УДК 616.379-008.64-092.9
https://doi.org/10.17816/JOWD682109-118

EXPERIMENTAL MODELS OF TYPE 1 DIABETES

© M.I. Yarmolinskaya[1, 2], N.Yu. Andreyeva[1], E.I. Abashova[1], E.V. Misharina[1]

[1] The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott, Saint Petersburg, Russia;
[2] North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

For citation: Yarmolinskaya MI, Andreyeva NYu, Abashova EI, Misharina EV. Experimental models of type 1 diabetes. *Journal of Obstetrics and Women's Diseases.* 2019;68(2):109-118. https://doi.org/10.17816/JOWD682109-118

Received: December 24, 2018 Revised: January 22, 2019 Accepted: March 18, 2019

▪ This article describes currently used experimental animal models of type 1 diabetes. The literature data on the pathogenesis of clinical and morphological patterns of the disease and the possibility of extrapolation have been summarized in the review. In addition, the advantages and disadvantages of each of the models have been evaluated. Based on the reported results, it can be concluded that preclinical research is essential as fundamental basis for the investigation of type 1 diabetes mellitus.

▪ **Keywords:** type 1 diabetes mellitus; experimental diabetes; alloxan; streptozotocin.

ЭКСПЕРИМЕНТАЛЬНЫЕ МОДЕЛИ САХАРНОГО ДИАБЕТА 1-го ТИПА

© М.И. Ярмолинская[1, 2], Н.Ю. Андреева[1], Е.И. Абашова[1], Е.В. Мишарина[1]

[1] ФГБНУ «Научно-исследовательский институт акушерства, гинекологии и репродуктологии им. Д.О. Отта», Санкт-Петербург;
[2] ФГБОУ ВО «Северо-Западный государственный медицинский университет им. И.И. Мечникова» Минздрава России, Санкт-Петербург

Для цитирования: Ярмолинская М.И., Андреева Н.Ю., Абашова Е.И., Мишарина Е.В. Экспериментальные модели сахарного диабета 1-го типа // Журнал акушерства и женских болезней. — 2019. — Т. 68. — № 2. — С. 109–118. https://doi.org/10.17816/JOWD682109-118

Поступила: 24.12.2018 Одобрена: 22.01.2019 Принята: 18.03.2019

▪ В настоящем обзоре рассмотрены основные методы моделирования сахарного диабета 1-го типа с использованием лабораторных животных. Обобщены данные литературы, связанные с представлениями о патогенезе развития клинической и морфологической картины заболевания, о возможности экстраполяции результатов, а также проанализированы преимущества и недостатки каждой из моделей. На основании представленных данных сделан вывод о необходимости проведения доклинических исследований в качестве фундаментальной базы для изучения сахарного диабета 1-го типа.

▪ **Ключевые слова:** сахарный диабет 1-го типа; экспериментальный диабет; аллоксан; стрептозотоцин.

Diabetes mellitus is a metabolic disorder that represents a serious problem for human health and has an extremely negative effect on the quality of life. Over the past few decades, the prevalence of this disease in the world is continuously increasing, and according to epidemiological prognosis, it will reach 7 %–8 % of the total world population by 2030 [1]. Approximately 10% of all cases of diabetes mellitus account for type 1 diabetes mellitus (DM1) [2], which develops as a result of the death of pancreatic β-cells, leading to the cessation of insulin production, which is a key regulator of carbohydrate and lipid metabolism in the body. Despite numerous studies aimed at studying this disease, it continues to progress, and an insufficient amount of means for DM1 prevention and treatment suggests the inclusion of these patients in high-risk groups for the development of a huge number of complications, which is confirmed by data on the overall risk of mortality among diabetic patients, which is twice higher than in their peers without diabetes [3]. The complications should be noted, such as the negative effect on the female reproductive system, manifested in the form of

menstrual irregularities, infertility, pathological pregnancy course, and delivery [4, 5].

Given the above facts, increasing insight into the complex mechanisms that underlie diabetes and its complications is necessary, as well as identifying new prospects for existing therapy and detecting application points to develop new methods of treatment and reduce the consequences associated with this disease, and therefore further research to study new aspects in DM1 pathogenesis. However, the solution of many medical problems is possible only through using invasive procedures or monitoring that are not applicable to people on ethical and moral principles.

Experimental models are most convenient for studying the pathophysiology of any disease. The use of animals in research can be a serious ethical problem, which includes the physical and/or psychological suffering of animals [6]. In this regard, one of the most important concepts is the concept of 3Rs (reduction, refinement, and replacement) developed by Russell and Birch in 1959 [7]. Compliance with this concept enables to reduce the number of animals used in the experiments and reduce their suffering and discomfort.

Over the past few decades, numerous animal models have been created to study diabetes and test antidiabetic agents, which include chemical, surgical, hormonal, virus-induced, and genetic interventions. Although approaches to the methodology for modeling diabetes have been known for a long time, experimental methods differ even within the framework of one model.

This article considers the most popular and affordable of them.

Surgical Models

One of the most obvious ways to study the effect of hyperglycemia in an animal is to excise the pancreas, partially or completely. The type of animal that will be used within the framework of this model depends on the study objectives. In general, the smaller the animal is, the more adapted is the model to various conditions and, accordingly, the cheaper is the cost of the experiment; therefore, rats and mice are most often used. Nevertheless, the use of rodents is seriously criticized because data obtained may inadequately reflect the disease relative to humans, so in this connection, larger animals such as cats, dogs, pigs, and nonhuman primates are required in some cases [8].

Despite the availability of the study from the point of view of the financial component, the development of hypoinsulinemia and hyperglycemia with total pancreatectomy already on day 1 after surgery, the following factors limit the widespread use of this model [9]:

- a high level of technical equipment and surgical skill of the researcher;
- a high mortality rate (the highest among all diabetes models);
- the development of diabetes after 9 months with 80% subtotal pancreatectomy [10];
- a high risk of infectious complications in the postoperative period;
- excretory pancreatic insufficiency, which requires replacement therapy; and
- the possibility of regeneration of pancreatic tissue cells in the presence of residual tissue after pancreatectomy.

Genetic Models

There are several types of rodents that can spontaneously develop diabetes, namely, nonobese diabetes (NOD) mice, biobreeding (BB) rats, LETL, KDP, and LEW-iddm. To date, the NOD mouse and BB rat are the most widely used experimental models of diabetes.

NOD is the so-called diabetes model without obesity. The NOD inbred line was created in Japan in the 1980s as a subtype of cataract-prone mice with a clear tendency to develop hyperglycemia. As in humans, DM1 in NOD mice develops as a result of chronic destruction of pancreatic β-cells by the immune system, which leads to a deficiency of insulin, hyperglycemia, and glucosuria; however, a characteristic aspect of this model is its high resistance to ketoacidosis. Mice can remain alive for 2–4 weeks after establishing the disease without insulin administration, and in case of the complete absence of therapy, death will be more likely the result of dehydration rather than ketoacidosis. Starting from the age of 3–4 weeks, NOD mice develop mononuclear cell infiltrate (insulite) around the pancreatic islets, which progresses for approximately 100 days until the onset of invasive insulin and complete destruction of β-cells [11]. Initially, antigen-presenting cells present an autoantigen to $CD4^+$ T-lymphocytes, which trigger a cascade of inflammatory reactions [12].

Further, the final damage to the β-cells producing insulin in the Langerhans islets is mainly mediated by $CD8^+$ T-lymphocytes [13]. It is worth noting that although NOD mice have an increased genetic susceptibility to DM1, the disease progression can be modulated by various environmental factors (including temperature, diet, and infectious agents). Therefore, not all NOD mice in the colo-

ny will develop a pattern similar to DM1. Under certain conditions, the incidence of DM reaches 80–95 % among female and 20–40 % among male mice aged 40 weeks [14]. Variability in morbidity is partly related to the pathogenic environment and susceptibility of the NOD mice's immune system. A higher incidence is registered in "very clean" specific (specific pathogen free [SPF] vivarium) and conditionally clean (specific opportunistic pathogen free) conditions, and the sex difference is leveled under axenic conditions in which NOD male mice develop a DM1 pattern at the same rate as in female mice [15, 16]. In a normal non-SPF-associated environment, diabetes incidence can be reduced by up to 10 % [17].

In addition to the development of DM1, NOD mice are also susceptible to the development of thyroiditis, sialoadenitis, autoimmune hemolytic anemia, and lupus-like syndrome.

In both NOD mice and humans, the most important genetic factor contributing to the susceptibility to DM1 is the alleles of the major histocompatibility complex. In some cases, genes such as *Ag7* or loci such as CTLA are considered homologous in humans and mice, which allows the use of this model, including using transgenic approaches, as a basis for studying factors that accelerate or slow down the development of DM1 [18, 19]. There are a large number of works proving the efficacy of targeted therapy for the prevention and treatment of DM1 in this animal model [20–22]. However, clinical studies do not fully support these findings. The authors emphasize the need for careful extrapolation of the results from a well-defined model of an inbred animal to a heterogeneous population of people [23]. To level differences between species, the so-called humanized NOD mice were introduced, which received introduced HLA molecules of class 1 or 2 to further analyze their prodiabetogenic or antidiabetogenic properties and identify T-cell epitopes related to the development of the disease in humans [24].

Thus, the advantages of this method include the following:

- the absence of the need for surgical procedures to create an experimental model of diabetes;
- the incidence can reach 90%–95%;
- the possibility of applying the model for long-term research; and
- the possibility of using this model as the basis for transgenic manipulations.

The disadvantages of this model are the following:

- the high cost (both rats in general and their maintenance);
- strict (SPF) conditions for a vivarium, which affect the incidence;
- the development of concomitant diseases (sialoadenitis, thyroiditis, and hemolytic disease);
- the development of the disease on average between weeks 18 and 20 (blood glucose > 250 mg/dL) [25]; and
- resistance to the development of ketoacidosis as the disease manifestation.

BB rats were obtained from Wistar outbred rats in 1974 in Canada [26]. These animals tend to develop symptoms of the disease, such as weight loss, polyuria, dipsesis, hyperglycemia, and insulinopenia, which lead to the development of severe ketoacidosis and death in the absence of replacement therapy with exogenous insulin. The clinical presentation appears on average at week 12 of an animal's life, often during puberty (weeks 8–16).

The incidence among rats is more than 90%, and it is equal among male and female rats [27]. Like NOD mice, Langerhans islets of BB rats are immune-attacked by T-lymphocytes, B-lymphocytes, macrophages, and natural killers, which cause the development of insulite. The "selective" morphological pattern of the development of insulite, which is closer to the human model, unlike total damage to beta cells in NOD mice, also relates to the advantages of this model [28]. As a rule, this model is characterized by the presence of deep T-cell lymphopenia, in particular, of cells that express receptors for ADP-ribosyltransferase ($ART2^+$) and have immunomodulating properties, since neither morphological nor clinical presentation of DM1 occurs during their preventive transfusion [29]. Lymphopenia is not a characteristic sign of DM1 in either humans or NOD mice. Therefore, this is perceived as a disadvantage in the use of BB rats as a model of DM1 [26]. T-lymphopenia is already present at the birth of rats, and its severity substantiates the development of immunodeficiency in a rodent. In addition, like NOD mice, BB rats are susceptible to the development of autoimmune sialoadenitis and thyroiditis.

The advantages of this method include the following:

- lack of need for surgical procedures;
- the incidence reaches 90–95%;
- the possibility of long-term research; and
- the possibility of using the model to study the induction of tolerance during transplantation of Langerhans islets.

The disadvantages of this model are the following:
- high cost;
- deep immunodeficiency due to congenital lymphopenia;
- the development of the clinical presentation of the disease on average at week 12;
- the development of concomitant diseases (sialoadenitis and thyroiditis); and
- stringent SPF vivarium conditions.

Chemical Models

Chemical agents are used to create a model of diabetes; as a result of which, selective damage to the pancreatic β-cells occurs, and a DM presentation develops. To induce the model, several substances with diabetogenic activity are used, such as streptozotocin (STZ) and alloxan, pirinuron [30], dithizone [31], and dialuric acid [32]. In addition to these substances, other compounds can imitate a specific complication in an animal with DM manifestations; for example, phlorhizin. Alloxan and STZ are most often used. Both are cytotoxic glucose analogs. Despite the differences in pharmacodynamics, the selective action mechanisms of these drugs on β-cells are identical.

Alloxan Diabetes Modeling

In 1838, Wöhler and Liebig synthesized a pyrimidine derivative (2,4,5,6-tetraoxypyrimidine, 5,6-dioxyuracil), which was later called alloxan [33]. The name came from the merger of two concepts — "allantoin" and "oxaluric acid." Allantoin is a uric acid product secreted by the allantoic sac of the poultry embryo, and oxaluric acid was obtained from oxalic acid and urea. In 1943, alloxan became an object of interest for diabetes specialists; thus, Dunn, Sheehan, and McLetchie reported that as a result of the use of this drug, specific pancreatic β-cell necrosis is registered [34, 35].

Drug-induced insulinopenia causes the state of experimental diabetes mellitus called "alloxan diabetes" [36]. In an aqueous solution, alloxan spontaneously decomposes into nondiabetogenic alloxanic acid within a few minutes [37]. At a body temperature of 37°C and a pH of 7.4, the half-life of alloxan is 1.5 min [38].

At lower temperatures, the half-life of alloxan is longer, and since alloxan is a weak acid, it is more stable at a lower pH. Alloxan is a hydrophilic unstable compound with a glucose-like structure. These properties are necessary for the development of diabetes. It is the hydrophilic behavior of the drug that prevents its penetration through the bilipid layer of the plasma membrane, whereas the glucose-like structure allows interaction with the type 2 glucose transporter (GLUT2) in the plasma membrane of β-cells [39]. According to the authors, alloxan does not inhibit the transporter function and, therefore, can enter selectively into β-cells in an unlimited amount [40]. The structural aspect of the compound consists of its central 5-carbonyl group, which reacts with thiol groups of various enzymes, especially with glucokinase (hexokinase IV), which is the most sensitive to it [41]. The result of drug interaction with the latter is a mediated decrease in adenosine triphosphate (ATP) production and suppression of glucose-induced insulin secretion [42]. Inhibition of glucokinase occurs 1 min after the drug administration [41]. Stimulation of insulin production occurs in a short period of time after inhibition of glucokinase because of inhibition of glucose phosphorylation. Within an hour after the model induction, substances such as leucine or tolbutamide can stimulate insulin secretion, since it is not mediated through glucokinase. Subsequently, because of progressive damage to β-cells, the possibility of inducing insulin secretion is completely lost [43]. There is evidence that cysteine and other thiols are able to reduce alloxan to dialuric acid, thus preventing the interaction with glucokinase, as well as glucose, although it is already a competitor to alloxan. At present, the cause of alloxan cytotoxic effect is recognized as the effect of reactive oxygen intermediates (ROIs) synthesized in the alloxan–dialuric acid cycle, which is individually not toxic to β-cells [44–46]. The reactions of this cycle require oxygen, which is converted to superoxide and then to hydroxyl radicals. Most likely, superoxide radicals are not responsible for the cytotoxicity of alloxan and dialuric acid, and most studies indicate hydroxyl radicals as the main cause of cytotoxicity [38]. The authors explain this assumption by the fact that catalase, which inactivates peroxide, provides better protection against the toxicity of alloxan and dialuric acid in comparison with superoxide dismutase [38].

It has been suggested that disorders in intracellular calcium homeostasis represent an important step in the diabetogenic effect of alloxan [47]. This concept was confirmed by *in vitro* and *in vivo* experiments, which demonstrate that alloxan increases by 2.5 times the content of cytosolic free Ca^{2+} in pancreatic cells, which is considered as one of the causes leading to breaks in DNA chains and cytotoxicity of the drug for β-cells [47–49]. In response to the drug administration, a process is launched in the animal's body that goes through several successive phases described by Lensen [38].

The maximum duration of phase 1 is 30 min, which occurs immediately after the injection of alloxan and is a transitional hypoglycemic phase. This short-term hypoglycemic response is the result of a temporary stimulation of insulin secretion in response to blocking of glucose phosphorylation mentioned earlier. At this stage, morphological changes in the pancreatic cells are minimal [50].

In phase 2, which occurs 1 h after the administration of alloxan, the blood glucose concentration increases. Moreover, at the same time, the concentration of plasma insulin decreases. This hyperglycemic phase 1 usually lasts for 2–4 h and is caused by inhibition of insulin secretion and leads to hypoinsulinemia. In this phase, β-cells exhibit the morphological signs, namely, intracellular vacuolization, smooth endoplasmic reticulum dilatation, Golgi complex area decrease, secretory granules decrease (in particular containing insulin), and mitochondrial swelling [50].

Transient hypoglycemic phase 3 usually develops 4–8 h after injection and lasts several hours, a maximum of 24 h [51]. At this stage, special attention should be paid to the animal, since the likelihood of death is extremely high. As a result of a drop in blood glucose levels, the convulsive syndrome can develop, and liver glycogen stores are rapidly depleted, which usually ends with a lethal outcome without glucose therapy [52]. A dramatic change in blood insulin levels is a consequence of alloxan-induced destruction of the plasma membrane of pancreatic β-cells. In addition, damage to other subcellular organelles is registered. In addition to these morphological changes, some β-cell nuclei become pyknotic, which indicates cell necrosis [53].

Stage 4 represents the final permanent diabetic hyperglycemic phase, which lasts 12–48 h and is characterized morphologically by complete degranulation and loss of β-cell integrity. Non-β-cells (α-, δ-, and pp-cells) of the pancreas remain intact and demonstrate selective nature of the toxic effect on β-cells. Cell debris originating from necrotic β-cells is absorbed by macrophages [38]. Many animals are sensitive to the diabetogenic properties of alloxan. Thus, alloxan can be used in rats, mice [54], rabbits [55], pigs [56], and dogs [50]. Animals such as guinea pigs and cats are resistant to alloxan, although the latter are sensitive to its side effects, especially to the drug nephrotoxic effect. It was noted that the drug has a diabetogenic effect with parenteral administration, that is, intravenously, intraperitoneally, or subcutaneously [57, 58]. In addition, the dose of alloxan required to induce diabetes depends on the animal type, administration route, and nutritional status.

Most often, rats are used to induce alloxan diabetes. The diabetogenic dose of alloxan for rats ranges between 100 and 200 mg/kg. However, a single intravenous administration of alloxan in the indicated doses is highly toxic and often leads to a lethal outcome. To reduce the overall mortality and toxicity, the dose of alloxan is recommended to reduce by two to three times [59]. In a study by Federiuk et al., it was revealed that the most successful method of inducing diabetes (with a mortality rate of 10% and 80%) is a single intraperitoneal administration of alloxan at a dose of 200 mg/kg [57].

Hyperglycemia arising after the destruction of β-cells by alloxan is unstable and may turn out to be a reversible process, which leads to normalization of blood glucose levels after a certain time [60]. Since alloxan and glucose are competitors for both GLUT2 and glucokinase, a lower plasma glucose concentration improves the interaction of alloxan with β-cells; therefore, an average fasting period of 12 to 24 h is required before alloxan injection [61].

High nephrotoxicity and hepatotoxicity are due to the presence of receptors to GLUT2 in the cells of the renal tubules and hepatocytes [62], which in turn induces uremic-diabetic syndrome during the first 5 days after surgery with an average mortality rate of 30% [63]. Unlike renal tissue, hepatocytes have higher antioxidant activity and often demonstrate their long-term reaction to the drug [64, 65].

In summary, the following advantages of the alloxan model of diabetes should be noted:
- the model cost is the lowest known at the moment;
- the incidence is 80%; and
- the clinical presentation of diabetes develops 48–72 h after the start of the experiment.

However, there are certain disadvantages:
- high nephrotoxicity;
- hepatotoxicity;
- toxicity to other organs is also possible [66];
- high mortality (at least 30%) [57]; and
- the development of "combined" diabetes due to the formation of insulin resistance [67].

STZ Diabetes Model

This analog of nitrosourea is a hydrophilic compound and, like alloxan, penetrates the β-cell with GLUT2 transport [68]. There are three known ways of β-cell destruction, which are implemented simultaneously. Thus, the main mechanism is the depletion of poly(ADP-ribose)-polymerase and

nicotinamide adenine dinucleotide in response to DNA alkylation, which leads to a decrease in ATP content and inhibition of insulin secretion [68]. The action of intracellular nitric oxide joins this process, which is formed as a result of the action of STZ, which suppresses the Krebs cycle, the formation of hydroxyl radicals, and causes oxygen starvation of mitochondria [69]. In addition, it was found that STZ generates ROIs, which also contribute to DNA fragmentation and cause other damage to cells [70]. Ultimately, DNA damage combined with energy depletion causes β-cell death. There are various schemes for administering the drug to an animal; however, according to the authors, a single administration of a diabetogenic dose of STZ is most effective for modeling DM1. When using STZ, not only interspecific but also intraspecific sensitivity to the action of the drug should be considered. Female rats are known to be more resistant to diabetes within the framework of this model [9]. Rats are considered the most sensitive with a single intravenous administration of STZ at a dose of 35 to 65 mg/kg [68]. For mice and rabbits, the average doses are 100–200 and 300 mg/kg, respectively [71]. STZ does not inhibit the action of glucokinase, unlike alloxan with its 5-carbonyl group, and therefore, there is no initial hypoglycemic phase. Further, with few exceptions, the same phase sequence (2–4) as with alloxan is noted, the clinical presentation develops on average 72 h after the drug intraperitoneal administration. The popularity of the STZ-induced diabetes model is justified primarily because of the lower hepatotoxicity and nephrotoxicity, as well as in connection with another mechanism of action, which enables to create a model with a long term. However, the likelihood of kidney or liver insulinomas depends directly on the experiment duration due to the oncogenic effect of STZ, and as a result, a spontaneous "recovery" is possible in the form of compensation for the diabetes presentation [72].

The advantages of the STZ model of diabetes are as follows:

- reasonable cost compared with genetic models;
- development of the diabetes clinical presentation after 72 h from the start of the experiment; and
- the ability to create a long-term model.

The disadvantages of this model of diabetes include the following:

- oncogenic effect of STZ;
- spontaneous "recovery";
- high cost compared with the alloxan model;
- species and gender specificity; and
- the development of "combined" diabetes due to the formation of insulin resistance [67].

Conclusions

Thus, the need to use experimental animal models in the study of DM1 is determined by the influence of this disease on the quality of life of both an individual and the population as a whole.

A significant amount of information regarding various aspects of pathogenesis and etiology was obtained as a result of preclinical studies. Unfortunately, despite the wide range of possible ways of diabetes induction, none of the models can fully demonstrate the essence of the disease and simulate all the aspects of human DM1. Nevertheless, the use and improvement of experimental models of diabetes in animals is necessary to develop new approaches to modeling of this disease, as well as to study the efficacy of various drugs.

References

1. Ostrauskas R. The prevalence of type 1 diabetes mellitus among 15-34-year-aged Lithuanian inhabitants during 1991-2010. *Prim Care Diabetes.* 2015;9(2):105-111. https://doi.org/10.1016/j.pcd.2014.07.009.
2. Дедов И.И., Шестакова М.В. Сахарный диабет и артериальная гипертензия. — М., 2006. — 344 с. [Dedov II, Shestakova MV. Sakharnyy diabet i arterial'naya gipertenziya. Moscow; 2006. 344 p. (In Russ.)]
3. Roglic G, Unwin N, Bennett PH, et al. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care.* 2005;28(9):2130-2135. https://doi.org/10.2337/diacare.28.9.2130.
4. Дедов И.И., Шестакова М.В. Сахарный диабет и репродуктивная система. — М., 2016. — 176 с. [Dedov II, Shestakova MV. Sakharnyy diabet i reproduktivnaya sistema. Moscow; 2016. 176 p. (In Russ.)]
5. Потин В.В., Боровик Н.В., Тиселько А.В. Сахарный диабет и репродуктивная система женщины // Журнал акушерства и женских болезней. — 2006. — Т. 55. — № 1. — С. 85–90. [Potin VV, Borovik NV, Tisel'ko AV. Diabetes Mellitus And Female Reproductive System. *Journal of Obstetrics and Women's Diseases.* 2006;55(1):85-90. (In Russ.)]
6. Levy N. The use of animal as models: ethical considerations. *Int J Stroke.* 2012;7(5):440-442. https://doi.org/10.1111/j.1747-4949.2012.00772.x.
7. Kroeger M. How omics technologies can contribute to the '3R' principles by introducing new strategies in animal testing. *Trends Biotechnol.* 2006;24(8):343-346. https://doi.org/10.1016/j.tibtech.2006.06.003.
8. Rees DA, Alcolado JC. Animal models of diabetes mellitus. *Diabet Med.* 2005;22(4):359-370. https://doi.org/10.1111/j.1464-5491.2005.01499.x.

9. Etuk E. Animals models for studying diabetes mellitus. *Agric Biol J N Am.* 2010;1(2):130-4.
10. Баранов В.Г. Экспериментальный сахарный диабет. — Л.: Наука, 1983. — 240 с. [Baranov VG. Eksperimental'nyy sakharnyy diabet. Leningrad: Nauka; 1983. 240 p. (In Russ.)]
11. Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol.* 2005;23:447-485. https://doi.org/10.1146/annurev.immunol.23.021704.115643.
12. Saxena V, Ondr JK, Magnusen AF, et al. The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the nonobese diabetic mouse. *J Immunol.* 2007;179(8):5041-5053. https://doi.org/10.4049/jimmunol.179.8.5041.
13. Jayasimhan A, Mansour KP, Slattery RM. Advances in our understanding of the pathophysiology of Type 1 diabetes: lessons from the NOD mouse. *Clin Sci (Lond).* 2014;126(1):1-18. https://doi.org/10.1042/CS20120627.
14. You S, Chatenoud L. Autoimmune diabetes: An overview of experimental models and novel therapeutics. *Methods Mol Biol.* 2016;1371:117-142. https://doi.org/10.1007/978-1-4939-3139-2_8.
15. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med.* 2002;347(12):911-920. https://doi.org/10.1056/NEJMra020100.
16. Markle JG, Frank DN, Mortin-Toth S, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science.* 2013;339(6123):1084-1088. https://doi.org/10.1126/science.1233521.
17. Pozzilli P, Signore A, Williams AJK, Beales PE. NOD mouse colonies around the world- recent facts and figures. *Immunol today.* 1993;14(5):193-196. https://doi.org/10.1016/0167-5699(93)90160-m.
18. Adorini L, Gregori S, Harrison LC. Understanding autoimmune diabetes: insights from mouse models. *Trends Mol Med.* 2002;8(1):31-38. https://doi.org/10.1016/s1471-4914(01)02193-1.
19. Baxter AG, Duckworth RC. Models of type 1 (autoimmune) diabetes. *Drug Discov Today Dis Models.* 2004;1(4):451-455. https://doi.org/10.1016/j.ddmod.2004.11.012.
20. Besançon A, Goncalves T, Valette F, et al. A selective CD28 antagonist and rapamycin synergise to protect against spontaneous autoimmune diabetes in NOD mice. *Diabetologia.* 2018;61(8):1811-1816. https://doi.org/10.1007/s00125-018-4638-7.
21. Montanucci P, Pescara T, Alunno A, et al. Remission of hyperglycemia in spontaneously diabetic NOD mice upon transplant of microencapsulated human umbilical cord Wharton jelly-derived mesenchymal stem cells (hUCMS). *Xenotransplantation.* 2018:e12476. https://doi.org/10.1111/xen.12476.
22. Fiorina P, Tahvili S, Törngren M, et al. Paquinimod prevents development of diabetes in the non-obese diabetic (NOD) mouse. *Plos One.* 2018;13(5):e0196598. https://doi.org/10.1371/journal.pone.0196598.
23. Mathews CE. Utility of murine models for the study of spontaneous autoimmune type 1 diabetes. *Pediatr Diabetes.* 2005;6(3):165-177. https://doi.org/10.1111/j.1399-543X.2005.00123.x.
24. Li Y, Zhou L, Li Y, et al. Identification of autoreactive $CD8^+$ T cell responses targeting chromogranin A in humanized NOD mice and type 1 diabetes patients. *Clin Immunol.* 2015;159(1):63-71. https://doi.org/10.1016/j.clim.2015.04.017.
25. Kachapati K, Adams D, Bednar K, Ridgway WM. The non-obese diabetic (NOD) mouse as a model of human type 1 diabetes. 2012:3-16. https://doi.org/10.1007/978-1-62703-068-7_1.
26. Mordes JP, Bortell R, Blankenhorn EP, et al. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. *ILAR J.* 2004;45(3):278-291. https://doi.org/10.1093/ilar.45.3.278.
27. Crisá L, Mordes JP, Rossini AA. Autoimmune diabetes mellitus in the BB rat. *Diabetes Metab Rev.* 1992;8(1):9-37. https://doi.org/10.1002/dmr.5610080104.
28. Lally FJ, Ratcliff H, Bone AJ. Apoptosis and disease progression in the spontaneously diabetic BB/S rat. *Diabetologia.* 2001;44(3):320-324. https://doi.org/10.1007/s001250051621.
29. Bortel R, Waite DJ, Whalen BJ, et al. Levels of $Art2^+$ cells but not soluble Art2 protein correlate with expression of autoimmune diabetes in the BB rat. *Autoimmunity.* 2001;33(3):199-211. https://doi.org/10.3109/08916930109008047.
30. Kim JM, Lee TH, Lee MC, et al. Endoneurial microangiopathy of sural nerve in experimental vacor-induced diabetes. *Ultrastruct Pathol.* 2002;26(6):393-401. https://doi.org/10.1080/01913120290104700.
31. Monago CC, Onwuka F, Osaro E. Effect of combined therapy of diabinese and nicotinic acid on liver enzymes in rabbits with dithizone-induced diabetes. *J Exp Pharmacol.* 2010;2:145-153. https://doi.org/10.2147/JEP.S11490.
32. Bailey CC, Bailey OT, Leech RS. Diabetes mellitus in rabbits injected with dialuric acid. *Proc Soc Exp Biol Med.* 1946;63(3):502-505. https://doi.org/10.3181/00379727-63-15651
33. Wöhler F, Liebig J. Untersuchungen über die Natur der Harnsäure. *Annalen der Pharmacie.* 1838;26(3):241-336. https://doi.org/10.1002/jlac.18380260302.
34. Shaw Dunn J, McLetchie NGB. Experimental alloxan diabetes in the rat. *Lancet.* 1943;242(6265):384-387. https://doi.org/10.1016/s0140-6736(00)87397-3.
35. Dunn JS, Kirkpatrick J, McLetchie NGB, Telfer SV. Necrosis of the islets of Langerhans produced experimentally. *J Pathol Bacteriol.* 1943;55(3):245-257. https://doi.org/10.1002/path.1700550302.
36. Goldner MG, Gomori G. Alloxan diabetes in the Dog1. *Endocrinology.* 1943;33(5):297-308. https://doi.org/10.1210/endo-33-5-297.
37. Lenzen S, Munday R. Thiol-group reactivity, hydrophilicity and stability of alloxan, its reduction products and its N-methyl derivatives and a comparison with ninhydrin.

Biochem Pharmacol. 1991;42(7):1385-1391. https://doi.org/10.1016/0006-2952(91)90449-f.

38. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia.* 2008;51(2):216-226. https://doi.org/10.1007/s00125-007-0886-7.
39. Gorus FK, Malaisse WJ, Pipeleers DG. Selective uptake of alloxan by pancreatic B-cells. *Biochem J.* 1982;208(2):513-515. https://doi.org/10.1042/bj2080513.
40. Boquist L, Nelson L, Lorentzon R. Uptake of labeled alloxan in mouse organs and mitochondria *in vivo* and *in vitro*. *Endocrinology*. 1983;113(3):943-948. https://doi.org/10.1210/endo-113-3-943.
41. Lenzen S, Panten U. Alloxan: history and mechanism of action. *Diabetologia.* 1988;31(6):337-342. https://doi.org/10.1007/bf02341500.
42. Tiedge M, Richter T, Lenzen S. Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase. *Arch Biochem Biophys.* 2000;375(2):251-260. https://doi.org/10.1006/abbi.1999.1666.
43. Borg LAH. Effects of alloxan on the islets of Langerhans inhibition of leucine metabolism and insulin secretion. *Biochim Biophys Acta Gen Subj.* 1981;677(2):257-262. https://doi.org/10.1016/0304-4165(81)90093-3.
44. Cohen G, Heikkila RE. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J Biol Chem.* 1974;249(8):2447-2452.
45. Winterbourn CC, Cowden WB, Sutton HC. Auto-oxidation of dialuric acid, divicine and isouramil. *Biochem Pharmacol.* 1989;38(4):611-618. https://doi.org/10.1016/0006-2952(89)90206-2.
46. Winterbourn CC, Munday R. Glutathione-mediated redox cycling of alloxan. *Biochem Pharmacol.* 1989;38(2):271-277. https://doi.org/10.1016/0006-2952(89)90037-3.
47. Kim H-R, Rho H-W, Park B-H, et al. Role of Ca^{2+} in alloxan-induced pancreatic β-cell damage. *Biochim Biophys Acta Mol Basis Dis.* 1994;1227(1-2):87-91. https://doi.org/10.1016/0925-4439(94)90111-2.
48. Park BH, Rho HW, Park JW, et al. Protective mechanism of glucose against alloxan-induced pancreatic beta-cell damage. *Biochem Biophys Res Commun.* 1995;210(1):1-6. https://doi.org/10.1006/bbrc.1995.1619.
49. Weaver DC, McDaniel ML, Naber SP, et al. Alloxan stimulation and inhibition of insulin release from isolated rat islets of Langerhans. *Diabetes.* 1978;27(12):1205-1214. https://doi.org/10.2337/diab.27.12.1205.
50. Wrenshall GA, Collins-Williams J, Best CH. Initial changes in the blood sugar of the fasted anesthetized dog after alloxan. *Am J Physiol.* 1950;160(2):228-246. https://doi.org/10.1152/ajplegacy.1950.160.2.228.
51. Tasaka Y, Inoue Y, Matsumoto H, Hirata Y. Changes in plasma glucagon, pancreatic polypeptide and insulin during development of alloxan diabetes mellitus in dog. *Endocrinol Jpn.* 1988;35(3):399-404. https://doi.org/10.1507/endocrj1954.35.399.
52. Macedo CS, Capelletti SM, Mercadante CS, et al. Experimental model of induction of diabetes mellitus in rats. In: Plastic surgery, laboratory of plastic surgery. Sao Paulo: Paulista School of Medicine; 2005. P. 2-15.
53. Lenzen S, Tiedge M, Jörns A, Munday R. Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan. In: Lessons from Animal Diabetes VI. Ed. by E. Shafrir. Boston: Birkhäuser; 1996. P. 113-122. https://doi.org/10.1007/978-1-4612-4112-6_8.
54. Favaro RR, Salgado RM, Covarrubias AC, et al. Long-term type 1 diabetes impairs decidualization and extracellular matrix remodeling during early embryonic development in mice. *Placenta.* 2013;34(12):1128-1135. https://doi.org/10.1016/j.placenta.2013.09.012.
55. Irshad N, Akhtar MS, Bashir S, et al. Hypoglycaemic effects of methanolic extract of *Canscora decussata* (Schult) whole-plant in normal and alloxan-induced diabetic rabbits. *Pak J Pharm Sci.* 2015;28(1):167-174.
56. Sodha NR, Boodhwani M, Clements RT, et al. Increased antiangiogenic protein expression in the skeletal muscle of diabetic swine and patients. *Arch Surg.* 2008;143(5):463-470. https://doi.org/10.1001/archsurg.143.5.463.
57. Federiuk IF, Casey HM, Quinn MJ, et al. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. *Comp Med.* 2004;54(3):252-257.
58. Sano T, Ozaki K, Terayama Y, et al. A novel diabetic murine model of *Candida albicans*-induced mucosal inflammation and proliferation. *J Diabetes Res.* 2014;2014:509325. https://doi.org/10.1155/2014/509325.
59. Reis FPd, Sementilli A, Gagliardi ARdT. Experimental diabetes exacerbates skin transplant rejection in rats. *Acta Cir Bras.* 2013;28(5):323-326. https://doi.org/10.1590/s0102-86502013000500001.
60. Kumar S, Singh R, Vasudeva N, Sharma S. Acute and chronic animal models for the evaluation of anti-diabetic agents. *Cardiovasc Diabetol.* 2012;11:9. https://doi.org/10.1186/1475-2840-11-9.
61. Radenkovic M, Stojanovic M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *J Pharmacol Toxicol Methods.* 2016;78:13-31. https://doi.org/10.1016/j.vascn.2015.11.004.
62. Chatzigeorgiou A, Halapas A, Kalafatakis K, Kamper E. The use of animal models in the study of diabetes mellitus. *In Vivo.* 2009;23(2):245-258.
63. Vargas L, Friederici HHR, Maibenco HC. Cortical sponge kidneys induced in rats by alloxan. *Diabetes.* 1970;19(1):33-44. https://doi.org/10.2337/diab.19.1.33.
64. Malaisse WJ, Malaisse-Lagae F, Sener A, Pipeleers DG. Determinants of the selective toxicity of alloxan to the pancreatic B cell. *PNAS.* 1982;79(3):927-930. https://doi.org/10.1073/pnas.79.3.927.

65. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes*. 1997;46(11):1733-1742. https://doi.org/10.2337/diab.46.11.1733.

66. Srinivasan K, Ramarao P. Animal model in type 2 diabetes research: An overview. *Ind J Med Res.* 2007;125(3):451.

67. Islam MS, Loots du T. Experimental rodent models of type 2 diabetes: a review. *Methods Find Exp Clin Pharmacol.* 2009;31(4):249-261. https://doi.org/10.1358/mf.2009.31.4.1362513.

68. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res.* 2001;50(6):537-546.

69. Turk J, Corbett JA, Ramanadham S, et al. Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. *Biochem Biophys Res Commun.* 1993;197(3):1458-1464. https://doi.org/10.1006/bbrc.1993.2641.

70. Bedoya FJ, Solano F, Lucas M. N-Monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. *Experientia.* 1996;52(4):344-347. https://doi.org/10.1007/bf01919538.

71. Dekel Y, Glucksam Y, Elron-Gross I, Margalit R. Insights into modeling streptozotocin-induced diabetes in ICR mice. *Lab Anim (NY).* 2009;38(2):55-60. https://doi.org/10.1038/laban0209-55.

72. Kazumi T, Yoshino G, Fujii S, Baba S. Tumorigenic action of streptozotocin on the pancreas and kidney in male Wistar rats. *Cancer Res.* 1978;38(7):2144-2147.

Information about the authors *(Информация об авторах)*

Maria I. Yarmolinskaya — MD, PhD, DSci (Medicine), Professor of the Russian Academy of Sciences, the Head of the Department of Endocrinology of Reproduction, the Head of the Diagnostics and Treatment of Endometriosis Center. The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott, Saint Petersburg, Russia; Professor. The Department of Obstetrics and Gynecology, North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia. **E-mail:** m.yarmolinskaya@gmail.com.

Nelly Yu. Andreyeva — Resident Doctor. The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott, Saint Petersburg, Russia. **E-mail:** nelly8352@yahoo.com.

Elena I. Abashova — MD, PhD, Senior Researcher. The Department of Endocrinology of Reproduction. The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott, Saint Petersburg, Russia. **E-mail:** abashova@yandex.ru.

Elena V. Misharina — MD, PhD, Senior Researcher. The Department of Endocrinology of Reproduction. The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott, Saint Petersburg, Russia. **E-mail:** mishellena@gmail.com.

Мария Игоревна Ярмолинская — д-р мед. наук, профессор РАН, руководитель отдела эндокринологии репродукции, руководитель центра «Диагностика и лечение эндометриоза». ФГБНУ «НИИ АГиР им. Д.О. Отта», Санкт-Петербург; профессор кафедры акушерства и гинекологии. ФГБОУ ВО «СЗГМУ им. И.И. Мечникова» Минздрава России, Санкт-Петербург. **E-mail:** m.yarmolinskaya@gmail.com.

Нелли Юрьевна Андреева — клинический ординатор. ФГБНУ «НИИ АГиР им. Д.О. Отта», Санкт-Петербург. **E-mail:** nelly8352@yahoo.com.

Елена Ивановна Абашова — канд. мед. наук, старший научный сотрудник отдела эндокринологии репродукции. ФГБНУ «НИИ АГиР им. Д.О. Отта», Санкт-Петербург. **E-mail:** abashova@yandex.ru.

Елена Владимировна Мишарина — канд. мед. наук, старший научный сотрудник отдела эндокринологии репродукции. ФГБНУ «НИИ АГиР им. Д.О. Отта», Санкт-Петербург. **E-mail:** mishellena@gmail.com.