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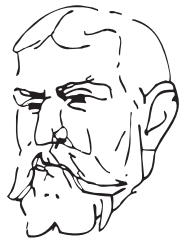

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ORIGINAL ARTICLES

ORIGINAL ARTICLE

STRUCTURAL PECULIARITIES OF RATS' TESTES DEVELOPMENT AFTER INTRODUCTION OF FEMALE HORMONES DURING PREGNANCY

DOI: 10.36740/WLek202302107

Olena A. Hryhorieva[1], Tetiana Topolenko[1], Kateryna Kovalchuk[1], Yuriy Guminskiy[2], Tetyana Matveishyna[1], Nataliya Grinivetska[1], Oleksandra Lazaryk[1]

[1]ZAPORIZHZHIA STATE MEDICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE

[2]PIROGOV NATIONAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim: To define regularities of testicular construction of the rats' offspring at 1-90 days of postnatal life after the introduction of female sex hormones to pregnant rats during the second and third periods of pregnancy

Materials and methods: The study was conducted on the testes of white laboratory rats' offspring during three months of life. Pregnant rats were exposed to intravaginal injection of Utrozhestan during the second and third periods of pregnancy. histological methods were used. Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J).

Results: Administration of female sex hormones to pregnant female rats leads to a reducing of the relative area, occupied by the convoluted seminiferous tubules with lumen, and increasing in relative area, occupied by extracellular matrix, starting from the 30th and up to the 90th observation day in the offsprings' testes. During the third month after birth, in experimental group a decreasing of the testicles' spermatids differentiation degree is determined

Conclusions: During the study, the following results and conclusions were obtained: decreasing of the relative area, occupied by convoluted seminiferous tubules, increasing in relative area, occupied by extracellular matrix, also decreasing Leydig cells relative amount and a delaying of spermatid differentiation process after exposing to female sex hormones during pregnancy, especially during third period, can lead to disruption of spermatogenesis and spermiogenesis in the future.

KEY WORDS: testes, female sex hormones, Utrozhestan, Leydig cells, spermatids

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INRTODUCTION

Nowadays the number of infertile couples in Ukraine increases [1] and according to statistics, this index is about 19%, while the particle of male infertility reaches 50% [2]. This fact is confirmed by WHO documents which underline that the reason of infertility is a man in half number of the cases [3]. In modern conditions, the male reproductive system is strongly influenced by various harmful factors associated with urbanization, environmental pollution, the use of chemical synthesis products [4]. In addition, one of the male infertility reasons lays in extreme sensitivity and susceptibility of the male reproductive system to the effects of various exogenous and endogenous factors that affect it during the fetal period. The negative effect of progesterone application during the prenatal period on the process of spermatogenesis has been experimentally proven [5]. This situation leads to the fact that newborn males who were breastfed were exposed to these hormones, which in turn led to disorders in puberty [6].

Nowadays, evidence the issue of morphogenesis and reactivity peculiarities of the male reproductive glands after changing hormonal balance in the mother – placenta – fetus system remains controversial [7] and look for further clarification and further study both in clinical practice and in experimental conditions.

THE AIM

The aim was to define peculiarities of testicular structure of the rats' offspring in postnatal life after the intravaginal introduction of female sex hormones during the second and the third periods of pregnancy.

MATERIALS AND METHODS

The object of the study is testes of 152 white laboratory rats. In the experiment, four groups of animals were analyzed: the first one includes intact 38 rats, the second one – control (38 rats) offspring animals, which were

exposed to intravaginal introduction of saline solution during the second and the third pregnancy periods; the third group is formed by offspring of rats (38 animals), which were exposed to intravaginal introduction of Utrozhestan during the second pregnancy period (from 8th to 14th day), and the fourth one *38 animals) – offspring of rats, which were exposed to intravaginal introduction of Utrozhestan during the third pregnancy period (from 15th to 21st days). The choice of Utrozhestan in experiment is explained by the simplicity of its use and the prevalence of its use in modern clinical practice. An experimental model of the effect of female sex hormones on the development of the fetus testes was created for the intravaginal injection of utrozestan and saline solution. Pregnant females were introducted with the natural medication of progesterone – «Utrozhestan» with a special injector for intravaginal administration throughout the second and the third weeks of pregnancy. A dose of Utrozhestan was calculated depending on the average weight of the mature female rat and drawn up 100 mg. Newborns were obtained from rats with a dated pregnancy established by vaginal smears stained with methylene blue; the presence of sperm in smears was the evidence of the 0 day of pregnancy. Rats were born full term at days 21-22 after conception. The testes were examined at the 1st, 5th, 14th, 30th, 45th, 60th and 90th days of postnatal life. Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

For histological analysis and morphometric examination, sections were stained with hematoxylin and eosin. In histological samples of testes (×280), a unit area occupied by the convoluted tubules with and without lumen, the extracellular matrix of the interstitium were studied. The size of the external diameter of the convoluted seminiferous tubules was studied. Under a microscope Granum L 60 with oil immersion technique (×700) the relative cells amount at the testicle interstitium were counted: Leydig cells, fibroblasts, fibrocytes. The degree indicator of spermatids differentiation (DISD) – the ratio of the average number of spermatids per unit area of the section of the convoluted seminiferous tubules at VIa and IXa stages of spermatogenesis by classification Leblond C.P. was determined [8]. Five tubules in stages VIa and IXa of spermatogenesis were examined in cross sections of the testes. The external diameter of the tubule (D) and the diameter of the lumen (d) were measured. Then, in the unit area (1000μm), which includes the entire thickness of the spermatogenic cells, the number of spermatids (P) was counted. For each tubule under study, this coution was performed in four fields of view. The calculation of degree indicator of spermatids differentiation (DISD) was made according to the patent (№ 35451A UA, 2001).

Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J). The statistical significance of the obtained differences of indicators in the comparison groups was evaluated using the Mann-Whitney U test and considered to be significant at $p<0,05$, that is generally accepted for biological and medical researches. The numerical data of the obtained results are presented as M ± m (arithmetic mean ± standard error of the mean) [9].

RESULTS

The lumen in the convoluted seminiferous tubules appeared at the 5th day of life, and its diameter tends to increase, reaching maximum values for puberty with a simultaneous decrease in the relative area occupied by the convoluted tubules without lumen, the diameter of the convoluted seminiferous tubules with lumen of pubescent rats is much larger [10].

During the period from the 1st to the 14th day of life, the same growth rate of the convoluted seminiferous tubules diameter of the testes in animals of all observed groups was found. However, after the introduction of female sex hormones in the fetal period, in offspring's testes a probable lag of growth rate of convoluted seminiferous tubules was revealed, especially after the 30th day of life. There is a likely decrease in the relative area of the convoluted seminiferous tubules with lumen in experimental rats, compared to control (56,01 ± 0,81 % and 55,23 ± 0,29 % in experimental groups instead of 61,89 ± 1,04 % in a control) and an increase in the relative area occupied by the fibers and the extracellular matrix of the interstitium (15,81 ± 0,41 % and 16,02 ± 0,45 % in experiment and 14,32 ± 0,28 % in control), from the 30th day of life up to the end of the observation period.

Testicular interstituim of intact and control rats was showed an increasing in the relative number of Leydig cells (especially at the 45th – 33,40 ± 0,75% and the 60th day – 30,70 ± 3,08%). The relative fibroblasts amount decreases with increasing observation period. The relative fibrocytes index gradually increases with increasing life expectancy, reaching a maximum by the 30th day (32,02 ± 4,60%). The results obtained are partially consistent with those of other studies [11, 3].

The relative number of Leydig cells after injection of Utrozhestan to females during pregnancy initially

Table I. The Dynamics of Spermatids Differentiation Degree of the Rats' offspring Testes at the Second and Third Months of Life (M ± m)

Group of Observation	Age of Animals (Day of Life)	
	60th	90th
Intact	0,92±0,08	0,98±0,02
Control	0,94±0,03	0,97±0,03
Experimental	0,89±0,03*	0,91±0,01*
Experimental	0,86±0,02*	0,89±0,02*

Notes:

1. Groups of animals: 3 experimental – offspring of rats, which were exposed to intravaginal injection of Utrozhestan during the second pregnancy period (from 8th to 14th day), 4 experimental – offspring of rats, which were exposed to intravaginal injection of Utrozhestan during the third pregnancy period (from 15th to 21st days);

2. the symbol * means that the result is statistically significant comparison with control group, $p \leq 0,05$.

outweighs their number in intact and control animals and begins to decrease significantly from the 30th day (18,80 ± 2,30% and 20,15 ± 3,08% in experimental animals instead of 30,70 ± 3,08% in the control group at the 60th day; 16,41 ± 2,30% and 18,17 ± 3,08% to 28,03 ± 3,08% appropriately at the 90th day). This is confirmed by previously obtained data on the reduction of the testicles absolute mass and facts of the relationship between low testicular mass and decreased glandular epithelium cells index [7].

The results also show a significant lag in the relative fibroblasts number in the initial lifespan and an increase in this indicator from the 45th to the 90th day of life. The relative fibrocytes number in experimental animals, unlike controls, increases with life expectancy (35,16 ± 2,30% and 37,34 ± 3,08% in the experimental groups versus 30,91 ± 3,05% in the control group at the 14 day; 39,53 ± 2,30% and 37,63 ± 3.80% in the experimental groups versus 29,14 ± 2,31% in the control group at the 60th day).

To calculate the spermatids differentiation degree were selected rats' testes samples from the sixtieth up to the ninetieth days after birth, that is, mature males. During this period of life, it is possible to determine all stages of spermatogenesis in the rats' testicular convoluted seminiferous tubules. Mature germ cells develop from spermatids. This period of their development is called spermiohistogenesis. Degree indicator of spermatids differentiation (DISD) in rats of intact and control groups at the 60th day of postnatal life was equal to 0,92 ± 0,08 and 0,94 ± 0,03, and at the 90th – 0,98 ± 0,02 and 0,97 ± 0,03. DISD is very sensitive to the effects of various factors, in particular, according to these researchers, to the effects of toxic agents (epoxy compounds). During exploration was also found a decreasing of DISD in offspring after the Utrozhestan introduction in the fetal period. The results obtained indicate a delay in the process of differentiation of spermatids after exposing to female sex hormones during pregnancy, especially during third period. At the 60th day in the third experimental group DISD was 0,89 ± 0,03, in the fourth – 0,86 ± 0,02, at the 90th day – 0,91 ± 0,01 and 0,89 ± 0,02 accordingly (Table I).

DISCUSSION

Consequently, the results indicate a delay in the offspring's spermatids differentiation process after exposing to female sex hormones during pregnancy, especially during its third period, and impaired spermato- and spermiogenesis in the future which coincides with the data of other researchers [12, 13].

Thus, during the VIa – IXa stages, the most important cytodifferentiation stages consistently take place, they include the essential and irreversible transformation of such cell organelles as the cell center and the Golgi complex, whose derivatives (tail compartment, acrosome) subsequently provide the two most important process functions – sperm motility and fertilization process. In addition, at stage IXa, the intercellular interactions between Sertoli cells and sperm cells are significantly altered – the previous generation of mature sperm loses its connection with Sertoli cells and moves into the lumen of the testicular tubule [14]. This establishes a closer morphofunctional relationship between the supporting cells and the new generation of differentiating spermatids. The previously obtained results are confirmed by determining the degree of differentiation of spermatids of adult rats, which is probably lower in animals of experimental groups compared with intact and control rats and indicates a delay in spermatogenesis in animals of these groups.

This is confirmed by the obtained data on the decrease of the total sperm count, viable sperm, motile sperm, also sperm membrane was severely altered. After the exposure to progesterone during prenatal period, in male mice the levels of serum sexual hormones was changed: especially, the level of serum testosterone

was decreased against of an increase the level of FSH and LH. This experiment showed that prenatal introduction of progesterone caused significant reduction in the number of spermatozoa and increase in the lumen of seminiferous tubule. There is an opinion that the impairment of male reproduction in mice exposed to progesterone during embryonic development could be mediated through the inhibition of testosterone production [15]. Lue Y. and Wang C. described the effect of synthetic progestins such as levonorgestrel (LNG) in combination with testosterone (T) in male contraceptive clinical trials. The aim was to study the effects of this combination progesterone with testosteron on spermatogenesis in adult rodents. Therefore, according to the results of research, combination LNG + T induced germ cell apoptosis of seminiferous epithelial against the background of testicular hormonal deprivation [16].

That is, assessing the status of sperm cells in stages VIa - IXa under conditions of exposure to female sex hormones (Utrozhestan), possible to evaluate the cellular and intercellular mechanisms of impaired spermatogenesis, in particular the process of spermatids differentiation, especially considering the increased sensitivity of the final stages of spermatogenesis to the effects of exogenous and endogenous factors.

The established effect of Utrozhestan in the second and third periods of pregnancy on the reproductive organs of males after birth is a specific effect characteristic of hormonal drugs. It should be specified in more detail in the instructions for use of the drug and taken into account when prescribing it to pregnant women who are carrying a male fetus.

CONCLUSIONS

1. In the offspring of rats after the introduction of female sex hormones in the second and third periods of pregnancy, compared with the control there is a decrease in the relative area of convoluted seminiferous tubules with lumen (56,01 ± 0,81% and 55,23 ± 0,29 % in the experimental groups versus 61,89 ± 1,04% in the control group, $p \leq 0,05$) and an increase in the area occupied by the extracellular matrix of the interstitium (15,81 ± 0,41% and 16,02 ± 0,45 % in the experiment and 14,32 ± 0,28% in the control, $p \leq 0,05$). The found changes are more pronounced for the offspring of animals, which were exposed to intravaginal injection of Utrozestan during the third pregnancy period.
2. In the testicle interstitium of experimental animals, an increase in the relative number of Leydig cells by the 14th day and a significant decrease in their number from the 30th day, especially pronounced at the 60th and 90th day of the life postnatal period were found (18,80 ± 2,30% and 20,15 ± 3,08% in the experimental groups versus 30,70 ± 3,08% in the control group at 60th day; 16,41 ± 2,30% and 18,17 ± 3,08% versus 28,03 ± 3,08%, additionally, at the 90th day, $p \leq 0,05$).
3. The index of spermatids differentiation of adult rats obtained after intravaginal injection of female sex hormones to pregnant females is significantly lower compared to control and intact rats (at the 60th day of life – 0,89 ± 0,01 and 0,86 ± 0,01 in the experiment and 0,94 ± 0,02 in control; at 90th day – 0,91 ± 0,01 and 0,89 ± 0,01 and 0,97 ± 0,02, additionally).

Prospects for further research are to investigate the effect of female sex hormones on the spermiogenesis of mature man offspring of rates.

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ORCID and contributionship:
Olena A. Hryhorieva: 0000-0002-6101-8322[A,D-F]
Tetiana Topolenko: 0000-0001-7832-7941[B,C]
Kateryna Kovalchuk: 0000-0002-4920-0615[B,D]
Yuriy Guminskiy: 0000-0002-8688-9829[E,F]
Tetyana Matveishyna: 0000-0002-9078-9580[B,C]
Nataliya Grinivetska: 0000-0001-9642-0285[B,C]
Oleksandra Lazaryk: 0000-0003-1937-1226[B,C]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olena A. Hryhorieva
Zaporizhzhia State Medical University
26 Mayakovstiy prospect, 69035 Zaporizhzhia, Ukraine
tel: +3800505450471
e-mail: elengrig212@gmail.com

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