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Features of the Generative Compartment of Male Sex Glands in the Offspring of Female Rats with Experimental Type 1 Diabetes Mellitus

Gennady V. Bryukhin[1], Sergei D. Antonov[2*]

[1]Doctor of Medical Sciences, Professor, Head of the Department of Histology, Embryology and Cytology of South-Ural State Medical University, Russia.

[2*]Applicant for the degree of Candidate of Medical Sciences at the Department of Histology, Embryology and Cytology of South-Ural State Medical University, Russia. E-mail: s.d.antonov@mail.ru

ABSTRACT

***Introduction*:** The relevance of this study is primarily conditioned by global upsurge in diabetes mellitus, including women of fertile age. The results of numerous clinical observations and experimental studies indicate that mothers with diabetes mellitus give birth to physiologically immature offspring with marked signs of diabetic fetopathy. At the same time, the role of mothers' diabetes mellitus in the male reproductive system disorders of their offspring is poorly explored.

***Objective*:** to study the features of spermatogenic cycle formation in the offspring of female rats with experimental type 1 diabetes mellitus.

***Materials and Methods*:** In adult female mature rats (the Wistar breed), type 1 diabetes mellitus was simulated using streptozotocin. The object of the study was the offspring of the animals with experimental diabetes, observed at various periods of the postnatal period. In the laboratory animals, there were several tests: cytological analysis of testicular germ cells in serial histological sections with determination of total number of spermatogenic cells and their subpopulation composition per one convoluted seminiferous tubule, determination of the number of epididymal spermatozoa and their atypical forms, as well as assessment of sexual behavior and fertility.

***Results*:** The cytological analysis made it possible to establish that the experimental animals have a disturbed formation of the testes generative function. That was manifested in a decrease in the total content of spermatogenic cells and a change in their subpopulation composition, as well as in a decrease in the spermatogenesis index, Sertoli cell index, germinative index and an increase in the maturation index, which are sensitive indicators of the spermatogenesis state. The revealed morphological changes ultimately determine a decrease in the total content of epididymal spermatozoa, sexual behavior disorders and a decrease in fertility.

***Conclusion*:** The obtained results show that the offspring of female rats with experimental diabetes mellitus have a disturbed formation of the generative compartment of the male reproductive system, which ultimately results in a decrease in the total content of epididymal spermatozoa and impaired reproductive function.

KEYWORDS

Diabetes Mellitus, Experiment, Rats, Testes, Spermatogenic Cycle, Sexual Behavior, Fertility.

Introduction

Diabetes mellitus has become one of the most acute and widespread medical problems over the past few decades and is currently one of the most common causes of death in the world [1].

Women with type 1 diabetes often have pathologies of the reproductive system; as a result, their fertility is reduced [2]. Late complications of diabetes and high levels of glycosylated hemoglobin may contribute to ovarian reserve decrease in patients with type 1 diabetes mellitus [2]. These patients experience premature ovarian aging, as evidenced by an earlier decrease in follicles compared to healthy women [3].

Younger-age diabetes mellitus all over the world and annual increase in the number of these patients leads to morbidity growth among women of fertile age [4], which boosts the urgency of problems associated with gestation management in diabetes cases [5, 6] . Women with type 1 or type 2 diabetes mellitus have higher risk of pregnancy complications such as congenital malformations (primarily of the heart or musculoskeletal system), pre-eclampsia, and premature birth.

The results of numerous clinical observations and experimental studies indicate that mothers with diabetes mellitus give birth to physiologically immature offspring with marked signs of diabetic fetopathy [7]. At present, the influence of mothers' diabetes mellitus on the female reproductive system of the offspring is being actively studied [8, 9, 10]. At the same time, the role of mothers' diabetes in the male reproductive system disorders of their offspring

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is poorly explored. It was found that reproductive health disorders do occur in sexually mature offspring of female rats with experimental type 1 diabetes mellitus; it is reflected in a decrease in the viability, concentration and motility of spermatozoa, and an increase in the number of atypical forms of germ cells [11].

To date, the complicated mechanisms of spermatogenesis disorders in the offspring of mothers with diabetes have not been studied. Better understanding of these mechanisms will help to develop optimal methods of prevention and treatment of male reproductive health.

Based on the above, it seems extremely relevant to explore the features of spermatogenic cycle formation in the offspring of female rats with experimental type 1 diabetes mellitus, which became the objective of this study.

Materials and Methods

The studies were carried out on white laboratory Wistar rats (females) and their offspring at various intervals of the postnatal period, including day 1 (neonatal period), day 15 (suckling period), days 30 and 45 (puberty period) and day 70 (sexual maturity period). To achieve the study objective, type 1 diabetes mellitus was modeled in sexually mature rats (females) before pregnancy according to a common method using streptozotocin (Biomedicals LLC, France) [12] that was injected intraperitoneally three times with an interval of 7 days (2.5 mg per 100 g of weight in the first and third weeks and 2 mg per 100 g of weight in the second week). For the entire course, 10 laboratory animals weighing from 230 to 256 g totally received 17 mg of streptozotocin. Under its influence the laboratory animals developed diabetes mellitus, as evidenced by a constant high level of glucose in the blood (32.56 ± 2.44 mmol/l), which persisted for at least three months (intact females glycemic indicators were 6.25 ± 0.22 mmol/l). Blood glucose concentration in the animals under experiment was determined at the Central Scientific Research Laboratory of the South Ural State Medical University (SUSMU) using a BioChem Analette (HTI) combined automatic biochemical and enzyme immunoassay analyzer. Blood for samples was taken from the tail vein of laboratory rats according to the standard technique. The level of glucose was determined in venous serum.

The transfer of females with experimental diabetes to intact males for mating was carried out one week after the last injection of streptozotocin. The rats born as a result of such mating were taken for the research (hereinafter referred to as ‘experimental animals’).

The works with laboratory animals was carried out in accordance with the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” of 18.03.1986. All animals under experiment were kept in the standard conditions of the SUSMU vivarium. Euthanasia of animals was carried out by decapitation method under ether anesthesia. This study was approved at the meeting No. 8 of the SUSMU Ethics Committee, held on 11.11.2018.

Cytological analysis of the testicular germ cells was carried out in laboratory animals of the intact and experimental groups. The assessment of spermatogenic epithelium was performed with serial histological sections stained with hematoxylin and eosin according to the generally accepted technique. The total number of spermatogenic cells and their subpopulation composition (including spermatogonia, spermatocytes, spermatids and spermatozoa) was determined per one convoluted seminiferous tubule. The assessment included 30 strictly transverse sections of convoluted seminiferous tubules; in each section there was a total count of spermatogenic epithelial cells. According to the literature, the most sensitive morphological criterion for the spermatogenesis activity is the content of spermatogonia in the convoluted seminiferous tubules [13]. Calculation of spermatogonia was carried out according to the standard method with determining the number of active (type A) and inactive (type B) spermatogonia. Each seminiferous tubule contains spermatogonia, which cannot be attributed to either active (A) or inactive (B) cells by morphological characteristics. Taking that into account, it is customary to isolate intermediate (In) forms of spermatogonia [14], which gives a more accurate assessment of the spermatogenic cycle features in the animals under experiment. The cytological analysis of spermatogenic epithelial cells was performed at a 400 magnification (eyepiece x 10; objective lens x 40).

To assess more complex spermatogenic cycle disorders in the experimental animals, the study included estimation of spermatogenesis index (the ratio of the number of spermatogenic epithelium layers to the number of counted

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tubules), Sertoli cell index (the ratio of the total content of different-type germ cells, including spermatogonia, spermatocytes, spermatids and spermatozoa, to the number of sustentocytes), germinative index and (the ratio of the total number of spermatogonia to the number of sustentocytes) and maturation index (the ratio of the total number of spermatogonia and spermatocytes to the total content of spermatids and spermatozoa). [15] The experimental group in this part of the study consisted of 52 rats, including 13 neonatal rats, 27 rats at the age of 15, 30 and 45 days (9 in each age sub-group), and 12 sexually mature 70-day-old rats. The control group (group C) consisted of 45 rats: 11 neonates, 24 rats at the age of 15, 30 and 45 days (8 in each age sub-group), and 10 sexually mature 70-day-old rats.

In order to count male germ cells in the ejaculate, mature spermatozoa was obtained from the testicular appendage, by cutting it lengthwise in a dosed amount (1 ml) of 5% glucose solution preheated to 37^0 C. With a piece of washed rubber tube, the spermatozoa from the epididymis were actively placed into the solution for 2 minutes. The number of spermatozoa per unit volume (1 ml) of the epididymal suspension was counted in the Goryaev chamber [16, 17].

We also studied the features of the animals' sexual behavior. Reproductive behavior was assessed in three-month-old animals of the control and experimental groups according to the standard method in the dark from 10.0 p.m. to 5.0 a.m. [18,19] A 40x40x30 cm test chamber was used under dim red light. The chamber was divided by a transparent perforated baffle plate; a male rat under study was placed in it. After 5 minutes, the male was brought into contact with an intact female in a state of estrous (that was determined using vaginal smear analysis). [20] The elements of behavioral and reproductive activity were recorded and assessed for 15 minutes in the tests with the baffle plate - without a female, with the baffle plate - with a female, and without the baffle plate - with a female. During the observation period, we considered such components of sexual behavior as the number of approaches to the plate in the presence of a female and the number of placements. The participating animals were 10 control and 11 experimental 90-day-old male rats.

To assess the fertility of the studied males, intact females in a state of estrous were placed to them at their age of 3 months, in a ratio of one male to two females. Fertility index, which is the ratio of the number of females mated with males to the total number of females placed to males, was estimated as a percentage. The fact of mating with a female was confirmed by the presence of spermatozoa in the female vaginal smears; these females were considered conditionally inseminated. Insemination index was calculated as the ratio of the number of pregnant females to the number of conditionally inseminated females, multiplied by 100% [21, 22].

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 19 software. The Shapiro-Wilk criterion was used to test the hypotheses that the distribution of features of the general population complies with the normal distribution law. Since the distribution of features differed from the normal distribution, the following indicators of descriptive statistics were calculated for quantitative features: the median (Me) and quartiles (Q1; Q3). The non-parametric Mann-Whitney test was used to compare two independent groups in terms of quantitative characteristics. All statistical hypotheses were tested at the critical significance level (p) of 0.05.

Results

First of all, we found that the offspring of female rats with experimental type 1 diabetes mellitus has a decrease in the total content of epididymal spermatozoa. While the content of spermatozoa in 1 ml of intact sex mature animals was 139 (121.75; 151.5) million units, this indicator in experimental rats was only 94.5 (81.5; 113.75) million units ($p = 0.001$). It is logical to suppose that the revealed decrease in the total spermatozoa content is caused by impaired spermatogenesis. In this regard, we analyzed the spermatogenic layer of the convoluted seminiferous tubules of the experimental animals' testes.

One of the most informative indicators of the morphofunctional state of the male sex gland is the characteristics of the spermatogenic layer. We found that in the process of postnatal development, the total content of spermatogenic cells gradually increases, reaching its maximum value by the period of sexual maturity. At the same time, in the offspring of female rats with experimental type 1 diabetes mellitus, the studied indicator is reduced in comparison with the control group at all periods of the study (Table 1).

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Table 1. Characteristics of the experimental animals' spermatogenic layer per one convoluted seminiferous tubule, Me (Q1; Q3)

Indicator	Control group					Experimental group				
	Day 1	Day 15	Day 30	Day 45	Day 70	Day 1	Day 15	Day 30	Day 45	Day 70
Spermatogenic cells	10.1 (9.89; 11.83)	52.2 (51.7; 57.4)	139.1 (129.0; 152.1)	268.5 (263.6; 276.7)	493.5 (480.3; 521.8)	7.8 (7.2; 8.4)*	36.2 (34.9; 36.6)*	93.6 (86.4; 98.8)*	176.5 (170.3; 182.1)*	338 (322; 350.8)*
Spermatogonia (total)	9.4 (8.98; 11.07)	18.7 (17.9; 20.4)	35.3 (33.7; 6.8)	62.0 (59.3; 65.0)	56.8 (52.7; 57.5)	7.07 (6.7; 7.63)*	18.7 (18.0; 19.8)	29.5 (28.0; 29.7)*	48.7 (44.7; 52.9)*	50.5 (47.6; 52.5)*
Spermatogonia A	6.83 (6.17; 7.78)	6.95 (5.35; 7.79)	8.33 (7.15; 10.7)	8.35 (6.84; 16.95)	12.0 (10.4; 13.3)	5.6 (4.57; 6.13)*	9.03 (8.7; 8.57)*	7.97 (7.6; 11.37)	14.0 (12.3; 16.6)	14.4 (11.8; 15.1)
Spermatogonia In	2.13 (2.09; 2.58)	2.77 (1.95; 3.37)	3.4 (2.9; 3.57)	3.25 (2.62; 4.8)	4.25 (3.82; 5.28)	1.63 (1.53; 1.93)*	2.0 (1.83: 2.5)	3.37 (3.23; 3.77)	3.0 (2.77; 3.2)	3.63 (3.28; 3.8)*
Spermatogonia B	0.43 (0.37; 0.52)	10.6 (8.02; 11.3)	24.2 (20.5; 26.6)	49.7 (46.8; 52.2)	40.1 (37.5; 42.2)	0.3 (0.3; 0.33)*	7.33 (7.2; 7.87)*	16.7 (14.7; 18.1)*	31.9 (29.6; 33.0)*	33.4 (32; 35.3)*
Spermatocytes	0.73 (0.7; 0.85)	32.1 (29.4; 38.2)	101.3 (93.1; 110.8)	55.3 (49.9; 63.1)	94.1 (91.7; 96.3)	0.63 (0.53; 0.73)*	17.4 (16.9; 17.5)*	64.7 (54.4; 67.7)*	70.5 (65.6; 74.0)*	73.9 (66.8; 80.7)*
Spermatids (total)	-	1.55 (1.07; 1.74)	2.77 (2.08; 3.1)	114.8 (110.0; 117.9)	198.7 (178.3; 219.7)	-	0.47 (0.33; 0.7)*	1.5 (0.97; 1.93)*	45.4 (43.5; 48.7)*	113.5 (105.3; 136.4)*
Early spermatids	-	1.55 (1.07; 1.74)	2.77 (2.08; 3.1)	106.2 (102.9; 109.0)	132.7 (128.2; 147.1)	-	0.47 (0.33; 0.7)*	1.5 (0.97; 1.93)*	44.5 (42.6; 47.3)*	82.6 (72.9; 96.9)*
Late spermatids	-	-	-	8.37 (4.12;4 12.1)	61.7 (49.2; 68.7)	-	-	-	1.3 (1.1; 1.47)*	34.5 (28.1; 41.5)*
Spermatozoa	-	-	-	42.6 (19.6; 45.7)	155.7 (144.3; 160.0)	-	-	-	8.7 (6.97; 8.9)*	90.5 (73.3; 106.9)*
Sustentocytes	4.17 (3.97; 4.19)	5.0 (4.67; 5.12)	7.27 (6.95; 7.61)	15.5 (14.6; 16.5)	22.0 (20.5; 23.5)	4.0 (3.7; 4.37)	4.63 (4.27; 4.87)*	6.43 (5.87; 6.7)*	12.2 (11.7; 13.6)*	21.6 (20.8; 24.1)
Spermatogenesis index	1.64 (1.6; 1.66)	2.53 (2.5; 2.59)	2.67 (2.62; 2.71)	3.34 (3.31; 3.38)	3.87 (3.82; 3.92)	1.49 (1.40; 1.53)*	2.22 (2.19; 2.27)*	2.47 (2.45; 2.51)*	3.18 (3.16; 3.23)*	3.6 (3.53; 3.68)*
Germinative index	2.26 (2.13; 2.72)	4.04 (3.57; 4.16)	4.84 (4.61; 5.3)	3.84 (3.78; 4.42)	2.55 (2.39; 2.72)	1.84 (1.61; 1.91)*	4.23 (3.88; 4.53)	4.4 (4.36; 4.82)	4.2 (3.53; 4.45)	2.28 (2.1; 2.4)*
Index of spermatogenesis relaxation	2.43 (2.36; 2.0)	11.0 (10.6; 11.4)	19.4 (17.6; 21.1)	17.2 (16.2; 18.4)	22.8 (21.5; 24.2)	1.95 (1.79; 2.1)*	7.96 (7.76; 8.16)*	14.3 (13.6; 17.2)*	13.9 (12.8; 15.8)*	15.2 (13.9; 15.8)*
Maturation index	-	-	-	0.75 (0.7; 0.96)	0.43 (0.41; 0.45)	-	-	-	2.26 (1.91; 2.42)*	0.59 (0.52; 0.65)*

* - the results are statistically significant in comparison with the control group ($p < 0.05$)

The data on the subpopulation composition of spermatogenic cells, taking into account their maturity, are of great interest. In this regard, we analyzed the content of spermatogonia, spermatocytes, spermatides and spermatozoa. First of all, it was established that the total spermatogonia content in intact rats after their birth gradually increases. A

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similar pattern was revealed in the experimental animals. However, it should be noted that the spermatogonia content in experimental animals was reduced in comparison with the control group in almost all periods of the study (Table 1). The table illustrates that the content of active spermatogonia (In and B) in the experimental animals was reduced in comparison with the control group in many periods of the study, but the number of inactive (A) spermatogonia was increased. In the period of postnatal development, there is also a change in the number of spermatocytes of the 1st and 2nd order in the animals of both groups (Table 1).

The study of the content of spermatids revealed the following pattern. For the first time, spermatids are detected in experimental animals on the 15th day of postnatal development. After birth, the number of spermatids in the animals of both groups gradually increases, reaching its maximum value on the 70th day of observation. At that, at all stages of the study, the total spermatid content in the offspring of females with experimental diabetes mellitus was reduced as compared to the control group. Table 1 shows that late spermatids (more differentiated ones) are found in the experimental animals' convoluted seminiferous tubules only on day 45.

In both groups, spermatozoa in the animals' convoluted seminiferous tubules appear only at the end of the period of puberty and reach their maximum value during the period of maturity. At that, the content of spermatozoa in the offspring of females with experimental diabetes is significantly reduced in comparison with intact animals (Table 1).

The most sensitive indicators of the generative function state of the testes are spermatogenesis index (the ratio of the number of spermatogenic epithelium layers to the number of counted tubules), Sertoli cell index (the ratio of the total number of spermatogenic cells to the number of sustentocytes), germinative index (the ratio of the number of spermatogonia to the number of sustentocytes) and the maturation index (the ratio of the total content of spermatogonia and spermatocytes to the content of spermatids and spermatozoa). An analysis of the obtained results allows us to state that in the offspring of female rats with experimental diabetes mellitus, a decrease in all indices takes place over most of the study periods, which convincingly indicates a disturbance of the spermatogenesis process. At that, the maturation index of male germ cells in the experimental animals, on the contrary, is greater than that in the control group, which is caused by the predominance of early forms in the spermatogenic epithelium. It also indicates an increased cell death at the stage of spermatids and spermatozoa in the offspring of female rats with diabetes mellitus.

One of the most important indicators of the male sex glands functional state is sexual activity, which is regulated by the neuroendocrine system through the combined action of neurotransmitters and neuropeptides, sexual steroids and metabolites. [23]

Table 2 presents the data obtained during the first stage of the study on reproductive behavior with a test with a baffle plate, taking into account that sexual motivation is manifested in movement towards an individual of the opposite sex [23]. After installing a transparent perforated plate in the chamber without a female behind it, the number of approaches of males in both groups did not show significant differences. After introduction of a female, this parameter changed in both groups of the experiment. In the control group, the number of approaches to the plate sharply increased when a female appeared behind it, while in the males born to mothers with experimental type 1 diabetes mellitus, there was a decrease in the number of approaches to the baffle plate. Similar data were obtained upon calculation of the time spent by males near the plate. In experimental animals, the time was significantly shorter (relative to the time spent near the plate without a female) in comparison with intact animals.

Table 2. Indicators of sexual behavior of the animals participating in the experiment, Me (Q1; Q3)

Indicator	Control group	Experimental group	p
Number of approaches to the baffle plate without a female	11.5 (7.0; 15.0)	9.0(6.5; 12.0)	0.512
Time near the plate without a female, s	59.5 (43.0; 69.0)	61.0 (48.5; 81.0)	0.654
Number of approaches to the plate with a female behind it	20.5 (15.0; 24.0)	11.0 (10.5; 13.5)	<0.001
Time near the plate with a female, s	193.5 (157.0; 218.0)	145.0 (124.0; 159.0)	0.002
Number of approaches to a female	44.5 (36.0; 53.0)	32.0 (30.0; 36.5)	0.004
Total time of the contact with a female, s	255.0 (233.0; 258.0)	152.0 (147.0; 165.0)	<0.001
Number of placements	2.5 (2.0; 4.0)	1.0 (0.0; 1.5)	0.005

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The second stage of the study on the male experimental animals' reproductive activity was a test observation over the direct interaction of partners (Table 2). It was found that during the observation period, the males of the experimental group made fewer approaches to the estrus female compared to intact animals. We also evaluated the duration of experimental animals' contacts. The total time of experimental animals' contact with females was less than the time in the comparison group.

One of the most indicative signs of a male's readiness for mating is the number of placements [23]. This indicator in the experimental group was lower than in the control group (Table 2).

The following data were obtained in the study of experimental animals' fertility. All the 18 females mated to the males of the control group were inseminated and became pregnant. Here, the fertility index (the ratio of the number of females mated with males to the total number of females introduced to males) was equal to 100%. In the experimental group, only 15 females out of 18 were conditionally inseminated (the fact of mating with the female was confirmed by the presence of sperm in vaginal smears). As a result, the fertility index was equal to 83.3%. Insemination index (the ratio of the number of pregnant females to the number of conditionally inseminated females, multiplied by 100%) also turned out to be significantly lower in the experimental group. It was 53.3% compared to 100% of the control group: out of 15 conditionally inseminated females, only 8 females inseminated by the males born to mothers with diabetes became pregnant.

Discussion

We understand the mechanism of testicular generative function dysfunction as follows. It is known that prenatal stress may lead to a complex of various micro- and ultrastructural morphological, neurochemical, endocrine, metabolic changes in offspring, which can cause not only teratogenic effects, but also microstructural and functional abnormalities. They can persist and have a long-term or permanent effect throughout entire subsequent life (the phenomenon of intrauterine disease programming). [24] At that, prenatal stress causes long-term disorders primarily in the system of neuroendocrine regulation of reproduction and hormonal adaptation, which can significantly affect the formation of spermatogenesis in offspring. [24] Maternal extragenital diseases, which are regarded as a type of significant prenatal pathological impact, have a significant effect on the offspring, including the reproductive system as well. For example, a number of researchers studied the effect of chronic experimental liver lesion on the sexual system of offspring. In particular, scientists revealed a decrease in the total content of spermatogenic cells, a decrease in the number of spermatocytes, spermatids and spermatozoa in males' seminiferous convoluted tubules, as well as disturbed folliculogenesis in females' ovaries. [25] According to the authors, these changes are caused by massive penetration of pathological products of mother's metabolism through the fetoplacental barrier into the fetus bloodstream and by disturbances in the fetal pituitary-thyroid-adrenal-sweat system, which ultimately leads to meiosis disruption and increased degenerative death of germ cells. [25]

The leading pathogenetic elements of prenatal stress caused by maternal diabetes mellitus are hyperglycemia and hyperketonemia. With the onset of pregnancy in women suffering from type 1 diabetes mellitus, formation of the embryo and the fetus is complicated by the development of diabetic fetopathy. This pathology occurs in more than 90% of fetuses and newborns born by women with diabetes. [26] Diabetic fetopathy often becomes the cause of fetal death or serves as a basis for various disorders of children's health in the postnatal period. [26] The fetus and newborns with diabetic fetopathy are characterized not only by macrosomia and anatomical disproportion of body parts, but also by delayed functional development of all systems, primarily of the nervous system. [26] This leads to the formation of perinatal encephalopathy in almost 100% of newborns to mothers with type 1 diabetes mellitus and in 15-20% of cases - to neuropsychiatric pathology [26], which includes, among other things, sexual behavior disorders and reduced reproductive activity.

Diabetic fetopathy is based on several factors: feto-placental insufficiency, hormonal placental dysfunction and maternal hyperglycemia [27]. Glucose penetrates through the placenta and continuously passes to the fetus from the mother's blood by facilitated diffusion. Ketone bodies also penetrate freely through the placenta [27, 28]. At the same time, insulin, glucagon and free fatty acids of the mother do not enter the fetal bloodstream. In the first 9-12 weeks of pregnancy, the fetal pancreas does not yet produce its own insulin. From the 12th week of intrauterine development, the fetal pancreas starts synthesizing insulin; thus, reactive hypertrophy and hyperplasia of β-cells of the fetal pancreas develop in response to hyperglycemia. [27] Hyperinsulinemia, in its turn, triggers a cascade of hormonal

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and metabolic changes in the fetus, contributing to an increase in the number of fat cells. Therefore, fetal hyperinsulinemia is considered to be the main cause of macrosomia development. [6]

As a result of β-cell hyperplasia and hyperinsulinemia, newborns are prone to severe and prolonged hypoglycemia. When the placenta is separated, glucose delivery to the fetus abruptly stops, but hyperinsulinemia does not decrease, as a result of which hypoglycemia develops during the first hours after birth. Normalization of blood sugar occurs approximately on days 6-7 [27].

Decompensation of carbohydrate metabolism in the mother during the first two trimesters of pregnancy may, on the contrary, lead to the depletion of fetal beta cells, to hypoinsulinemia and subsequently to the development of intrauterine growth restriction syndrome. [28]

Hyperglycemia itself leads to morphological changes in the emerging placenta. The placenta increases in size due to proliferation of cytotrofoblast, edema and fibrosis of villous stroma, branching and increase in villous total surface. Decrease in the volume of intervillous space leads to blood flow decrease in the feto-placental complex and to chronic hypoxia of the fetus [28].

The features of the fetoplacental complex and placental insufficiency in diabetes mellitus lead to disorganization of the fetus hormonal status. Dyshormonogenesis in combination with chronic intrauterine hypoxia is the cause of impaired generation, differentiation and formation of fetal organs and tissues, including the male reproductive system. It is also known that the health problems of children born to mothers with diabetes mellitus persist in the subsequent years of life. [28]

Developing fetal hyperglycemia and hyperinsulinism and newborn hypoglycemia are very serious complications of the antenatal period. They are conditions for histogenesis processes disorders [29], including proliferation and differentiation of testicular tissue elements. In addition, there is a decrease in the expression of insulin receptors in cells in the convoluted seminiferous tubules. This can lead to suppression of insulin-mediated proliferation of spermatogonia, causing a decrease in their number. [30]

Ketone bodies penetrate through the placenta simultaneously with glucose. [29] As a result, hyperketonemia disrupts the conditions of antenatal development. It can be one of the main pathogenetic factors of histogenesis disturbance, including proliferation and differentiation of testicular tissue elements in the offspring of female rats with experimental type 1 diabetes mellitus.

The results of our serialized research on the endocrine part of male glands confirmed the presence of disorders in the formation of testosterone-producing endocrinocytes. In particular, we revealed a decrease in the number of Leydig cells and a change in the ratio of their morphofunctional types in most of the observation periods, which ultimately leads to a decrease in testosterone concentration in puberty. [31]

It is known that the stimulating effect of spermatogonia proliferative activity of is associated with an increase in testosterone concentration of (to a greater extent) and luteinizing hormone. Steroidogenesis is under the control of the hypothalamic-pituitary-gonadal axis; the main role in its regulation belongs to the luteinizing hormone, which enhances metabolic processes in Leydig cells. [32]

It should be noted that in addition to spermatogenic cells, testosterone regulates the functional activity of sustentocytes, which are no less important components of the spermatogenic layer. [32] It is known that sustentocytes (Sertoli cells) perform not only a supporting function in relation to the cells of the spermatogenic epithelium, but also participate in the endocrine regulation of spermatogenesis. Sertoli cells create a special microenvironment necessary for a directed differentiation of spermatogenic stem cells lying on the basement membrane of the seminiferous tubules. [33] In particular, sustentocytes secrete a number of peptides acting on Leydig cells: inhibin, activin, insulin-like growth factor-I, transforming growth factors alpha and beta and others - they increase expression of luteinizing hormone receptors on Leydig cells and thereby induce steroidogenesis processes. [34] Under the influence of the pituitary follicle-stimulating hormone, sustentocytes synthesize androgen-binding protein, which ensures the transport of sex hormones to spermatogenic cells. Thus, sustentocytes, being an important and specific component of the microenvironment, are actively involved in the spermatogenesis regulation. [35]

At the same time, glucose and lactate serve as the main energy substrates for germ cells. However, the ability of germ cells to use glucose is very low, and lactate is the main survival factor for these cells under hypoxic conditions. [36] The supplier of lactate for germ cells is Sertoli cells. It is logical to assume that the decrease in the stentocytes concentration, which was revealed in many periods of study in the offspring of female rats with experimental type 1 diabetes mellitus, ultimately leads to lactate deficiency.

Some studies show that excessive production of active oxygen forms by the placenta (the state of oxidative stress) is observed during pregnancy complicated with diabetes mellitus, gestosis , epilepsy, and is accompanied by an overload of the antioxidant defense system. [37] It can be assumed that it is these factors that influence the change in epigenetic factors of cell homeostasis and, as a consequence, the proliferative activity of testicular germ cells in the offspring of mothers with diabetes. Besides, it cannot be excluded that lipid peroxidation products play a certain role in the pathogenesis of endocrine compartment dysfunction in experimental animals. [38]

Apoptosis is considered to be a mechanism for avoiding attacks of certain influences [39], for example, prenatal or oxidative stress. The reason for the negative effect of active oxygen forms on the DNA of spermatogenic cells is believed to be the direct action of active radicals on the DNA areas not protected by protamines. The consequence after damage is endonuclease-mediated induction of apoptosis. [40] It is logical to assume that in experimental animals, the increased level of apoptosis in both spermatogenic and endocrine cells is associated with these mechanisms.

The research data testifying testosterone level decrease are in full compliance with the found disturbances of copulative (sexual) behavior of the offspring of female rats with experimental type 1 diabetes mellitus. That is, impaired animals' fertility is aggravated by reduced possibility to realize individuals' reproductive activity.

Conclusion

In general, the obtained results allow us to state that maternal type 1 diabetes mellitus causes disturbance in the formation of the generative function of the testes in the offspring. This formation is associated with a complex of compound mechanisms of neuroendocrine, biochemical, morphological and ultrastructural disorders, which ultimately leads to a decrease in fertility.

Additional Information

The authors declare no conflict of interest.

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