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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

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Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

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In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

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1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

PUBERTY GENESIS OF FEMALES-OFFSPRING RATS BORN TO MOTHERS WITH FETOPLACENTAL INSUFFICIENCY

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In every country of the world women's reproductive health is very important stage of population development. But nowadays, there are a lot of women suffering from infertility [14]. A large number of factors at different stages of life influence the condition of mature female reproductive system. These factors may include such negative points as bad feeding, drugs using, bad habits, mother's acute and chronic diseases, condition of fetoplacental complex during pregnancy, mother's age etc [9]. Due to inadequate interaction between harmful factors and fetus, the fetoplacental complex may react in the wrong way that may cause development of symptoms complex, named fetoplacental insufficiency (FPI), in mother's organism as well as in fetus. It is well known, that FPI influences the pregnancy and delivery characteristics. During FPI remote consequences studying, the different disturbances of physical and intellectual development, the increasing of somatic and infectious diseases in newborns and first year babies have been observed [6]. These disturbances may lead to developing of such pathologies, as arterial hypertension, diabetes mellitus, metabolic syndrome [2, 3, 7, 8]. Other authors have proved that FPI causes perinatal lesions: central nervous system hypoxia and traumatic damage [11], brain blood circulation disturbances, joint dysplasia, cardiac diseases, newborn respiratory distress syndrome etc. [16, 18]. Moreover, it is proved that children born to mothers with FPI have disbalance of immunological parameters which impacts on functioning of their immune system in the future [13].

Nowadays, it is not clear in what way FPI influences the matured females-offspring reproductive system condition and its functioning. It is also known, that in last few decades women have been giving birth at a later age due to numerous reasons. Puberty is a transitional period of reproductive system development, an important step before sexual maturity. Puberty genesis is a difficult, multistage process that leads to the integration of the divided parts into united, functionally active hypothalamic-pituitary-gonadal axis.

Therefore, the purpose of this scientific work was to investigate the puberty development of females-offspring born to mothers of different ages with FPI and to evaluate the efficacy of base and complex pharmacological therapy during pregnancy.

Material and methods. The investigation has been carried out according to the "National General Principles for Animal Researches Ethics" (Ukraine, 2001), which corresponds to the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1985)" [1] and to the "Principles of Bioethics committees", 2012 [4].

Healthy, mature Vistar rat's females of young (3-4 months) and mature (8-10 months) reproductive age with normal four-to-five day's estrus cycle have been used in the experiment. The presence of sperm cells in morning vaginal swabs has been considered to be the first day of pregnancy. Eights groups for 7 pregnant females in each group have been formed: groups 1 and 2 – intact animals of young and mature reproductive age; groups 3 and 4 – young and mature females with experimental FPI accordingly; groups 5 and 6 – young and mature animals with experimental FPI which have obtained from 11th to 19th day of pregnancy the pharmaceutical composition (PhC) containing nontoxic active pharmaceutical ingredients of the base FPI therapeutic group. The PhC has consisted of amino acid (L-arginine) and dicarbonic acid (succinic acid), vitamins (folic acid) and vasoactive drug (Dipyridamole). Groups 7 and 8 – young and mature animals with experimental FPI which have obtained drug of comparison – Dipyridamole mixed with food. Modeling of FPI has been carried out by daily subcutaneous introduction of the 50% tetrachlormethane oil solution in dose of 2 ml/kg of body weight from 12th to 18th day of pregnancy [10].

The first part of animals-offspring has been killed on the 50th day of life (puberty period) by quick decapitation. Offspring were weighted every 10 days for physical development estimation. Females' reproductive organs (uterus and ovaries). thymus gland and adrenal gland have been removed and weighted. Serum blood samples have been used for assessment of estradiol (E2), testosterone (T) and progesterone (P) concentrations. These samples have been stored at temperature -18 °C before analysis. Levels of ex hormones have been evaluated using test-sets "Estradiol-IFA". "Testosterone-IFA" and "Progesterone-IFA" (LLC "Chema", Kyiv). The second part of offspring has been grown up to sexual maturity and has been further researched. The sexual development signs of the second part of animals, namely the anogenital distance and vaginal opening have been investigated.

For ovaries histological researching by one organ from each animal (right and left ovaries are not differed by size and structure components) has been taken [5]. The whole ovary has been fixed in 10% formalin, and then they have been immersed in increasing concentrations of alcohol and infiltrated with paraffin wax. The histological samples have been stained with hematoxylin and eosin. Taking into account, that the most part of animals had closed vagina and didn't have estral cycles, only ovarian offspring histology has been researched. The microscopic analysis of histological specimens has been carried out using light microscope "Granum L 30 (03)", microscopic pictures have been photographed by digital camera "Granum DCM 310". The pictures have been processed in PC Pentium 2,4Ghz using Toup View program.

The normality of samples distribution has been estimated by Kolmogorov-Smirnov test. The statistical analysis has been carried out using parametric and nonparametric methods (Student's test, Mann-Whitney U-test, Newman-Keuls method) depending on the character of data distribution in samples and on the χ^2. Me – median; S – standard deviation; P – statistical significance of differences among groups within the same sex according to criterion. The testing of statistical hypotheses has been carried out at the level of significance ($p<0.05$).

Results and discussion. The evaluation of animals body mass which is integral index of physical condition, has demonstrated females-offspring born to young mothers with experimental FPI haven't had differences in body masses compared with intact group. On the contrary, offspring born to mature mothers with FPI have shown reliable body masses increasing of 18%, group of offspring with FPI+ Dipyridamole – of 34% (Table 1).

Table 1. Females-offspring body mass at the age of 50 days, born to mothers of different reproductive age, g

Group of offspring	Young females, (n=10)	Mature females, (n=9)
1. Intact	51.8±2.6	58.4±2.3
2. Group with FPI	57.0±2.9	69.4±1.8*
3. Group with FPI+ Dip.	53.8±1.3	77.7±1.8*
4. Group with FPI + PhC	68.0±5.5	62.2±5.0

*Notes: * Probability of differences comparing with intact group, p<0.05*

Table 2. Sexual maturing of females-offspring born to mothers with complicated pregnancy

Offspring group	Anogenital distance, mm (50th day of life)		Vaginal opening, day of life	
	young females, n=10	matured females, n=9	young females, n=19	matured females, n=17
1. Intact	7.7±0.2	8.2±0.2	68.3±2.2	73.9±2.7
2 Group with FPI	9.6±0.7*	9.6±0.4*	63.1±1.1*	55.7±0.8*
3. Group with FPI+ Dip.	8.6±0.4	9.3±0.3*	74.3±2.9	52.4±0.7*
4. Group with FPI + PhC	9.7±0.8*	10.0±0.4*	64.3±1.8	61.5±0.5*

Notes: Probability of differences comparing with intact group, p<0.05*

Table 3. Mass indices of reproductive organs of females born to mothers with complicated pregnancy

Offspring group	Uterus, mg		Ovaries, mg	
	young females, n=10	matured females, n=9	young females, n=10	matured females, n=9
1. Intact	78.5±11.8	59.8±2.8	56.7±4.0	51.3±2.4
2 Group with FPI	154.8±30.5*	182.1±63.0*	74.3±2.9*	55.5±3.6
3. Group with FPI+ Dip.	83.9±13.4	116.9±58.5	63.1±8.3	48.3±5.0
4. Group with FPI + PhC	91.9±23.0	188.9±60.6*	55.3±4.7	69.7±7.9

Notes: Probability of differences comparing with intact group of appropriate age, p<0.05*

Sexual maturing comes when body has gained a sufficient amount of energy as a fatty tissue with high levels of leptin and insulin. But other investigations have shown these hormones high levels are not required for maturing onset [20]. Our investigation has demonstrated that offspring born to mothers of mature reproductive age with FPI have had an increased body mass which may be explained by estrogen-depending decreasing of fatty acids transportation to the liver and by lowering of triglycerides blood levels [19]. Estrogen decreasing or loss at the menopausal period as well as due to age or ovarioectomy are caused by fatty acids gaining, lipogenic gene expression rising and insulin resistance [12]. That, in turn, creates the risk of prompt perinatal complication and may lead to the long-term adaptive consequences in offspring such as predisposition to the obesity, type 2 diabetes mellitus and metabolic syndrome in the future life [15, 21].

The start of sexual maturing in rats females is connected with sudden increasing of estrogens blood levels, following by vaginal membrane rupturing and opening, then, first ovulation occurs. At the moment for slaughter (50th day of life), all the rats offspring born to reproductive matured mothers with FPI have had closed vagina, but, 100% of animals with FPI treated with drug of comparison Dipyridamole have had open vagina. The terms of vaginal opening were delayed by PhC using: vaginal opening has been determined in 40% of animals. It may evidence that Dipyridamole causes the earlier sexual maturing of females-offspring, which may have negative consequences for matured female's reproductive system to be infertile and mammary glands cancer developing [17].

Anogenital distance in mammals is one of the sign of sexual dimorphism: in males it is longer than in females. Obtained results have pointed out a certain discrepancy of anogenital lengthening in the almost all offspring born to mothers with FPI, which is typical for estrogen deficiency with accelerated sexual maturing that reflected in term of vaginal opening (Table 2). Moreover, offspring born to mature mothers have demonstrated vaginal opening on average 17 days earlier.

The studying of reproductive organs masses has shown offspring born to young mothers have had uterus 98% heavier than intact animals; offspring born to reproductively matured mothers with FPI have demonstrated uterus 300% heavier than intact group of animals of proper age. The uterus masses in young offspring-females treated by medicines have reached the same levels of masses as intact animals, in contrast to the group of offspring born to matured mothers (Table 3). It may be explained by accelerated sexual maturing of offspring born to matured rats; the size and mass of uterus in matured animals are larger than in infantile ones.

The weight of ovaries has had differences only in offspring born to young females and ovaries weight was increased close to 30%.

The determination of sex hormones levels in rats-offspring born to mothers of young reproductive age with FPI hasn't demonstrated any data differences. On the contrary, offspring born to matured mothers with FPI have had E_2 levels were 57% decreased accordingly; the T/E ratio was 75% increased. Neither PhC nor drug of comparison didn't facilitate the increasing of E_2 levels to intact group born to mature mothers (see table 4). The estimation of progesterone levels hasn't shown any changes in all the groups.

Table 4. Sex hormones levels of 50th-day offspring born to intact and experimental animals, n=9, Me [Min – Max]

Group of offspring	Estradiol, nmol/l	Testosterone, nmol/l	T/E_2 Ratio, SU
1. Born to young mothers	0.60 [0.40-0.70]	0.90 [0.80-1.00]	1.50 [1.14-2.50]
2. Born to young mothers with FPI	0.50 [0.30-0.50]	0.80 [0.70-2.40]	2.67 [1.40-6.00]
3. Born to young mothers with FPI +Dipyridamole	0.40 [0.40-0.50]	0.70 [0.60-0.80]	1.75 [1.50-2.00]
4. Born to young mothers with FPI+PhC	0.50 [0.30-0.70]	1.50 [0.70-3.00]	3.00 [1.14-7.50]
5. Born to intact mature mothers	0.70 [0.50-0.70]	0.80 [0.70-0.90]	1.14 [1.00-1.80]
6. Born to mature mothers with FPI	0.40 [0.30-0.60] P_{5-6}<0.05	0.80 [0.70-0.90] P_{5-6} >0.05	2.00 [1.17-3.00] P_{5-6}<0.05
7. Born to mature mothers with FPI + Dipyridamole	0.30 [0.20-0.04] P_{5-7}<0.05	2.00 [0.90-2.80] P_{5-7} >0.05	5.00 [3.00-14.00] P_{5-7}<0.05
8. Born to mature mothers with FPI +PhC	0.3 [0.20-0.50] P_{5-8}<0.05 P_{6-8} >0.05	2,00 [0.70-0.28] P_{5-8} >0.05 P_{6-8} >0.05	6.67 [1.75-14.00] P_{5-8}<0.05 P_{6-8}<0.05

Notes: n– the number of animal in each group; Me – median; Min – minimal value; Max – maximum value; P – statistical significance of differences among groups of the same sex according to Newman criterion

The histological investigation has determined good separated cortex and medulla of ovary in offspring born to reproductively young intact females. The follicles of all stage of development: primary (primordial), two- and multilayered secondary, early and late, tertiary including late follicles have been detected. The reserve of folliculogenesis – quantity of primordial follicles within normal, has been counted to be up to 8 –9 (Fig. 1). Approximately 1/3 part of all identified follicles has been in the state of physiological atresia or seemed to be atretical bodies in which they have transformed.

Physiologically normal histological structure has been observed in group of offspring born to intact reproductively matured mothers: the quantity of primordial follicles has reached 7-8 in the vision field of microscope; many enough early secondary and mature tertian follicles have been observed (Fig. 2), and atretical follicles were fewer comparing with offspring born to young reproductive females. However, the presence of corpora lutea due to ovulation – the final stage of effective folliculogenesis – hasn't been observed in this group of offspring.

The decreasing of primary follicles number has been detected in groups of offspring born to reproductively young and matured females with FPI. The loss of density of early and late secondary follicles has been determined (Fig. 3, 4). As for tertian follicles, their increasing has been observed, but the most part of them was atretical. No one of females has had ovulation – the effective final stage of folliculogenesis.

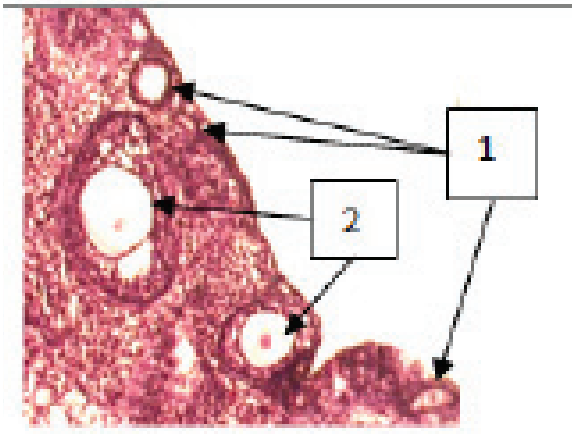

Fig. 1 Ovary of offspring born to young female: secondary follicles early (1) and late (2), Hematoxilin-eosin. ×200

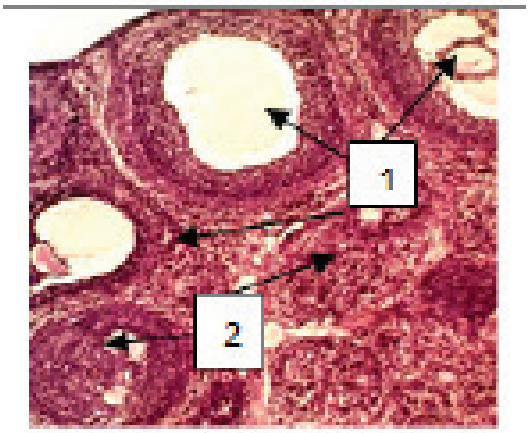

Fig .2 Ovary of offspring born to matured female – tertian mature (1) and secondary late (2) follicles. Hematoxilin-eosin. × 100

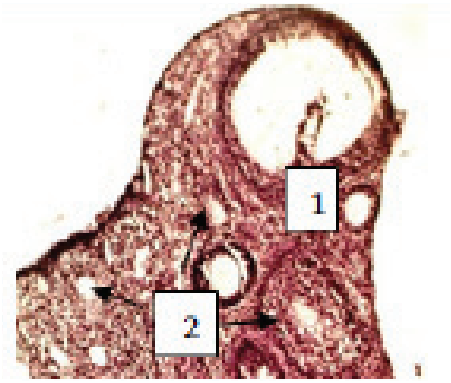

Fig. 3. The ovary of offspring born to young female with FPI. Increasing of follicles atresia: vacuolization of oocyte cytoplasm (1); atretical body on the site of follicle with proliferation of teca cells and shriveling of smooth membrane. Hematoxilin-eosin. ×200

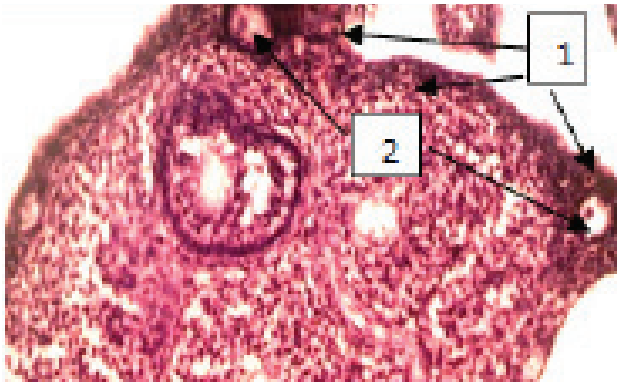

Fig. 4. The ovary of offspring born to mature female with FPI. An emphatic decreasing of primordial (1) and early secondary follicles. Hematoxilin-eosin. ×200.

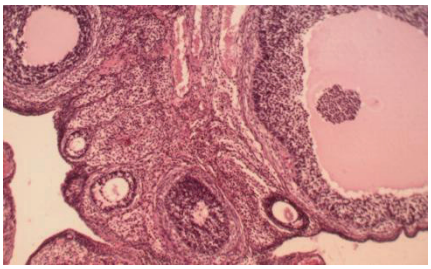

Fig 5. Ovary of rat-offspring born to young female with FPI+PC. Increased follicles density. Hematoxilin-eosin. ×100

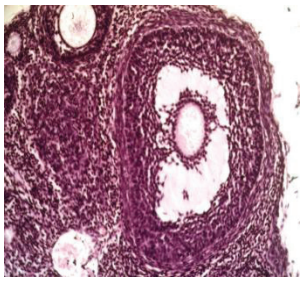

FIg.6. Ovary of rat-offspring born to matured female with FPI+PC. Decreased number of atretical follicles amid normal quantity of follicles of different stage of maturing. Hematoxilin-eosin. ×200

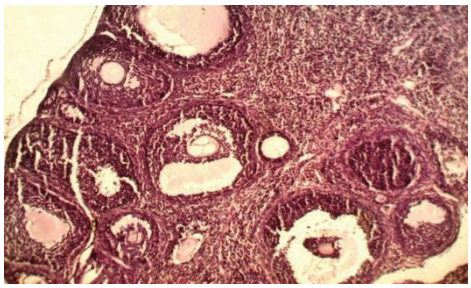

Fig. 7. Ovary of rat-offspring born to young female with FPI+"Dipyridamole". Increased follicles density. Hematoxilin-eosin. ×100

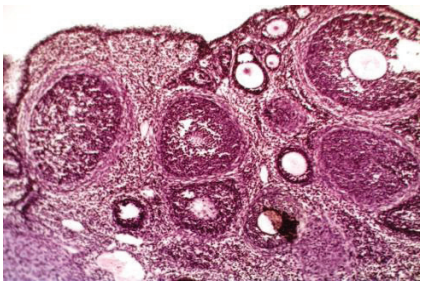

Fig. 8. Ovary of rat-offspring born to matured female with FPI+ Dipyridamole". Normal density of follicles of different stages of maturing. Hematoxilin-eosin. ×100

The introduction of PhC to young females with FPI has led to marked visual increasing of follicles density in their pubertal offspring (Fig. 5). The reserve of folliculogenesis has also been significantly increased – the quantity of primordial follicles in the microscope field of vision has reached 15 and more; the quantity of atretical follicles has declined.

The introduction of PhC to reproductively matured females with FPI has positively influenced their offspring folliculogenesis. Thus, folliculogenesis has reached its final stage – ovulation and appearing of yellow body of estrus in 40% of animals. The number of primordial follicles has decreased in all offspring of this group compared with offspring of young females, but increased compared with control pathology group – 7-8 microscope field of vision. More follicles have reached maturity due to atresia declining (Fig. 6).

Due to introduction of drug of comparison "Dipyridamole" to reproductively young females with FPI, the increasing of follicles density of their offspring's ovaries has been determined (Fig. 7). However, the level of atretical follicles has remained high enough, especially related to late secondary ones. At the same time, the recovering of primordial follicles number (up to 8-9 the microscope field of vision) to intact control level has been observed; the quantity of primary early follicles has been visually increased.

The normal follicles density and recovering of ratio of follicles of different stage of maturity have been visually observed in offspring born to reproductively matured mothers with FPI treated with drug of comparison "Dipyridamole" (Fig. 8). However, the reserve of folliculogenesis has remained a few low – not more than 3-4 follicles in the microscope field of vision. The degree of follicles atresia hasn't visually exceeded the same one in young females' offspring. All the offspring of this experimental group have demonstrated final result of effective folliculogenesis – the presence of "yellow bodies" of estrus.

Therefore, taking into account obtained histological results of puberty rats' ovaries, it may be argued that the decreasing of ovarian follicles density has been determined in offspring born to young females with FPI as well as in offspring born to reproductively matured females with FPI. The disturbance of follicles types' ratio has been detected. There were more early secondary follicles than others. Besides, the reserve of folliculogenesis has declined and number of atretical follicles has significantly increased.

The introduction of PhC to young as well as reproductively matured females with FPI has led to follicles density increasing, recovering the ratio of follicles of different stages of maturing, follicles reserve increasing and follicles atresia declining compared with control pathology in offspring of puberty age.

Although drug of comparison "Dipyridamole" has increased ovarian follicles density, it hasn't completely recovered distribution balance of follicles of different stages of maturing comparing with PhC. "Dipyridamole" has activated the increasing of reserve of folliculogenesis and has less influenced follicles atresia.

Conclusions

1. Fetoplacental insufficiency negatively influences the reproductive system's development of pubertal females-offspring born to mothers of different age.
2. In offspring born to young mothers amid fetoplacental insufficiency the increased anogenital distance is detected, which is the sign of estrogen deficiency.
3. In offspring-females born to reproductively matured mothers with fetoplacental insufficiency more negative changes in reproductive system development have been observed. In particular, growing of body mass and increased anogenital distance amid accelerated sexual development have been determined. The increasing of testosterone level causes inadequate ovaries stimulation which leads to steroid genesis disturbances.
4. During histological investigation of ovarian structure of pubertal rats born to mothers of both groups of age, the decreasing of follicles density, the disturbance in follicles types ratio – early secondary follicles were prevailed, declining folliculogenesis reserve and increased number of atretical follicles have been observed.
5. The introduction of pharmaceutical composition to pregnant rats of both groups of age amid fetoplacental insufficiency leads to stronger normalization of reproductive system development in females-offspring than using of drug of comparison.

REFERENCES

1. Верховна Рада України. Європейська конвенція про захист хребетних тварин, що використовуються для дослідження або інших наукових цілей від 18.03.1986. http://zakon4.rada.gov.ua/laws/ main?find=1&sp=i&user=c393&text=%F2%E2%E0.
2. Макаров О. В., Волкова Е. В., Лысюк Е. Ю., Копылова Ю. В. Фетоплацентарный ангиогенез у беременных с плацентарной недостаточностью // Акушерство гинекология репродукция 2013, том 7, №3, с.13-19.
3. Макацария А. Д., Бицадзе В. О., Баймурадова С. М., и др. Профилактика повторных ослонений беременности в условиях тромбофилии. // Руковод. для врачей. М. 2008; 152.
4. Наказ № 1287 від 19.11.2012. Положення про комитет з питань етики (биоетики) http://www.mon.gov.ua/ua/activity/63/64/ normativno-pravova-baza.
5. Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ, М., 2005, с. 41-46.
6. Савельева Г. М., Федорова М. В., Клименко П. А. и др. Плацентарная недостаточность. – М.: Медицина, 1991. – 272.
7. Савельева Г. М., Курцер М. А., Шалина Р. И. Материнская смертность и пути ее снижения // Акушерство и гинекология. 2009; 3: 11-15.
8. Стрижаков А. Н., Мусаев З. М., Тимохина Т. Ф., Наумчик Б. И., Буданцева А. В. Системные нарушения гемодинамики при синдроме задержки роста плода как фактор риска гипоксически-ишемических поражений ЦНС и отклонений психомоторного развития детей // Акушерство и гинекология.2003; 1: 11-6.
9. Ціборовський О. М. Здоров'я населення і фактори ризику, що впливають на його стан, як об'єкт управління (огляд літератури) // Україна. Здоров'я нації. 2015. № 2 (34) с.13-19.
10. Яковлєва Л. В., Зайченко Г. В., Ципкун А. Г. [та ін.]. Доклінічне вивчення лікарських засобів, призначених для лікування плацентарної дисфункції [Текст] : метод. рекомендації / ДФЦ МОЗ України ; – К., 2009.
11. Яловчук А. В. Віддалені результати порушень нервової системи у немовлят, народжених від матерів з ускладненим перебігом вагітності // Буковинський медичний вісник Том 10, №2, 2006 с. 83-86.
12. Barros RPA, Gustafsson JÅ. Estrogen receptors and the metabolic network. //Cell Metab 14: 289–299, 2011. doi:10.1016/j.cmet.2011.08.005.
13. Bekmukhambetov Y, Mamyrbayev A, Dzharkenov T. at. all. Metabolic and immunologic aspects of fetoplacental insufficiency. // Am J Reprod Immunol. 2016;76(4):299-306. doi: 10.1111/aji.12544.
14. Chelsea B. Polis, Carie M. Cox, Özge Tunçalp, Alexander C. McLain, Marie E. Thoma, Estimating infertility prevalence in low-to-middle-income countries: an application of a current duration approach to Demographic and Health Survey data. // Human Reproduction, 2017;32(5):1064–1074. doi.org/10.1093/humrep/dex025.
15. Cheong JN, Wlodek ME, Moritz KM, Cuffe JSM. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. // J Physiol 594: 4727–4740, 2016. doi:10.1113/JP271745.
16. Giussani DA. The fetal brain sparing response to hypoxia: physiological mechanisms. // J Physiol. (2016) 594:1215–30. doi: 10.1113/JP271099.
17. Lee H.B., Han W. Unique features of young age breast cancer and its management // J. Breast Cancer. – 2014. 17(4). – P. 301-7. doi: 10.4048/jbc.2014.17.4.301.
18. Malhotra A, Allison BJ, Castillo-Melendez M, Jenkin G, Polglase GR, Miller SL. Neonatal Morbidities of Fetal Growth Restriction: Pathophysiology and Impact. // Front Endocrinol (Lausanne). 2019 Feb 7;10:55. doi: 10.3389/fendo.2019.00055.
19. Palmisano BT, Zhu L, Stafford JM. Estrogens in the regulation of liver lipid metabolism. // Adv Exp Med Biol 1043: 227–256, 2017. doi:10.1007/978-3-319-70178-3_12.
20. Rahim Ullah, Ali Raza, Naveed Rauf, Yi Shen, Yu-Dong Zhou, Junfen Fu. Postnatal Feeding With a Fat Rich Diet Induces Precocious Puberty Independent of Body Weight, Body Fat, and Leptin Levels in Female Mice. Front. Endocrinol., 08 November 2019 | https://doi.org/10.3389/fendo.2019.00758.
21. Stojanovska V, Sharma N, Dijkstra DJ, Scherjon SA, Jäger A, Schorle H, Plösch T. Placental insufficiency contributes to fatty acid metabolism alterations in aged female mouse offspring. // Am J Physiol Regul Integr Comp Physiol. 2018 Dec 1;315(6):R1107-R1114. doi: 10.1152/ajpregu.00420.2017.

SUMMARY

PUBERTY GENESIS OF FEMALES-OFFSPRING RATS BORN TO MOTHERS WITH FETOPLACENTAL INSUFFICIENCY

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The purpose of this scientific work was to investigate the development of puberty in females-offspring born to mothers of different age with fetoplacental insufficiency (FPI) and to evaluate efficacy of base and combined drug therapy during pregnancy.

Negative influence of FPI on the puberty genesis of females-offspring born to mothers of different reproductive age is considered to be the results of the investigation. In particular, the increased anogenital distance, which is the sign of estrogen deficiency, has been observed in females-offspring born to reproductively young mothers with FPI. Females-offspring born to reproductively matured mothers with FPI have demonstrated more negative changes of reproductive system development. That is, body mass and anogenital distance increasing amid accelerated sexual development have been detected. The increasing of testosterone level has caused inadequate ovaries stimulation which has led to steroid genesis disturbances. During histological investigation of ovarian structure of pubertal rats born to mothers of both groups of age, the decreasing of follicles density, the disturbance in follicles types ratio – early secondary follicles were prevailed, declining folliculogenesis reserve and increased number of atretical follicles have been observed. The introduction of pharmaceutical composition to pregnant rats of both groups of age amid fetoplacental insufficiency leads to stronger normalization of reproductive system development in females-offspring than using of drug of comparison.

Keywords: fetoplacental insufficiency, mother's age, ovaries' histology, pharmaceutical correction.

РЕЗЮМЕ

ПУБЕРТАТОГЕНЕЗ САМОК ПОТОМКОВ КРЫС, РОЖДЕННЫХ ОТ МАТЕРЕЙ С ФЕТОПЛАЦЕНТАРНОЙ НЕДОСТАТОЧНОСТЬЮ

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Целью исследование явилось изучение течения пубертатного периода самок потомков, рожденных от матерей различного возраста с фетоплацентарной недостаточностью, и оценка эффекта базовой и комплексной медикаментозной терапии во время беременности.

Результаты исследования выявили негативное влияние фетоплацентарной недостаточности на пубертатогенез самок потомков, рожденных от матерей различного репродуктивного возраста. У потомков, родившихся от репродуктивно молодых матерей на фоне фетоплацентарной недостаточности, наблюдалось большее аногенитальное расстояние, что является признаком эстрогенодефицита. У самок потомков, родившихся от репродуктивно зрелых матерей с фетоплацентарной недостаточностью, наблюдались более негативные изменения в становлении репродуктивной системы, в частности выявлено повышение массы тела, увеличение аногенитального расстояния на фоне ускоренного полового созревания; увеличенное соотношение половых гормонов в сторону тестостерона, который способствует неадекватной стимуляции яичников и как следствие, нарушению стероидогенеза. При исследовании гистологической структуры яичников крыс пубертатного периода, рожденных от матерей двух возрастных групп, отмечалось уменьшение объемной плотности всех фолликулов, нарушение соотношения их типов - преимущественно наблюдались вторичные ранние фолликулы, уменьшение резерва фолликулогенеза, увеличение уровня атрезии фолликулов. Применение фармкомпозиции у матерей двух возрастных групп в течение беременности на фоне фетоплацентарной недостаточности ведет к большей нормализации репродуктивной функции потомков женского пола, чем препарат сравнения «Дипиридамол».

რეზიუმე

ფეტოპლაცენტური უკმარისობით დედების ნაყარი მდედრი ვირთაგვების პუბერტატოგენეზი

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კვლევის მიზანს წარმოადგენდა ფეტოპლაცენტური უკმარისობით მქონე სხვადასხვა ასაკის დედების ნაყარი მდედრი ვირთაგვების პუბერტატული პერიოდის მიმდინარეობის და მაკეობის პერიოდში ბაზისური და კომპლექსური მედიკამენტური მკურნალობის ეფექტების შეფასება.

კვლევის შედეგებით გამოვლენილია ფეტოპლაცენტური უკმარისობის ნეგატიური გავლენა სხვადასხვა რეპროდუქციული ასაკის დედების ნაყარი მდედრი ვირთაგვების პუბერტატოგენეზზე. სახელდობრ, მდედრობითი სქესის ნაყარს, დაბადებულს ახალგაზრდა რეპროდუქციული ასაკის დედებიდან ფეტოპლაცენტური უკმარისობის ფონზე, აღენიშნებოდა მეტი ანოგენიტალური მანძილი, რაც ესტროგენდეფიციტის ნიშანს წარმოადგენს; მდედრობითი სქესის ნაყარს, დაბადებულს ზრდასრული რეპროდუქციული ასაკის დედებიდან ფეტოპლაცენტური უკმარისობის ფონზე, აღენიშნა უფრო ნეგატიური ცვლილებები რეპროდუქციული სისტემის ჩამოყალიბებაში, კერძოდ, დადგენილია სხეულის მასის მომატება, ანოგენიტალური მანძილის გაზრდა სქესობრივი მომწიფების დაჩქარების ფონზე. სასქესო ჰორმონების თანაფარდობის შეცვლა ტესტოსტერონის სასარგებლოდ ხელს უწყობს საკვერცხეების არაადეკვატურ სტიმულაციას და შედეგად – სტეროიდოგენეზის დარღვევას. ორი ასაკობრივი