser LM, et al. Diabetes mellitus and birth defects. Am J Obstet Gynecol. 2008; 199(3):237.e1–237.e9. [PubMed: 18674752]

Cousins L. Congenital anomalies among infants of diabetic mothers: tiology, prevention, prenatal diagnosis. Am J Obstet Gynecol. 1983; 147:333–338. [PubMed: 6353925]

DCCT Research Group. Pregnancy outcomes in the Diabetes Control and Complications Trial. Am J Obstet Gynecol. 1996; 174:1343–1353. [PubMed: 8623868]

Deuchar EM. Embryonic malformations in rats, resulting from maternal diabetes: preliminary observations. J Embryol Exp Morphol. 1977; 41:93–99. [PubMed: 145455]

Doria A, Warram JH, Krolewski AS, et al. Genetic susceptibility to nephropathy in insulin-dependent diabetes: from epidemiology to molecular genetics. Diabetes-Metab Rev. 1995; 11:287–314. [PubMed: 8718493]

Dressler GR, Gruss P. Do multigene families regulate vertebrate development? TIG. 1988; 4:214–219. [PubMed: 2907693]

el-Hage S, Singh SM. Temporal expression of genes encoding free radical-metabolizing enzymes is associated with higher mRNA levels during in utero development in mice. Dev Genet. 1990; 11:149–159. [PubMed: 2379326]

Engstrom E, Haglund A, Eriksson UJ, et al. Effects of maternal diabetes or in vitro hyperglycemia on uptake of palmitic and arachidonic acid by rat embryos. Pediatr Res. 1991; 30:150–153. [PubMed: 1910160]

Epstein DJ, Vogan KJ, Trasler DG, et al. A mutation within intron 3 of the Pax-3 gene produces aberrantly spliced mRNA transcripts in the splotch (Sp) mouse mutant. Proc Natl Acad Sci USA. 1993; 90:532–536. [PubMed: 8421686]

Eriksson UJ. Importance of genetic predisposition and maternal environment for the occurrence of congenital malformations in off-spring of diabetic rats. Teratology. 1988; 37:365–374. [PubMed: 3394110]

Eriksson UJ, Bone AJ, Turnbull DM, et al. Timed interruption of insulin therapy in diabetic BB/E rat pregnancy: effect on maternal metabolism and fetal outcome. Acta Endocrinol (Cph). 1989; 120(6):800–810.

Eriksson UJ, Borg LAH. Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations in vitro. Diabetologia. 1991; 34:325–331. [PubMed: 1864487]

Eriksson UJ, Borg LAH. Diabetes and embryonic malformations: role of substrate-induced free-oxygen radical production for dysmorphogenesis in cultured rat embryos. Diabetes. 1993; 42:411–419. [PubMed: 8432412]

Eriksson UJ, Dahlstrom E, Larsson KS, et al. Increased incidence of congenital malformations in the offspring of diabetic rats and their prevention by maternal insulin therapy. Diabetes. 1982; 31:1–6.

Eriksson UJ, Naeser P, Brolin SE, et al. Increased accumulation of sorbitol in offspring of manifest diabetic rats. Diabetes. 1986; 35:1356–1363. [PubMed: 3770312]

Evers IM, de Valk HW, Visser GH, et al. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. BMJ. 2004; 328(7445):915–919. [PubMed: 15066886]

Fine E, Horal M, Chang T, et al. Evidence that hyperglycemia causes altered gene expression, apoptosis, and neural tube defects in a mouse model of diabetic pregnancy. Diabetes. 1999; 48:2454–2462. [PubMed: 10580436]

Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J Reprod Fertil. 1993; 99(2):673–679. [PubMed: 8107053]

Forsberg H, Borg LA, Cagliero E, et al. Altered levels of scavenging enzymes in embryos subjected to a diabetic environment. Free Radic Res. 1996; 24:451–459. [PubMed: 8804988]

Freinkel N, Cockroft DL, Lewis NJ, et al. The 1986 McCollum Award lecture. Fuel-mediated teratogenesis during early organogene-sis: the effects of increased concentrations of glucose, ketones, or somatomedin inhibitor during rat embryo culture. Am J Clin Nutr. 1986; 44:986–995. [PubMed: 3788845]

Freinkel, N.; Ogata, E.; Metzger, BE. The offspring of the mother with diabetes. In: Rifkin, H.; Porte, D., Jr, editors. Diabetes mellitus: theory and practice. New York: Elsevier Science Publishers; 1990. p. 651-660.

Fuhrmann K, Reiher H, Semmler K, et al. The effect of intensified conventional insulin therapy before and during pregnancy on the malformation rate in offspring of diabetic mothers. Exp Clin Endocrinol. 1984; 83:173–177. [PubMed: 6373320]

Gabbe SG. Pregnancy in women with diabetes mellitus: the beginning. Clinics Perinatol. 1993; 20:507–515.

Gareskog M, Wentzel P. Altered protein kinase C activation associated with rat embryonic dysmorphogenesis. Pediatr Res. 2004; 56(6):849–857. [PubMed: 15496608]

Gilboa SM, Correa A, Botto LD, et al. Association between prepregnancy body mass index and congenital heart defects. Am J Obstet Gynecol. 2010; 202(1):51.e1–51.e10. [PubMed: 19796755]

Goldman AS, Baker L, Piddington R, et al. Hyperglycemic-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. Proc Natl Acad Sci USA. 1985; 82:8227–8231. [PubMed: 3934670]

Goldman AS, Goto MP. Biochemical basis of the diabetic embryopathy. Israel J Med Sci. 1991; 27:469–477. [PubMed: 1960043]

Goulding M, Sterrer S, Fleming J, et al. Analysis of the Pax-3 gene in the mouse mutant splotch. Genomics. 1993; 17:355–363. [PubMed: 8406486]

Goulding MD, Chalepakis G, Deutsch U, et al. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J. 1991; 10:1135–1147. [PubMed: 2022185]

Greene MF, Hare JW, Cloherty JP, et al. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. Teratology. 1989; 39:225–231. [PubMed: 2727930]

Gurdon JB. The generation of diversity and pattern in animal development. Cell. 1992; 68:185–199. [PubMed: 1733498]

Hagay ZJ, Weiss Y, Zusman I, et al. Prevention of diabetes-associated embryopathy by overexpression of the free radical scavenger copper zinc superoxide dismutase in transgenic mouse embryos. Am J Obstet Gynecol. 1995; 173:1036–1041. [PubMed: 7485290]

Hall J, Solehdin F. Folic acid for the prevention of congenital anomalies. Eur J Pediatr. 1998; 157(6): 445–450. [PubMed: 9667396]

Hawthorne G. Preconception care in diabetes. Semin Fetal Neonatal Med. 2005; 10(4):325–332. [PubMed: 15921968]

Hiramatsu Y, Sekiguchi N, Hayashi M, et al. Diacylglycerol production and protein kinase C activity are increased in a mouse model of diabetic embryopathy. Diabetes. 2002; 51:2804–2810. [PubMed: 12196474]

Hod M, Star S, Passonneau JV, et al. Effect of hyperglycemia on sorbitol and myoinositol content of cultured rat conceptus: failure of aldose reductase inhibitors to modify myo-inositol depletion and dysmorphogenesis. Biochem Biophys Res Commun. 1986; 140:974–980. [PubMed: 3096334]

Hogan A, Heyner S, Charon MJ, et al. Glucose transporter gene expression in early mouse embryos. Development. 1991; 113:363–372. [PubMed: 1765007]

Horal M, Zhang Z, Virkamaki A, et al. Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: Involvement in diabetic teratogenesis. Birth Defects Res A Clin Mol Teratol. 2004; 70:519–527. [PubMed: 15329829]

Horii KI, Watanabe GI, Ingalls TH, et al. Experimental diabetes in pregnant mice: prevention of congenital malformations in offspring by insulin. Diabetes. 1966; 15(3):194–204. [PubMed: 5948641]

Horton WE Jr, Sadler TW. Effects of maternal diabetes on early embryogenesis. Alterations in morphogenesis produced by the ketone body, B-hydroxybutyrate. Diabetes. 1983; 32(7):610–616. [PubMed: 6862109]

Hua H, Munk S, Goldberg H, et al. High glucose-suppressed endothelin-1 Ca2+ signaling via NADPH oxidase and diacylglycerol-sensitive protein kinase C isozymes in mesangial cells. J Biol Chem. 2003; 278(36):33951–33962. [PubMed: 12821678]

Hunter, ES3rd; Sadler, TW.; Wynn, RE. A potential mechanism of DL-beta-hydroxybutyrate-induced malformations in mouse embryos. Am J Physiol. 1987; 253(1 Pt 1):E72–E80. [PubMed: 3111272]

Hunter ESI, Sadler TW. Fuel-mediated teratogenesis: biochemical effects of hypoglycemia during neurulation in mouse embryos in vitro. Am J Physiol. 1989; 257:E269–E276. [PubMed: 2764103]

Ishibashi M, Akazawa S, Sakamaki H, et al. Oxygen-induced embryopathy and the significance of glutathione-dependent antioxidant system in the rat embryo during early organogenesis. Free Radic Biol Med. 1997; 22:447–454. [PubMed: 8981036]

Jain SK, Levine SN, Duett J, et al. Reduced vitamin E and increased lipofuscin products in erythrocytes of diabetic rats. Diabetes. 1991; 40(10):1241–1244. [PubMed: 1936587]

Jain SK, McVie R, Duett J, et al. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes. 1989; 38:1539–1543. [PubMed: 2583378]

Jonasson JM, Brismar K, Sparen P, et al. Fertility in women with type 1 diabetes: a population-based cohort study in Sweden. Diabetes Care. 2007; 30(9):2271–2276. [PubMed: 17563340]

Joslin EP. Pregnancy and diabetes mellitus. Boston Med Surg J. 1915; 23:841–849.

Kalter H. Case reports of malformations associated with maternal diabetes: history and critique. Clin Genet. 1993; 43(4):174–179. [PubMed: 8330451]

Kessel M, Gruss P. Murine developmental control genes. Science. 1990; 249:374–379. [PubMed: 1974085]

Kitzmiller JL, Cloherty JP, Younger MD, et al. Diabetic pregnancy and perinatal morbidity. Am J Obstet Gynecol. 1978; 131:560–580. [PubMed: 354386]

Kondoh H, Lleonart ME, Gil J, et al. Glycolytic enzymes can modulate cellular life span. Cancer Res. 2005; 65(1):177–185. [PubMed: 15665293]

Kondoh H, Lleonart ME, Nakashima Y, et al. A high glycolytic flux supports the proliferative potential of murine embryonic stem cells. Antioxid Redox Signal. 2007; 9(3):293–299. [PubMed: 17184172]

Kotch LE, Iyer NV, Laughner E, et al. Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. Dev Biol. 1999; 209(2):254–267. [PubMed: 10328919]

Kucera J. Rate and type of congenital anomalies among offspring of diabetic women. J Reprod Med. 1971; 7:61–70.

Kumar SD, Dheen ST, Tay SS, et al. Maternal diabetes induces congenital heart defects in mice by altering the expression of genes involved in cardiovascular development. Cardiovasc Diabetol. 2007; 6:34. [PubMed: 17967198]

Lawrence JM, Contreras R, Chen W, et al. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. Diabetes Care. 2008; 31(5):899–904. [PubMed: 18223030]

Leung MB, Choy KW, Copp AJ, et al. Hyperglycaemia potentiates the teratogenicity of retinoic acid in diabetic pregnancy in mice. Dia-betologia. 2004; 47(3):515–522.

Li R, Chase M, Jung SK, et al. Hypoxic stress in diabetic pregnancy contributes to impaired embryo gene expression and defective development by inducing oxidative stress. Am J Physiol Endocrinol Metab. 2005; 289(4):E591–E599. [PubMed: 15928021]

Li R, Thorens B, Loeken MR, et al. Expression of the gene encoding the high Km glucose transporter 2 by the early postimplantation mouse embryo is essential for neural tube defects associated with diabetic embryopathy. Diabetologia. 2007; 50(3):682–689. [PubMed: 17235524]

Loffredo CA, Wilson PD, Ferencz C, et al. Maternal diabetes: an independent risk factor for major cardiovascular malformations with increased mortality of affected infants. Teratology. 2001; 64(2): 98–106. [PubMed: 11460261]

Ma W, Sung HJ, Park JY, et al. A pivotal role for p53: balancing aerobic respiration and glycolysis. J Bioenerg Biomembr. 2007; 39(3):243–246. [PubMed: 17551815]

Maltepe E, Schmidt JV, Baunoch D, et al. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature. 1997; 386:403–407. [PubMed: 9121557]

Melvin EC, George TM, Worley G, et al. Genetic studies in neural tube defects. NTD Collaborative Group. Pediatr Neurosurg. 2000; 32(1):1–9. [PubMed: 10765131]

Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin A1c in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med. 1981; 304:1331–1334. [PubMed: 7012627]

Mills JL. Malformations in infants of diabetic mothers. Teratology. 1982; 25:385–394. [PubMed: 7051398]

Mills JL, Baker L, Goldman AS, et al. Malformations in infants of diabetic mothers occur before the seventh gestational week: implications for treatment. Diabetes. 1979; 28:292–293. [PubMed: 437367]

Mills JL, Simpson JL, Driscoll SG, et al. Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. New Engl J Med. 1988; 319:1617–1623. [PubMed: 3200277]

Miodovnik M, Mimouni F, Siddiqi TA, et al. Spontaneous abortions in repeat diabetic pregnancies: relationship with glycemic control. Obstet Gynecol. 1990; 75:75–77. [PubMed: 2296426]

Miodovnik M, Mimouni F, Tsang RC, et al. Glycemic control and spontaneous abortion in insulin-dependent diabetic women. Obstet Gynecol. 1986; 68:366–369. [PubMed: 3737060]

Mokdad AH, Bowman BA, Ford ES, et al. The continuing epidemics of obesity and diabetes in the United States. JAMA. 2001; 286(10):1195–1200. [PubMed: 11559264]

Moore LL, Singer MR, Bradlee ML, et al. A prospective study of the risk of congenital defects associated with maternal obesity and diabetes mellitus. Epidemiology. 2000; 11(6):689–694. [PubMed: 11055631]

Moretti A, Caron L, Nakano A, et al. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. Cell. 2006; 127(6):1151–1165. [PubMed: 17123592]

Morgan SC, Lee H-Y, Relaix F, et al. Cardiac outflow tract septation failure in Pax3-deficient embryos is due to p53-dependent regulation of migrating cardiac neural crest. Mech Dev. 2008a; 125(9–10): 757–767. [PubMed: 18672055]

Morgan SC, Relaix F, Sandell LL, et al. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. Birth Defects Res A Clin Mol Teratol. 2008b; 82(6):453–463. [PubMed: 18435457]

MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the MRC Vitamin Study. Lancet. 1991; 338:132–137.

Muller YL, Yueh YG, Yaworsky PJ, et al. Caudal dysgenesis in Islet-1 transgenic mice. FASEB J. 2003; 17(10):1349–1351. [PubMed: 12738808]

New DA, Coppola PT, Terry S, et al. Culture of explanted rat embryos in rotating tubes. J Reprod Fertil. 1973; 35(1):135–138. [PubMed: 4742154]

Oyama K, Sugimura Y, Murase T, et al. Folic acid prevents congenital malformations in the offspring of diabetic mice. Endocr J. 2008; 56(1):29–37. [PubMed: 18781038]

Paddenberg R, Ishaq B, Goldenberg A, et al. Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature. Am J Physiol Lung Cell Mol Physiol. 2003; 284(5):L710–L719. [PubMed: 12676762]

Pani L, Horal M, Loeken MR, et al. Polymorphic susceptibility to the molecular causes of neural tube defects during diabetic embryopathy. Diabetes. 2002a; 51(9):2871–2874. [PubMed: 12196484]

Pani L, Horal M, Loeken MR, et al. Rescue of neural tube defects in Pax-3-deficient embryos by p53 loss of function: implications for Pax-3- dependent development and tumorigenesis. Genes Dev. 2002b; 16(6):676–680. [PubMed: 11914272]

Pavlinkova G, Salbaum JM, Kappen C, et al. Maternal diabetes alters transcriptional programs in the developing embryo. BMC Genomics. 2009; 10:274. [PubMed: 19538749]

Phelan SA, Ito M, Loeken MR, et al. Neural tube defects in embryos of diabetic mice: role of the Pax-3 gene and apoptosis. Diabetes. 1997; 46(7):1189–1197. [PubMed: 9200655]

Reece EA, Ji I, Wu YK, et al. Characterization of differential gene expression profiles in diabetic embryopathy using DNA microarray analysis. Am J Obstet Gynecol. 2006; 195(4):1075–1080. [PubMed: 17000240]

Robert MF, Neff RK, Hubbell JP, et al. Association between maternal diabetes and the respiratory-distress syndrome in the newborn. N Engl J Med. 1976; 294(7):357–360. [PubMed: 1246288]

Rodesch F, Simon P, Donner C, et al. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol. 1992; 80(2):283–285. [PubMed: 1635745]

Roest PA, van Iperen L, Vis S, et al. Exposure of neural crest cells to elevated glucose leads to congenital heart defects, an effect that can be prevented by N-acetylcysteine. Birth Defects Res A Clin Mol Teratol. 2007; 79(3):231–235. [PubMed: 17183584]

Sadler T. Effects of maternal diabetes on early embryogenesis: II. Hyperglycemia-induced exencephaly. Teratology. 1980a; 21(3):349–356. [PubMed: 7455924]

Sadler TW. Effects of maternal diabetes on early embryogenesis: I. The teratogenic potential of diabetic serum. Teratology. 1980b; 21(3):339–347. [PubMed: 7455923]

Sakamaki H, Akazawa S, Ishibashi M, et al. Significance of glutathione-dependent antioxidant system in diabetes-induced embryonic malformations. Diabetes. 1999; 48:1138–1144. [PubMed: 10331421]

Sato N, Sugimura Y, Hayashi Y, et al. Identification of genes differentially expressed in mouse fetuses from streptozotocin-induced diabetic pregnancy by cDNA subtraction. Endocr J. 2008; 55(2): 317–323. [PubMed: 18323671]

Sauka-Spengler T, Bronner-Fraser M. Development and evolution of the migratory neural crest: a gene regulatory perspective. Curr Opin Genet Dev. 2006; 16(4):360–366. [PubMed: 16793256]

Shaw GM, Velie EM, Schaffer D, et al. Risk of neural tube defect-affected pregnancies among obese women. JAMA. 1996; 275:1093–1096. [PubMed: 8601928]

Shepard TH, Tanimura T, Robkin MA, et al. Energy metabolism in early mammalian embryos. Symp Soc Dev Biol. 1970; 29:42–58. [PubMed: 4950153]

Shum L, Sadler TW. Biochemical basis for D,L,-beta-hydroxybutyrate-induced teratogenesis. Teratology. 1990; 42:553–563. [PubMed: 2278030]

Siman CM, Eriksson UJ. Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. Diabetologia. 1997a; 40:1416–1424. [PubMed: 9447949]

Siman CM, Eriksson UJ. Vitamin E decreases the occurrence of malformations in the offspring of diabetic rats. Diabetes. 1997b; 46:1054–1061. [PubMed: 9166679]

Siman CM, Gittenberger-De Groot AC, Wisse B, et al. Malformations in offspring of diabetic rats: morphometric analysis of neural crest-derived organs and effects of maternal vitamin E treatment. Teratology. 2000; 61(5):355–367. [PubMed: 10777831]

Sivan E, Lee YC, Wu YK, et al. Free radical scavenging enzymes in fetal dysmorphogenesis among offspring of diabetic rats. Teratology. 1997; 56(6):343–349. [PubMed: 9485543]

Smoak IW, Sadler TW. Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro. Am J Obstet Gynecol. 1990; 163:619–624. [PubMed: 2386154]

Styrud J, Eriksson UJ. Effects of D-glucose and beta-hydroxybutyric acid on the in vitro development of (pre)chondrocytes from embryos of normal and diabetic rats. Acta Endocrinol. 1990; 122:487–498. [PubMed: 2333734]

Styrud J, Eriksson UJ. In vitro effects of glucose and growth factors on limb bud and mandibular arch chondrocytes maintained at various serum concentrations. Teratology. 1991; 44:65–75. [PubMed: 1957265]

Sussman I, Matschinsky FM. Diabetes affects sorbitol and myoinositol levels of neuroectodermal tissue during embryogenesis in rat. Diabetes. 1988; 37:974–981. [PubMed: 3384192]

Suzuki N, Svensson K, Eriksson UJ, et al. High glucose concentration inhibits migration of rat cranial neural crest cells in vitro. Diabe-tologia. 1996; 39:401–411.

Takao Y, Akazawa S, Matsumoto K, et al. Glucose transporter gene expression in rat conceptus during high glucose culture. Diabetologia. 1993; 36(8):696–706. [PubMed: 8405736]

Thorens B. Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. Am J Physiol. 1996; 270:G541–G553. [PubMed: 8928783]

Tian H, Hammer RE, Matsumoto A, et al. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. Genes Dev. 1998; 12:3320–3324. [PubMed: 9808618]

Trocino RA, Akazawa S, Ishibashi M, et al. Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. Diabetes. 1995; 44:992–998. [PubMed: 7622006]

Trocino RA, Akazawa S, Takino H, et al. Cellular-tissue localization and regulation of the GLUT-1 protein in both the embryo and the visceral yolk sac from normal and experimental diabetic rats during the early postimplantation period. Endocrinology. 1994; 134(2):869–878. [PubMed: 8299581]

Uldry M, Ibberson M, Hosokawa M, et al. GLUT2 is a high affinity glucosamine transporter. FEBS Lett. 2002; 524(1–3):199–203. [PubMed: 12135767]

Viana M, Herrera E, Bonet B, et al. Terotogenic effects of diabetes mellitus in the rat: prevention by vitamin E. Diabetologia. 1996; 39:1041–1046. [PubMed: 8877287]

Warburg O. On respiratory impairment in cancer cells. Science. 1956a; 124(3215):269–270. [PubMed: 13351639]

Warburg O. On the origin of cancer cells. Science. 1956b; 123(3191):309–314. [PubMed: 13298683]

Watanabe G, Ingalls TH. Congenital malformations in the offspring of alloxan-diabetic mice. Diabetes. 1963; 12:66–72. [PubMed: 13999085]

Watkins ML, Rasmussen SA, Honein MA, et al. Maternal obesity and risk for birth defects. Pediatrics. 2003; 111(5 Part 2):1152–1158. [PubMed: 12728129]

Wentzel P, Eriksson UJ. Insulin treatment fails to abolish the teratogenic potential of serum from diabetic rats. Eur J Endocrinol. 1996; 134:459–466. [PubMed: 8640298]

Wentzel P, Eriksson UJ. A diabetes-like environment increases malformation rate and diminishes prostaglandin E(2) in rat embryos: reversal by administration of vitamin E and folic acid. Birth Defects Res A Clin Mol Teratol. 2005; 73(7):506–511. [PubMed: 15959876]

Wentzel P, Gareskog M, Eriksson UJ, et al. Decreased cardiac glutathione peroxidase levels and enhanced mandibular apoptosis in malformed embryos of diabetic rats. Diabetes. 2008; 57(12): 3344–3352. [PubMed: 18728230]

Wentzel P, Thunberg L, Eriksson UJ, et al. The teratogenic effect of diabetic serum is prevented by supplementation of superoxide dismutatse and N-acetylcysteine in rat embryo culture. Diabetologia. 1997; 40:7–14. [PubMed: 9028712]

Wentzel P, Welsh N, Eriksson UJ, et al. Developmental damage, increased lipid peroxidation, diminished cyclooxygenase-2 gene expression, and lowered prostaglandin E_2 levels in rat embryos exposed to a diabetic environment. Diabetes. 1999; 48:813–820. [PubMed: 10102698]

Werler MW, Louik C, Shapiro S, et al. Prepregnant weight in relation to risk of neural tube defects. JAMA. 1996; 275:1089–1092. [PubMed: 8601927]

White, P. Pregnancy complicating diabetes. In: Joslin, EP.; Root, HF.; White, P.; Marble, A., editors. The treatment of diabetes mellitus. Philadelphia: Lea & Febiger; 1937. p. 633-638.

White P. Pregnancy complicating diabetes. Am J Med. 1949; 7(5):609–616. [PubMed: 15396063]

WHO/IDF Europe. Diabetes care and research in Europe: the Saint Vincent declaration. Diabet Med. 1990; 7(4):360. [PubMed: 2140091]

Widness JA, Goldman AS, Susa JB, et al. Impermeability of the rat placenta to insulin during organogenesis. Teratology. 1983; 28(3):327–332. [PubMed: 6364437]

Yao TP, Oh SP, Fuchs M, et al. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell. 1998; 93:361–372. [PubMed: 9590171]

Ylinen K, Aula P, Stenman UH, et al. Risk of minor and major fetal malformations in diabetics with high haemoglobin A1c values in early pregnancy. Br Med J (Clin Res Ed). 1984; 289(6441):345–346.

Zabihi S, Eriksson UJ, Wentzel P, et al. Folic acid supplementation affects ROS scavenging enzymes, enhances Vegf-A, and diminishes apoptotic state in yolk sacs of embryos of diabetic rats. Reprod Toxicol. 2007; 23(4):486–498. [PubMed: 17482424]

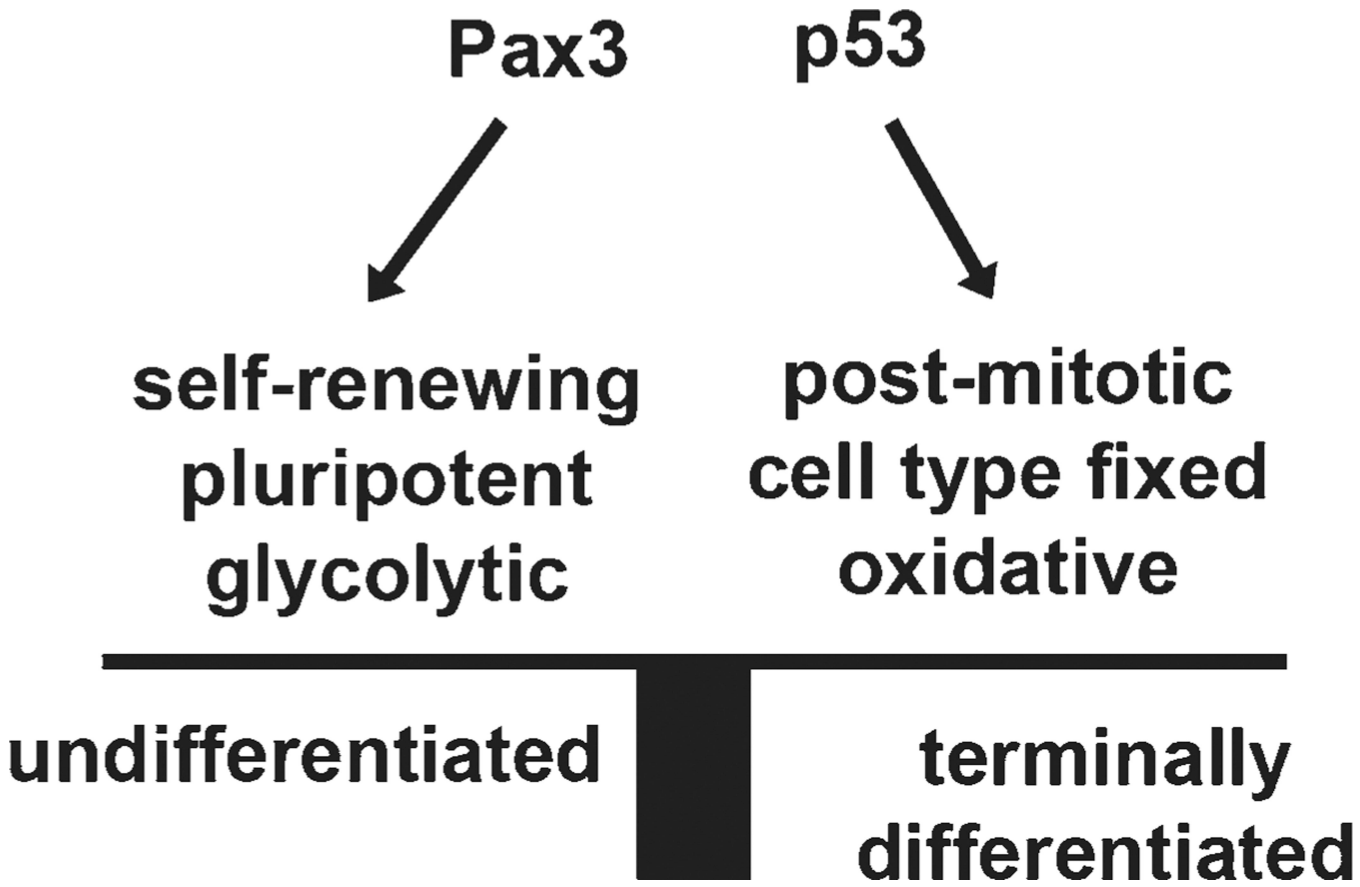

Figure 1.

Balance of p53 activity by *Pax3*. Before differentiation, early embryonic cells are proliferative (self-renewing), have the potential to differentiate into many cell types (pluripotent), and fuel metabolism is predominantly anaerobic (glycolytic). Upon terminal differentiation, cells are nonproliferative (post-mitotic) and can no longer differentiate into other cell types (cell type fixed), and fuel metabolism is predominantly aerobic (oxidative). Activation of p53 during differentiation promotes a differentiated phenotype. *Pax3* may be necessary to balance p53 activity by preserving undifferentiated cell characteristics until the stage of development at which terminal differentiation should occur. There may be other regulators of an undifferentiated cell phenotype in the progenitor cells of other embryonic organs besides neuroepithelium and neural crest.

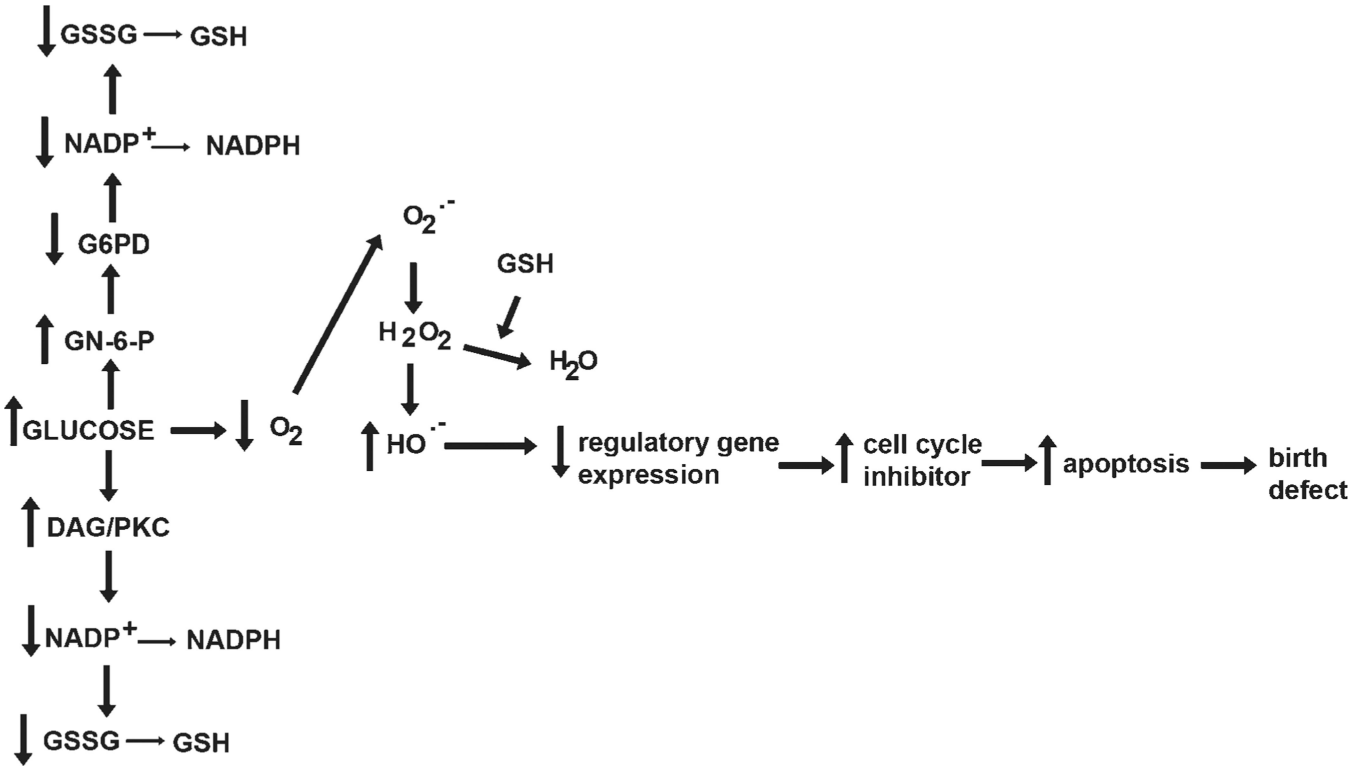

Figure 2.

Biochemical/molecular pathway by which maternal hyperglycemia may cause birth defects. Excess glucose transported to the embryo is metabolized. Increased glycolytic flux stimulates glucosamine-6-PO_4 synthesis (GN-6-P), which inhibits G6PD activity, which decreases NADPH synthesis, which decreases GSH synthesis. Increased glycolytic flux also stimulates the DAG/PKC pathway, which may also inhibit NADPH and GSH synthesis. Increased glucose metabolism, some of which is aerobic, increases oxygen consumption faster than it can be delivered, which stimulates superoxide production. Increased superoxide production increases hydrogen peroxide production, which, due to decreased GSH availability, leads to increased production of the hydroxyl radical, rather than water. The resulting oxidative stress inhibits expression of critical regulatory genes (such as *Pax3*), which leads to derepression of a cell cycle regulator (such as p53), which leads to apoptosis. Loss of a critical mass of progenitor cells by a newly forming organ or structure will lead to a birth defect.