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REVIEW

Research Progress on the Experimental Animal Model of Gestational Diabetes Mellitus

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Abstract: Gestational diabetes mellitus (GDM) refers to different degrees of abnormal glucose metabolism during pregnancy, where blood glucose levels do not reach the level of overt diabetes, accounting for 80–90% of pregnancy with hyperglycemia. Hyperglycemia affects the pregnancy process, leading to a series of adverse maternal outcomes that have a profound impact on the future of the offspring. The establishing of an appropriate GDM model will provide theoretical basis for study GDM pathogenesis involves, the choice of resonable drugs and the observation the disease trends and outcomes. At present, there are many methods for establishing experimental GDM animal models and animal choices. This paper examines the different GDM models and their induction methods.

Keywords: gestational diabetes mellitus, animal model, chemical induction, high-sugar and high-fat diet, genetic manipulation

Introduction

With the gradual improvement of people's living standards around the world, the incidence of diabetes has increased year on year, with the number of hyperglycemia in pregnancy increasing significantly. The prevalence of GDM varies greatly between countries and even between regions within a country. Epidemiological studies show an incidence of more than 9% GDM in the United States and between 3.0% and 21.2% in Asian countries.[1] Hyperglycemia during pregnancy will cause various complications for pregnant women and fetuses, even endanger life and health, and will have a close impact on the outcome of pregnancy. It adversely affects the long-term blood glucose and blood lipid levels in the offspring. However, the pathogenesis of GDM has not yet been fully clarified. The establishment of ideal GDM animal models will therefore provide a theoretical basis for early detection and prevention of GDM and subsequent clinical medication and drug evaluation, in order to better control the occurrence and development of GDM and better improvement of the outcome of mothers and children.

In recent years, the main methods for establishing GDM animal models include experimental animal model, spontaneous animal model and transgenic animal model. Currently, many researchers select experimental animal models that is they use physical, chemical, and biological pathogenic factors to cause certain damage to animal tissues, organs and even the body as a whole to induce some functions, metabolism, or toxic side effects similar to those of human diseases. Researchers have adopted a variety of methods in the GDM study, and have used various animals for the experiment, including dogs, sheep, pigs, primates, and

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rodents.[2] This study provides a brief review of the animals used in current researches, and the most commonly used methods of induction.

Introduction of GDM

GDM first appeared in the medical literature in the early 1970s. In 1979, the World Health Organization (WHO) identified GDM as an independent type of diabetes.[3] The state of hyperglycemia during pregnancy is divided into gestational diabetes mellitus (GDM), overt diabetes mellitus (ODM) and pre-gestational diabetes mellitus (PGDM). Among these hyperglycemic variations, GDM refers to abnormal glucose metabolism in which blood glucose does not reach the level of overt diabetes during pregnancy, accounting for 80–90% of hyperglycemia during pregnancy.[4] If the control of blood glucose in pregnant women is not good, it will not only increase the maternal risk of preeclampsia, abortion, stillbirth, premature birth, but will also lead to neonatal hypoglycemia, hyperbilirubinemia, overweight and other situations, and even cause long-term adverse effects on the blood glucose and the blood lipid of the offspring, and even endanger life and health in serious cases.[5] At present, the diagnostic criteria for GDM in the different guidelines are different, as shown in Table 1.[4]

Relationship Between Animal Model and GDM

The animal model of human disease refers to the animal with human disease simulation performance established in various medical scientific research. There are three main animal models mentioned above: experimental animal model, spontaneous animal model and transgenic animal model. The spontaneous animal diseases are very similar to the corresponding human diseases in the occurrence, and development that occurs under natural conditions, so the application value of spontaneous animal models is very high, but the source of such models is limited and it is impossible to apply them in large quantities.[6] Transgenic animal models are expensive with a long reproduction time, but the effective control of a single factor makes the experiment more scientific, rigorous, and reliable. The transgenic animal models can therefore help to find the cause inaccurately, and are more suitable for the mechanism of diabetes or drug targeted research. As a result, in the current research field of gestational diabetes, the method of constructing experimental animal models is

often used, that is, researchers use physical, chemical and biological pathogenic factors to cause certain damage to the tissues, organs and even the entire body of animals, and some functions, metabolism or side effects similar to human diseases. Experimental animal models have the characteristics of high reproducibility, appropriate conditions, short experimental period, and easy control of disease characteristics, therefore they are commonly used in medical research.

At present, the research focus on the pathogenesis of GDM is mainly reflected in the following aspects: insulin resistance; insulin antagonistic hormone release; abnormal insulin signal transduction pathway; cells, inflammatory factors; genetic factors. In the field which has been developed, animal experiments have made an indelible contribution. For example, when it comes to insulin resistance, through the study of rodents, we find that β-cell changes in pregnancy are similar to human beings. In other words, pancreatic β cells use a variety of adaptive mechanisms in early pregnancy, including increasing the quality, quantity and insulin secretion capacity, increasing the number of beta cells, and strengthening their functions to cope with the increase of insulin resistance in late pregnancy.[7,8] Although the mechanism is not the same, it provides guidance for exploring the inducement and mechanism of human GDM, and also provides a signal for early prevention and late follow-up. Establishing an ideal animal model of GDM is helpful in better exploring the mechanism, causes and development process of GDM, helping to predict, prevent and treat GDM, and provide experimental basis for new methods of treatment for Chinese and Western medicine. The specific animal types and construction methods of the model are presented below and the criteria for successful modeling are shown in Table 2.

Animal Model Analysis
Rodents
Chemical Induction
Streptozotocin (STZ)

Streptozotocin is an antibiotic extracted from streptomycin, which has a highly selective toxic effect on the islet β cells of experimental animals, which can make insulin secretion insufficient and increase blood glucose, and is therefore one of the most commonly used drugs in animal models for diabetic induction.[9] Since Rakieten et al first reported the effect of STZ on DM in 1963, many researchers used different doses of STZ to replicate DM animal

Table 1 Different Guidelines for GDM Diagnostic Criteria

Guidelines	Diagnostic criteria			
	Screening time	FPG (mmol/L)	75g OGTT 1h (mmol/L)	75g OGTT 2h (mmol/L)
ADA guidelines (2020)[55]	Gestation 24-28 weeks	5.1-6.9	10.0-11.0	8.5-11.0
ACOG guidelines (2018)[56]	The same as ADA guidelines			
FIGO guidelines (2015)[57]	Any time during pregnancy	5.9-6.1	10.0-11.0	8.5-11.0
IADPSG guidelines (2010)[58]	The first pregnancy test	5.1-6.9		
	Gestation 24-28 weeks	5.1-6.9	10.0-11.0	8.5-11.0
WHO guidelines (2014)[59]	The same as FIGO guidelines			
CDS guidelines (2017)[60]	Any time during pregnancy	5.1-6.9	10.0-11.0	8.5-11.0
Chinese Society of Obstetrics and Gynecology guidelines (2017)[61]	Gestation 24-28 weeks and after 28 weeks	5.1-6.9	10.0-11.0	8.5-11.0

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; ADA, American Diabetes Association; IADPSG, International Association of Diabetes and Pregnancy Study Groups; WHO, World Health Organization Guideline; ACOG, American College of Obstetricians and Gynecologists; CDS, Chinese Diabetes Society.

models, and this method is still widely used in DM-related animal studies.[10] STZ can induce a stable and rapid DM animal models, has low animal category selectivity even in small doses, and relatively has low tissue toxicity, and good stability. It may cause diabetes by damaging islet β cells with nitric oxide (NO) and free radicals, impairing the function of islet β cells, and reducing insulin synthesis. In the same species, the degree of cell damage caused by STZ depends on the amount of STZ, ie, low-dose STZ induces β cell apoptosis, and high-dose STZ causes β cell necrosis, which is very similar to human diabetes pathogenesis.[11] Different doses are suitable for the construction of T1DM and T2DM models. The model induced by single large dose of STZ is more consistent with T1DM, while the model induced by small dose STZ is more consistent with T2DM.[12] Based on this modeling method, it also has a certain guiding role in the construction of the GDM model. However, due to the embryonic factors caused by pregnancy, the application of STZ dose in the GDM model is limited, so the STZ dosage will also be discussed in the modeling process.

Previously, Hao et al had conducted experiments to compare the modeling rate and stability of the one-time high-dose STZ injection and multiple low-dose injection methods for the development of the gestational diabetic mice model. The membrane rate of 30mg/kg.bw (body weight) was 70%. Compared with the control group, the state of hyperglycemia lasted a long time and the weight

gain decreased significantly. The conclusion was STZ 30mg/kg.bw, intraperitoneal injection 24 hours apart, three times injection is a relatively good method for establishing a model of gestational diabetes mice with has good stability.[13] Li et al randomly divided 46 SD pregnant rats into low-dose, medium-dose and high-dose STZ groups in the preparation of the GDM models, injected 35, 45, 60 mg/kg.bw of STZ intraperitoneally on the day of pregnancy confirmation, measured the fasting blood glucose on the 3rd, 9th, 14th, and 19th days of pregnancy, observed the changes of bodyweight, water intake, food intake and urine volume. Pregnant rats in the 45 mg/kg STZ group were found to have 3 P's of diabetes after medication, with the highest modeling success rate of 83.3% and the lowest rate of negative conversion.[14] Meanwhile, in recent years, experimental data showed that the GDM rat model could be established by one-time intraperitoneal injection of 25 mg/kg.bw STZ to SD pregnant rats, the blood glucose of pregnant rats after modeling fluctuated between 6.7 and 8.3 mmol/L, and the modeling success rate was 100%. In an ideal clinical situation, overly high blood glucose levels during pregnancy can often be controlled, and the limited growth rate of the offspring in the uterus is significantly reduced, so at present, a single small dose 25 mg/kg.bw intraperitoneal injection of STZ in the establishment of a pregnant rat model is more in line with the clinical characteristics of human GDM and is one of the ideal methods for

Table 2 Summary of Animal Model Construction

Strategy and Method	Animal Species	Author and Year of Literature	Experimental Dose or Operation Method	Blood Glucose Diagnostic Criteria of Model	Other Diagnostic Criteria of Model	Major Advantage	Major Disadvantage	Research Direction	Notes
PPX	Dogs	Markowitz et al,1927[45]	Total pancreatectomy in the late pregnancy	Blood glucose increased (No specific criteria)	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Can be used when other methods are not applicable	Difficult to operate	Construction of ancient GDM model	Rarely used in recent years
Chemical Induction (STZ)	Rats	Jia Li et al,2009[14], Jingyi Zhuang et al,2014[15]	one-time intraperitoneal injection of 25 mg/kg STZ	Day 4 of gestation: FPG ≥ 11.1mmol / L; Non -FPG ≥ 6.7mmol / L	Weight loss, increased water and food intake, increased urine output; Aggravation of insulin resistance phenomenon	Small dosage and low toxicity	Hurting more to mother and offspring	Suitable for studying symptoms, prognosis and drug effect test, but not suitable for studying the mechanism of GDM	Most of the drug-induced models were consistent with hyperglycemia during pregnancy, but blood glucose did not recover at the end of pregnancy
Chemical Induction (STZ)	Mice	Qiuyun Hao et al,2019[13]	three-time intraperitoneal per day of 30mg /kg STZ	FPG > 5.1mmol / L	Weight loss, increased water and food intake, increased urine output	Convenient modeling method	Expensive		
Chemical Induction (STZ)	Sheep	Dickinson JE et al,1991[48], Lips JP et al,1988[50]	STZ injection every 4 days from day 85 to day 90 of pregnancy	Blood glucose increased (No specific criteria)	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Effective	High toxicity	Suitable for studying symptoms, prognosis and drug effect test, but not suitable for studying the mechanism of GDM	No specific experimental data standard, pay attention to the follow-up negative situation
Chemical Induction (STZ)	Pigs	Ezekwe MO et al,1984[52]	STZ injection on day 80 of pregnancy	Blood glucose increased (No specific criteria)	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Effective	Leading to miscarriage		

Model	Animal	Reference	Method	Diagnostic Criteria	Symptoms	Advantages	Disadvantages	Applicability	Notes
Chemical Induction (ALX)	Rats	Jianhua Liang et al,2006[18]; Yingchun Shui et al,2015[19]	one-time intraperiton eal injection 40 mg / kg ALX	Day4 gestation: FPG >11.1mmo l/L, Random blood glucose > 17.92 mmol/L	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Cheap, High success rate, low mortality rate	High toxicity	Suitable for studying symptomatology, prognosis and drug experiment, but not suitable for studying the specific mechanism of GDM	No mice GDM model was established by ALX
Chemical Induction (ALX)	Sheep	Miodovnik M et al,1989[49]	ALX infusion in early pregnancy	Hyperglycemia	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Effective	High toxicity	Suitable for studying symptomatology and drug experiment, but not suitable for studying the specific mechanism of GDM	No specific experimental data for large animals
Chemical Induction (ALX)	Pigs	Ramsay TG et al,1994[51]	ALX injection on the 75th day of pregnancy	Hyperglycemia	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Effective	Leading to miscarriage	Suitable for studying symptomatology, but not suitable for studying the specific mechanism of GDM	No specific experimental data for large animals
Single HFSD	Rodents	Jie Zhang et al, 2013[21]; Huang B et al, 2019[22]; Vuong B et al, 2017[23]	HFSD	Day 18 of gestation: FPG $\geq$ 5.1mmol / L	Insulin resistance increase	Little harm to mother and offspring Low cost of replication Short replication cycle	Single mechanism	Suitable for studying the changes and symptoms of disease process	
Single HFSD	Dogs	Mary Courtney Moore et al, 2011[21]	HFSD feeding at 4-5 weeks of gestation	Hyperglycemia	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Little harm to mother and offspring	Single mechanism	Suitable for studying the changes and symptoms of disease	

(*Continued*)

Table 2 (Continued).

Strategy and Method	Animal Species	Author and Year of Literature	Experimental Dose or Operation Method	Blood Glucose Diagnostic Criteria of Model	Other Diagnostic Criteria of Model	Major Advantage	Major Disadvantage	Research Direction	Notes
HFSD	Sheep	Ford SP et al, 2009[47]	HFSD feeding in pregnancy	Hyperglycemia	Weight loss, increased water and food intake, increased urine output (No specific criteria)	Little harm to mother and offspring	Single mechanism		
HFSD combined with STZ	Rodents	Lin Chen et al, 2016[30], Mengyu Tang et al, 2018[31]	HFSD feeding for 8 weeks; Day 1 of gestation: intraperitoneal 25 g / kg STZ	Random blood glucose ≥ 11.1 mmol / L; Three days after STZ injection FPG 6.67–16.67 mmol /L	Weight increase	Hurt little to mother and offspring	Leading to abnormal increase of blood glucose	Study the changes and symptoms of disease process	
Hypertonic glucose injection	Rats	Linxia Li et al, 2006[20]	Continuous infusion of 30% glucose at a rate of 2 ml / h	Hyperglycemia	Food intake increase	Can be used when other methods are not available	Cumbersome infusion method	Suitable for studying symptoms, maternal prognosis and offspring	
Multifactor synthesis	Rats	Abdul AS et al, 2016[33]	Through the regulation of nutrition, chemical drug induction and lifestyle control and other factors	Hyperglycemia	Insulin decrease	Similar to human GDM mechanism	Complex program and time-consuming	Suitable to score the influencing factors of GDM and conduct directional research on the mechanism	
Genetic manipulation	Rodents		Many ways	Hyperglycemia	No specific criteria	Scientific, rigorous and effective	Time-consuming, difficult, strict on species	Suitable for mechanism or drug targeting research	Specific analysis is shown in Table 3

establishing experimental GDM rat models.[15] Although STZ is expensive and has more applications in the construction of DM animal models, it has the advantages of small dosage, simple modeling method, low drug toxicity, and low mortality of modeling animals.

Alloxan (ALX)

Alloxan is a β cell cytotoxic agent synthesized by the oxidation of uric acid. It is chemically unstable compound that generates oxygen free radicals and reacts with the SH group (mainly cysteine). It induces hyperpolarization of the cytoplasmic membrane potential, inhibits glucose-induced membrane impulses, which in turn inhibits voltage-dependent $Ca2^+$ influx, destroys intracellular free $Ca2+$ concentration fluctuations, and affects the cell metabolism. Alloxan selectively damages the pancreatic islet β cells of various animals because the animals have high levels of SH groups in islet β cells, which impair the function of insulin-producing β-cells resulting in hyperglycemia and diabetes.[16] Alloxan also damages β cells by inhibiting the expression of glucose transfer factor and glucokinase mRNA, which are necessary substances for the transport and metabolism of glucose.[17]

The effects of ALX on GDM induction are different with the route and dose in animals. Pregnant rats were randomly divided into groups administered intravenously and intraperitoneally. Pregnant rats were also divided randomly as intravenous high dose 60 mg/kg.bw, medium dose 40 mg/kg.bw and low dose 20 mg/kg.bw subgroups. The intraperitoneal administered pregnant rats were divided into high dose 120 mg/kg.bw and medium dose 100 mg/kg.bw subgroups. The effects of different doses of ALX and different routes of drug administration on the modeling success rate have been studied. It was observed that the 40 mg/kg.bw intravenous injection of ALX had a good modeling success rate of 91.7%, a low mortality rate of 8.3%, and a low negative conversion rate of 9.1%, and that the best dose for induction of GDM was chosen in pregnant rat models.[18] ALX is highly toxic inducing great damage to animals for modeling, but is inexpensive in establishing a stable model with a high modeling success rate and low mortality. Therefore, alloxan-induced animal diabetes models are widely used in the development and selection of hypoglycemic drugs.[19]

Hypertonic Glucose Injection

Continuous intravenous injection of hypertonic glucose induces hyperglycemia during pregnancy, and is performed through intubation and fixation of the internal jugular veins after 16.5 days of mating. Since both STZ and ALX have a relatively high mortality rate in animals as well as pregnancy failure along with an unstable blood glucose levels in fetal rats,[20] it was possible to establish a gestational hyperglycemia by continuous injection of 30% glucose into pregnant rats at a rate of 2 mL/h. However, the above-mentioned method of hypertonic glucose injection is slightly cumbersome and inconvenient to operate in actual experiments, and the method of hypertonic glucose injection is not very simple, whereas the GDM mechanism found to be more complicated so that it is not widely used in actual animal research.

Simple High-Sugar and High-Fat Diet Induction

The intake of sugars, lipids, and proteins increases during pregnancy. High lipid content in the diet pattern during pregnancy causes not only obesity but also GDM during pregnancy. Excessive intake of high-calorie foods in the diet increases the accumulation of fat in the body, promotes the secretion of obesity-causing adipokines, leading to insulin resistance through various related pathways. Combining the clinical features of gestational hyperglycemia and insulin resistance pathogenesis, dietary induction methods have been developed.

During the establishment of high sugar and high fat diet (HFSD) induced diabetes animal models, experimental animals are given a specific high-sugar and high-fat diet to significantly increase their fasting insulin and decrease their insulin sensitivity index, and thereby induce insulin resistance, which is one of the characteristics of GDM. A successful GDM was established in pregnant mice with a high sugar and high-fat diet. The modelling success rate of pregnant rats was 53.3%, and the fasting blood glucose level of the GDM model group was higher than that of the normal control group from the 10th day of pregnancy. On the 18th day of pregnancy, the fasting blood glucose level of the GDM model group was still higher than that of the normal control group. These were similar to the changes in the characteristics of human GDM blood glucose levels. At the same time, the FINS was measured and the insulin resistance index was calculated on the 18th day of pregnancy and the result indicated insulin resistance, which is consistent with the GDM pathophysiological mechanism.[21] Huang et al also used this method to establish a GDM mouse model and conducted in-depth research on it.[22] Vuong et al established a model and further studied the effect of GDM on the activation of microglia in the cerebellum and the chronic inflammatory

response of the offspring.[23] This kind of experiments eliminate the embryonic influence of drugs and to some extent, damages the mother's organ function and restore the process of human GDM. In addition, high glucose and high-fat diet induction can lead to visceral fat increase, lipid metabolism disorder is one of the important pathophysiological characteristics of GDM.[24] There is a certain degree of rationality.

High-Fat Diet Combined with Streptozotocin

The levels of CRP factors, leptin, and adiponectin have undergone special changes in the GDM process, which may become a new identification point for the discovery and prevention of GDM.[25–29] Some researchers reported that after high-fat diet induction was combined with low-dose STZ intraperitoneal injection, the food intake, water intake and urine volume of mice increased significantly, the blood glucose level, insulin level, serum C-reactive protein content, leptin level increased significantly, while the adiponectin level was decreased significantly. However, the glucose level returned to normal after delivery, and the modeling success rate was approximately 60% with no mortality compared to control group suggesting that it was the ideal method for establishing gestational diabetes model.[30] Tang et al explored the optimal HFSD scheme combined with STZ to establish gestational diabetes rat model, and determined the optimal dose of STZ. The model rate of 25 mg/kg group was 62.5%, the highest in the three groups, and the mortality rate was 12.5%, the lowest in the three groups. Blood glucose of pregnant rats increased significantly after STZ injection, and was stable in the 25 mg/kg group.[31] The combination of low-dose STZ and HFSD can induce an animal model similar to diabetes. Compared with the simple HFSD model, the model setting rate is improved. Compared with the simple STZ induced model, it is more abundant and close to the actual human condition, which improves the animal model rate of this kind. It has more important medical research significance in the evaluation of drug efficacy in the treatment of diabetes.[32]

Combination of Multiple Factors

In recent years, Siti Hajar Abdul Aziz has provided a new method. A common diet was given to rats in the control group, and since the 10th week, rats in the GDM group have been fed HFSD. The mating behavior was then performed. At day 0 of pregnancy, 120 mg/kg.bw (body weight) of niacinamide was injected intraperitoneally, and 15 minutes

later, 35 mg/kg.bw of STZ was dissolved in 0.1 mol/L citrate buffer (pH 4.5) and injected intraperitoneally. At the same time, the sleep mode of rats was controlled in order to induce stress. Various parameters of rats, including the blood glucose parameters, lipid profiles and genes that affect insulin signaling, have been measured. The results showed that GDM rats showed significantly higher body weight and blood glucose level, as well as lower plasma insulin levels and C-peptide compared with the controls.[33] Although this method is cumbersome and time-consuming, it is closer to the real human GDM situation in the GDM mechanism. Based on these results, it can be concluded that an appropriate GDM animal model can be developed through a combination of factors such as nutrition, chemical drug induction, and lifestyle control. This kind of model can be used to further understand the pathophysiology of GDM and thus find new methods for GDM therapy.

Genetic Manipulation

Genetic factors are a very important part in the pathogenesis of GDM. Many studies have conducted in-depth researches on genes and genetics shown in Table 3.

Db/db mice are leptin receptor mutants (ObR), which are characterized by the inability to fully inhibit eating behavior, and their female mice are infertile, but the heterozygous females with mutant allele (db/+) are fertile and fasting blood glucose and insulin levels are normal in non-pregnant states. Excessive appetite of db/+ pregnant rats, however, leads to obesity, develops insulin resistance, and increase blood glucose, and phenotypic GDM is characterized by moderate glucose tolerance.[34–36]

The increase in prolactin is one of many physiological changes during the pregnancy of female mice and human beings. One of the key consequences of prolactin elevation is that the PRLR signal increases cell proliferation, which leads to an increase in pancreatic value in the presence of PRLR, and secretes more insulin to resist body changes and maintain blood glucose homeostasis in the body.[37,38] In PRLR related experimental mice, heterozygous PRLR (PRLR ±) female mice are fertile. The destruction of PRLR gene leads to the failure of β cells to expand properly during pregnancy, which leads to the decrease in blood insulin levels and maternal glucose tolerance and damage to glucose homeostasis in vivo. Many people reported that after PRLR inactivation, β - cell volume decreased, glucose tolerance decreased and insulin secretion decreased.[39,40] At present, some scholars have refined

to study PRLR pdx1-d/d, and have successfully developed a mouse model for gestational diabetes mellitus.[41]

Studies have shown that the expression of hepatocyte growth factor (HGF) and c-Met will also be up-regulated during pregnancy. HGF is an insulin promoter of beta cells. In the study of PancMet KO mice, it was found that the pancreatic condition and blood glucose homeostasis of nonpregnant PancMet Ko female mice were normal, but glucose intolerance occurred after pregnancy, and the replication and apoptosis of islet β cells decreased, followed by increased blood glucose and impaired glucose tolerance. This indicates that the HGF/c-Met signal plays an important role in pregnant women, and its deletion or weakening may cause gestational diabetes mellitus.[42]

5-hydroxytryptamine (5-HT) acts on the downstream of prolactin signal to stimulate the proliferation of beta cells. During pregnancy, the expression of 5-hydroxytryptamine synthetase tryptophan hydroxylase-1 (Tph1) and the production of 5-HT in β - cells increase dramatically. Blocking 5-HTR2b signal in pregnant mice makes 5-HT unable to play a role, resulting in the reduction of β - cell proliferation and glucose intolerance. This study revealed a signaling pathway linking beta cells to hormonal changes during pregnancy, demonstrating the role of 5-HTR2b related genes in GDM.[7,43]

A new candidate gene PAX-8 has recently been identified. PAX-8 was induced in the islets of pregnant mice. It should be noted that under basic conditions, overexpression of PAX-8 in mouse islets could reduce the apoptosis by 40% and reduce cytokine-induced apoptosis. A similar decrease in caspase-3 cleaved by the apoptotic marker in the transfected β cells was also confirmed by immunostaining. Overall, these data suggest that PAX-8 promotes the survival of islet cell sand establishes a link between PAX-8 and GDM research.[44]

Dogs
Partial Pancreatectomy (PPX)
PPX is the most direct way to induce high blood glucose levels. After surgery, insulin-producing cells and various other pancreatic cells are significantly reduced, which further impairs the body's ability to control blood glucose homeostasis. In a series of experiments at the beginning of the twentieth century, Markowitz et al performed total pancreatectomy for dogs in late pregnancy which caused hyperglycemia and diabetes in a few days, while Carlson et al pointed out that the similar results did not appear after similar operations. Moreover, the sample size of these early experiment is often relatively small, usually including 1–3 animals, and due to the occurrence of the

Table 3 GDM Genetic Manipulation Mouse Model

Mouse ID	Target Gene	Changes of Pregnancy Characteristics After Knockout	Author and Year of Literature	Notes
db/db	ObR	Eating too much leads to obesity, elevated blood sugar and insulin resistance	Yamashita H et al,2001[36]	
PrlR±	PrlR	The number of β - cell decreased, glucose tolerance decreased, insulin secretion decreased, and the homeostasis of glucose regulation was impaired	Banerjee RR et al,2016[39]; Arumugam R et al,2014[40]; Nteeba J et al,2019[41]	Loss of pancreatic PRLR signal affects fetal growth and placental gene expression[41]
PancMet KO	c-Met	β - cell proliferation decreased, apoptosis increased, insulin secretion decreased, glucose homeostasis impaired	Demirci C et al,2012[42]	Incomplete proliferation of maternal beta cells in early postpartum PancMet KO mice[42]
HTR2b-/-	5-HTR2b	The proliferation of β - cells decreased and glucose intolerance was observed	Baeyens L et al,2016[7]; Kim H et al,2010[43]	Related to PRL signaling pathway
PAX-8 KO-/-	PAX-8	Promoting beta cell survival	Martin-Montalvo A et al,2019[44]	

pregnancy death, and spontaneous abortion, the experimental results are not ideal, and the conclusions are not convincing. Although PPX can successfully lead to abnormal blood glucose level during pregnancy, this is not specific. In-depth studies of the GDM mechanism in recent years have shown that human GDM is a spontaneous event caused by a combination of genetic, environmental, dietary, and other factors, rather than a sudden pancreatic dysfunction.[45] Therefore, the PPX model cannot conduct in-depth research into the causes of GDM, but it has a certain role to play in studying the outcome of GDM.

HFSD

Mary Courtney Moore et al compared changes in the blood glucose between the normal meat diet group and the HFSD group during the 4–5th week of pregnancy. All dogs with a HFSD showed impaired glucose tolerance (IGT) or gestational diabetes (GDM).[46] In the second trimester of pregnancy, the dog's pancreas did not have an islet hypertrophy, hyperplasia or regeneration, combined with lack of pancreatic adaptability. In this study, systemic insulin sensitivity was reduced by 20–30% in the HFSD group. Although the role of genetic factors, environmental factors and other factors in GDM is not considered, this model provides a useful large animal model for the study of pathophysiology and treatment of GDM.

Sheep
HFSD

From 60 days before pregnancy to 75 days after pregnancy, many ewes were fed with high-fat diet. On day 75, the concentration of blood glucose and ewe insulin in the experimental group was significantly increased.[47] This model is similar to other animal models based on high-fat diets or glucose injection, which ignores the genetic and environmental factors and achieves different pregnancy outcomes and effects on offspring for dietary factors.

Chemical Induction
STZ

STZ was used on the 85th day and 90th day of gestation for 14 pregnant ewes. The intravenous glucose tolerance test was performed before to the first administration of the drug, before the second administration of the drug, and 4 weeks after the last administration of the drug, and it was observed that the fasting blood glucose of the pregnant ewe was significantly increased. Insulin response from the experimental group showed that one STZ lost the second

stage insulin response to glucose load, while the first stage insulin resistance to glucose load was observed after two doses.[48] In this experiment, the STZ-induced destruction of pancreatic β-cells led to a reduction in insulin and weight gain, resulting in changes in maternal glucose and insulin responses. The study provided a large animal model suited to gestational diabetes research.

ALX

Miodovnik et al took intravenous injection of 40 mg/kg.bw ALX in the maternal inferior vena. Before ALX injection and 1, 3, and 5 days after ALX administration, the plasma glucose, insulin and glucagon of pregnant ewes were measured, and a series of symptoms of GDM in pregnant sheep were found. However, two animals had miscarriage between day 1 and day 3 following the administration of ALX.[49] In the case of an increase in the number of experimental animals, J P lips and others also tried to construct such models. The results were similar in that all non-abortion ewes had symptoms of hyperglycemia and insulin resistance, but the rate of abortion was also relatively high.[50] Therefore, this model is more suitable for the acute study of GDM. If we want to conduct a long-term study, we need to give appropriate amount of insulin to prevent serious acidosis and death of the mother.

Pigs
Chemical Induction
STZ

On the 80th day of gestation, STZ was injected into pregnant pigs. In the high-dose group, serum glucose concentration increased to hyperglycemia level and the insulin concentration decreased.[51] But again, this method is more suitable for the study of the consequences and treatment of GDM.

ALX

On the 75th day of gestation, the pregnant pigs were divided into two groups and injected with buffer or ALX and the blood glucose level of pregnant pigs injected with ALX increased by 300%, confirming the successful induction of GDM model.[52] This model is similar to other chemically induced animal models, but the systems of small pigs are highly similar to those of humans, and thus pigs have many applications in the establishment of diabetes models, which provides a certain basis for the establishment of GDM animal models.

Summary

In conclusion, animal models of different types and conditions can be used for different research purposes, and the modeling standards are also different. There are many methods used for different factors affecting GDM, such as high-fat diet or glucose injection, which can be used in dietary factors research; methods such as gene knockout can be used during the research on genetic factors. It is worth mentioning that in the way of chemical drug induction, such as STZ, ALX and other induced models, drugs will lead to permanent destruction of beta cells, resulting in GDM symptoms that still exist after pregnancy has ended. It won't be like human GDM progress, when the end of pregnancy, the high glucose level and insulin resistance phenomenon are relieved. The current solution is to transplant normal islets into experimental animals, but this method requires high experimental technology and cost.[53,54] Even if it is successful in rodents, it may not be able to find new specific directions for research due to different physiological mechanisms. However, large animals are more similar to humans, which may be applicable in large animal experiments and further exploration is needed. In the drug experiment of STZ, the time of injection of STZ is still worth discussing. At present, STZ is usually used to construct the animal model of GDM after animal pregnancy in domestic and foreign experiments, and it is used more in rodent experiments. The time of STZ injection is mostly 0–6 days of gestation. Some literatures indicate that if STZ is injected prematurely, the disorder of glucose metabolism may directly lead to abortion or termination of pregnancy in rats. Therefore, STZ injection on the 6th day of gestation can relatively guarantee embryos survival rate.[11] Due to the complexity of GDM pathogenesis, in the future, the research direction will gradually shift to a combination of multiple factors. There is still a lack of experimental data for large animals, and HFSD and chemical induction methods are still the mainstream. However, because of the incidence of large animals is more similar to the actual situation of human GDM, in the follow-up study, there are still many problems to be solved, such as the islet function which cannot be recovered after pregnancy, and the mechanism of the model constructed by some method is too single. If conditions are allowed, large animals, including pigs, rabbits, sheep, non-human primates, etc., should be used for the establishment of GDM animal models in future research with a view tender standing of human GDM.

Statement of Ethics

This article does not contain any studies with human or animals performed by any of the authors.

Consent for Publication

Does not apply for this manuscript.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare no conflicts of interest in this work.

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