CHANGES IN THE REPRODUCTIVE SYSTEM OF RATS AND ITS GETS IN ANTENATAL PERIOD AFTER SESAME SEEDS OIL MEAL SUPPLEMENT

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The article describes reactive changes in the reproductive system of rats after sesame seeds oil meal supplement. The experiment involved 80 female and 32 male rats one month of birth, weighing 190–210 g, equally divided into 8 groups. Animals were administered suspension of sesame seeds oil meal according to the group affiliation. Oil meal suspension was prepared on distilled water. The animals received suspension daily intragastrically at a dose of 10 mg/100 g body weight, 1 ml of volume, in accordance with the group affiliation and schedule. The control animals received 1 ml of distilled water. All animals were daily monitored. The following parameters were assessed depending on the duration of sesame seeds oil meal intake: embryonic (pre- and post-implantation) mortality; backwardness in a form of decreased body weight and cranio-caudal sizes of a fetus, as well as condition of the reproductive system in the males and in the females. The experiment revealed that additional supplement of sesame seeds oil meal causes gonadotropic effect: an increase of testes and prostate mass ratio in males, a decrease of pathological sperm cells number; the females demonstrated decreased number of the atretic bodies and increased number of Graafian follicles in the ovaries. Depending on the duration of the oil meal intake, the females showed an increase in the average number of the pups in a litter and an increase in the number of implantation sites per one female rat, a reduction of total embryonic mortality, fetal weight and cranio-caudal size, as well as placenta weight. In addition, the study showed decreased probability of hemorrhage in the fetal meninges and non-significantly increased probability of local bleeding, possibly due to hypercoagulability.

Keywords: sesame seeds oil meal, rats, reproductive system, pre-implantation mortality, post-implantation mortality, cranio-caudal sizes, fetal-placental index

All functional activity of the body is focused on the maintaining of the reproductive system and on the optimization of the birth process. Condition of the reproductive system is a direct reflection of the internal dynamic stability, as well as properties and stability of the basic physiological body functions.

For this system, the indicators of the normal activity include: mass ratios of the testes, parorchis, and prostate; sperm quality; number of the atretic bodies and Graafian follicles, follicles with a single granulosa cells layer, follicles with two or more granulosa cells layers.

The reproduction parameters include: number of the fetuses and the corpora lutea per female rat; pre-implantation embryonic mortality; post-implantation embryonic mortality; total mortality; average weight of a fetus; cranio-caudal sizes of a fetus; weight / length ratio of a fetus; average weight of placenta; fetal – placental index [5].

Administration of the biologically active substances, for example, sesame seeds oil meal, is one of the methods to correct the functionality of these systems.

Sesame seeds oil meal with its specific chemical composition is an optimal substation for these purposes.

It contains saturated and polyunsaturated fatty acids, triglycerides and glyceryl esters, A, B_1, E, C, PP vitamins, affecting the blood composition, rate of growth, condition of the nervous and digestive systems [1, 2, 3, 4].

The oil meal contains different mineral components including calcium, phosphorus, iron, potassium, magnesium, making it ideal for bones and joints. Composition of the sesame seeds oil meal also includes phytin which helps to restore mineral body balance; among other components there are dietary fibers, lecithin and sesamin – an antioxidant which uses for lots of diseases, including cancer, and which is able to reduce blood cholesterol level. β-phytosterol, also found in the oil meal, has similar cholesterol reduction effect. Oil meal anti-inflammatory effect is associated with β-phytosterol which reduces the risk of atherosclerosis [1, 2, 3, 4].

Sesame seeds oil meal is a waste of food production, thus, it is cheaper raw material than whole sesame seeds or oil. That is why the oil meal is economically more interesting, as small concentrations of the active substrates is enough to achieve therapeutic effect [3].

Seeing that sesame seeds oil meal can be used to correct different pathological conditions and to maintain the homeostasis in a constantly changing environment, **the aim of this work** was to study the reproductive system in rats and its get in the antenatal period to determine possible biological effects of oil meal.

To achieve this aim, the following indicators had to be analyzed: embryonic (pre-and post-implantation) mortality depending on the duration of sesame seeds oil meal intake; backwardness in a form of decreased body weight

and decreased cranio-caudal sizes of a fetus, fetal-placental index, as well as condition of the males and females reproductive system.

Materials and methods of research

The study included healthy mature outbred rats kept in a vivarium under standard conditions.

The study involved 80 females and 32 males one month of birth, weighing 190–210 g. The rats were equally divided into 8 groups (Table 1) and received suspension of sesame seeds oil meal in accordance with the group affiliation.

Suspension of sesame seeds oil meal was prepared on distilled water. The animals received suspension on daily basis intragastrically at a dose of 10 mg/100 g body weight, 1 ml of volume, in accordance with the group affiliation and schedule. The control animals received 1 ml of distilled water. All animals were strictly monitored.

In order the females to have known date gestation, the researchers used 4–4,5 months rats The males (1 male per 2–3 females) were brought to the females in the evening, and in the morning the females were taken the vaginal smears. Since rats usually have breeding in 1–2 am, the day of sperm detection in the smear was considered as the first pregnancy day. After that the males were taken from the females. Pregnant rats were kept in the individual cages with bedding necessary for making a nest. The animals were monitored from the first pregnancy day. The researches registered females' condition and behavior, the dynamics of the body weight, duration of pregnancy and the process of labor.

The study results were analyzed after the pregnant females were killed by cervical dislocation on 20th pregnancy day. The following parameters were examined at autopsy: number of the corpora lutea in the ovaries, number of the implantation site in the uterus, number of alive and dead fetuses. Condition of the placenta was also analyzed. Embryonic material was carefully examined with special attention to the anatomical structure of the fetuses, its weight and cranio-caudal sizes [5].

The signs of sesame seeds oil meal negative effects considered to be embryonic (pre- and post-implantation) mortality and backwardness in a form of decreased body weight and cranio-caudal sizes of the fetus.

Pre-implantation embryonic mortality was calculated as a difference between the number of the corpora lutea and the number of the implantation sites in the uterus.

Post-implantation mortality was calculated as a difference between the number of the implantation sites and the number of the alive fetuses.

Table 1

Groups of the experimental animals

Group number	Study materials
1	Suspension of sesame seeds oil meal was administered to the males and the females for 21 days before the fertilization.
2	Suspension of sesame seeds oil meal was administered to the females for 21 days before the fertilization; the males received distilled water.
3	Suspension of sesame seeds oil meal was administered to the males for 21 days before the fertilization; the females received distilled water.
4	Suspension of sesame seeds oil meal was administered to the females for 21 days before pregnancy, as well as from the 1st to the 13th day of pregnancy.
5	Suspension of sesame seeds oil meal was administered to the females for 21 days before pregnancy, as well as from the 14th to the 20th day of pregnancy.
6	Suspension of sesame seeds oil meal was administered to the females from the 1st to the 13th day of pregnancy.
7	Suspension of sesame seeds oil meal was administered to the females from the 14th to the 20th day of pregnancy.
8	Control group of animals
9	Suspension of sesame seeds oil meal was administered to the males for 41 days.

Other assessed parameters included the overall embryonic mortality, litter size, number of the alive fetuses [5].

Digital material was statistically analyzed with Student's t-test using the program Sigma Stat 6.0 [5].

Results of research and their discussion

During the entire pregnancy, no significant differences in the dynamics of the body weight in the pregnant rats were revealed between the experimental and the control groups (Table 2).

Table 3 shows the results of the reproductive function quantitative assessment in rats and its gets in antenatal period after sesame seeds oil meal supplement.

The results presented in the Table 3 shows that only in the 7th experimental group the average number of the pups in a litter corresponded to a level observed in the control group, while in the other groups the average number of the pups was significantly greater: 22,4 % more in the 1st group, 26,2 % more in the 2nd group, 18,7 % more in the 3rd group, 31,8 % more in the 4th group, 24,3 % more in the 5th group and 13,1 % more in the 6th group.

Table 2

History of body weight in pregnant rats

Gestation age	Animals group							
	1	2	3	4	5	6	7	8
Before the study	209,6 ± 8,17	197,1 ± 7,29	205,5 ± 6,98	192,8 ± 7,52	201,3 ± 7,85	213,4 ± 6,62	202,2 ± 6,67	208,7 ± 7,51
7 days	245,6 ± 7,85	247,1 ± 7,17	229,9 ± 8,05	231,8 ± 8,34	232,3 ± 7,20	246,4 ± 9,36	229,1 ± 7,10	237,3 ± 7,59
14 days	281,6 ± 10,14	284,3 ± 11,09	257,9 ± 8,78	272,5 ± 10,08	265,3 ± 8,22	283,4 ± 10,20	252,1 ± 8,32	268,7 ± 9,14
20 days	310,6 ± 9,94	316,3 ± 11,07	289,1 ± 9,54	309,2 ± 9,59	304,7 ± 10,06	306,3 ± 9,80	288,6 ± 10,68	301,6 ± 10,56

Table 3

The reproductive system of rats and its gets in antenatal period after sesame seeds oil meal supplement

Indicators		Control group (8)	Experimental groups received sesame seeds oil meal						
			1	2	3	4	5	6	7
Number of	Female rats	10	10	10	10	10	10	10	10
	Fetuses / per one female rat	10,7 ± 0,38	13,1 ± 0,41[1]	13,5 ± 0,45[1]	12,7 ± 0,37[1]	14,1 ± 0,39[1]	13,3 ± 0,47[1]	12,1 ± 0,43[1]	11,2 ± 0,37
	Corpora lutea / per one female rat	13,8 ± 0,44	15,1 ± 0,49	14,9 ± 0,54	14,3 ± 0,36	16,7 ± 0,57[1]	15,5 ± 0,51[1]	13,9 ± 0,43	13,1 ± 0,46
	Implantation sites / per one female rat	11,1 ± 0,35	13,3 ± 0,44[1]	13,8 ± 0,39[1]	12,9 ± 0,45	15,7 ± 0,46[1]	14,2 ± 0,41[1]	12,5 ± 0,41[1]	11,9 ± 0,32
Pre-implantation embryonic mortality	Absolute number	2,7 ± 0,08	1,8 ± 0,06[1]	1,1 ± 0,07[1]	2,2 ± 0,07[1]	1,0 ± 0,04[1]	1,3 ± 0,05[1]	1,4 ± 0,05[1]	1,2 ± 0,08[1]
Post-implantation embryonic mortality	Absolute number	0,4 ± 0,012	0,2 ± 0,006[1]	0,3 ± 0,009[1]	0,2 ± 0,005[1]	1,6 ± 0,051[1]	0,9 ± 0,032[1]	0,4 ± 0,013	0,7 ± 0,020[1]
Total mortality	Absolute number	3,1 ± 0,08	2,0 ± 0,07[1]	1,4 ± 0,04[1]	2,4 ± 0,08[1]	2,6 ± 0,09[1]	2,2 ± 0,08[1]	1,8 ± 0,06[1]	1,9 ± 0,07[1]
Average fetal body weight, g	20th day of the embryonic development	1,37 ± 0,046	1,62 ± 0,053[1]	1,58 ± 0,046[1]	1,35 ± 0,038	1,61 ± 0,047[1]	1,55 ± 0,060[1]	1,48 ± 0,052	1,51 ± 0,051
Cranio-caudal sizes of a fetus, cm		2,62 ± 0,091	3,03 ± 0,085[1]	3,10 ± 0,099[1]	2,81 ± 0,101	3,14 ± 0,097[1]	3,09 ± 0,108[1]	2,65 ± 0,098	2,70 ± 0,086
Weight / length ratio of a fetus		0,52 ± 0,017	0,53 ± 0,015	0,51 ± 0,018	0,48 ± 0,013	0,51 ± 0,017	0,50 ± 0,016	0,56 ± 0,021	0,56 ± 0,016
Average weight of placenta, g	Fetal-placental index	0,38 ± 0,013	0,43 ± 0,012[1]	0,41 ± 0,013	0,34 ± 0,011[1]	0,49 ± 0,018[1]	0,47 ± 0,015[1]	0,38 ± 0,011	0,40 ± 0,012
Fetal – placental index		0,27 ± 0,009	0,27 ± 0,007	0,26 ± 0,008	0,25 ± 0,008	0,30 ± 0,008[1]	0,30 ± 0,007[1]	0,26 ± 0,011	0,26 ± 0,010
Results of the fetuses visual examination									
Number of the examined fetuses	Absolute number	113	137	142	124	149	126	118	110
Number of the fetuses with malformations	Total number / %	3 / 2,6	0	0	1 / 0,8	0	2 / 1,6	2 / 1,7	0
Results of the internal organs examination in the fetuses									
Number of the examined fetuses	Absolute number	113	137	142	124	149	126	118	110
Number of the fetuses with malformations	Total number / %	7 / 6,1	0	1 / 0,7	2 / 1,6	0	1 / 0,8	3 / 2,5	0
Results of the skeletal development examination in fetuses after sesame seeds oil meal supplement									
Number of the examined fetuses	Absolute number	113	137	142	124	149	126	118	110
Breastbone ossification delay	Total number / %	0	0	0	0	0	0	0	1 / 0,9
Hyoid bone ossification delay	Total number / %	2 / 1,7	0	0	2 / 1,6	0	0	2 / 1,7	0
Metacarpal and metatarsal bones ossification delay	Total number / %	0	0	1 / 0,7	0	0	1 / 0,8	1 / 0,8	0
Diamelia	Total number / %	0	0	0	0	0	0	0	0

N o t e . The differences are significant for $P < 0,05$: [1] – as compared to the control group of animals.

In the 1st, 2nd, 3rd, 6th and 7th experimental groups, the number of the corpora lutea per one female was about the same and corresponded to the level in the control group. Herewith, in the 4th group, the average number of the corpora lutea per one female was 21,0 % more and in the 5th group – 12,3 % more than in the control group.

In the 3rd and 7th groups, the number of the implantation sites per one female corresponded to the level in the control group, while in the 1st, 2nd, 4th, 5th, and 6th groups it was more than in the control group on 19,82 %, 24,32 %, 41,44 %, 27,93 %, and 12,62 % respectively.

In all experimental groups, pre-implantation embryonic mortality was significantly less than in the control group: 33,33 % less in the 1st group, 59,26 % less in the 2nd, 18,52 % less in the 3rd, 62,96 % less in the 4th, 51,85 % less in the 5th, 48,15 % less in the 6th and 55,56 % less in the 7th group.

In the 6th experimental group, post-implantation embryonic mortality corresponded to the level of the control group. In the 1st and 3rd groups, it was 50 % less than in the control one, while in the 2nd experimental group it was 25 % less. In the 4th, 5th and 7th experimental groups, post-implantation embryonic mortality was 300, 125 and 75 % more than the control group respectively.

Thus, the overall embryonic mortality in all the experimental groups was significantly less than in the control group: in the first group it was 35,48 % less, in the second – 54,84 % less, in the third – 22,59 % less, in the fourth – 16,13 % less, in the fifth – 29,03 % less, in the 6th – 41,94 % less, and in the 7th group it was 38,71 % less than in the control group.

The average weight of the fetuses in the 3rd, 6th and 7th groups almost did not differ from the control level. In other experimental groups, the average weight of the fetuses was significantly more than the control group: in the 1st group – 18,3 % more, in the 2nd – 15,3 % more, in 4th group – 17,5 % more, and in the 5th group – 13,1 % more than in the control group.

Cranio-caudal size of the fetuses in the 3rd, 6th and 7th groups corresponded to the control level, and in the 1st, 2nd, 4th and 5th groups it was significantly more than in the control group – on 15,6; 18,3; 19,8 % and 12, 6 %, respectively.

In all groups, the weight / length ratio of the fetuses was approximately the same and corresponded to the control level.

In the 2nd, 6th and 7th groups, the average weight of the placenta corresponded to the control level. In the 1st, 4th and 5th groups, placenta weighted 13,2; 28,9 and 23,7 % more respectively than in the control group, and in the 3rd group, it was 10,5 % less than in the control group.

In all the experimental groups, except for the 4th group, the fetal-placental index was approximately the same and matched the control. In the 4th group, it was 11,1 % more than the control.

Visual examination showed no malformations in the 1st, 2nd, 4th and 7th groups. Herewith, there were 3 pups with malformations (2,6 %) in the control group, 1 pup in the 3rd group (0,8 %), 2 pups in the 5th group (1,6 %) and 2 pups in the 6th group (1,7 %).

The results of the fetuses' internal organs examination revealed malformations in 7 pups (6,1 %) from the control group, in 1 pup from the 2nd and 1 pup from the 5th group (0,7 and 0,8 %), in 2 pups from the 3rd group (1,6 %), and in 3 pups from the 6th group (2.5 %).

The results of the fetuses' skeletal system examination after sesame seeds oil meal supplement revealed breastbone ossification delay in 1 pup (0,9 %) from the 7th experimental group.

Hyoid bone ossification delay was registered in 2 pups from the control group (1,7 %), as well as in the 3rd and 6th experimental groups (1,6 and 1,7 % respectively). Metacarpal and metatarsal bones ossification delay was revealed in 1 fetus from the 2nd, 5th and 6th experimental groups (0,7; 0,8 and 0,8 %, respectively). No diamelia was found.

The results of the internal organs examination showed 1 pup (0,8 %) with vascular congestion in the liver in the 6th group. Hemorrhages in the liver were found in 1 fetus (0,8 %) from the 3rd group. Subcutaneous hemorrhages were revealed in 1 fetus from the 2nd and 6th groups (0,7 and 0,8 % respectively). Hemorrhages in the kidneys were revealed in 1 fetus (0,8 %) from the 6th experimental group. Hemorrhages in the intestine and stomach were registered in 1 fetus (0,8 %) from the 3rd and from the 5th groups. Hemorrhages in the meninges were revealed in 7 fetuses (6,1 %) from the control group.

The results of the reproductive system assessment in the males showed (Table 5), that testes mass ratio in the 1st experimental group was similar to the control group, while in the 9th group, it was significantly higher (on 10,6 %). In the experimental groups, the parorchis mass ratio did not differ from the control level. The prostate gland mass ratio in the 9th experimental group was 12,1 % more than the control group, and in the 1st group it was equal to the control. In rats received sesame seeds oil meal, a number of the abnormal sperm cells were less than in the control group (on 19,4 % in the 1st experimental group and on 16,0 % in the 9th group).

Table 5

Functional condition of the testes in rats after sesame seeds oil meal supplement

Indicators	The control group (8)	The groups received sesame seeds oil meal	
		1	9
Testes mass ratio	8,5 ± 0,29	8,7 ± 0,27	9,4 ± 0,31[1]
Parorchis mass ratio	3,9 ± 0,13	4,3 ± 0,15	4,2 ± 0,14
Prostate gland mass ratio	3,3 ± 0,12	3,3 ± 0,10	3,7 ± 0,13[1]
Pathological forms of the sperm cells, %	38,7 ± 1,35	31,2 ± 1,06[1]	32,5 ± 1,14[1]

N o t e . In this table the differences are significant for P < 0,05: [1] – as compared to the control group of animals.

Table 6

The results of the ovarian structures quantitative assessment after sesame seeds oil meal supplement

Ovarian structures	The control group (8)	The 1st group
Atretic bodies in the ovaries	1193,5 ± 42,93	947,4 ± 32,21[1]
Graafian follicles	6,5 ± 0,21	7,7 ± 0,24[1]
Follicles with a single granulosa cells layer	657,8 ± 24,95	629,9 ± 17,55
Follicles with two and more granulosa cells layers	82,3 ± 2,72	106,2 ± 3,82[1]

N o t e . In this table the differences are significant for P < 0,05: [1] – as compared to the control group of animals.

The results of the reproductive system assessment in the females showed (table 6), that in the 1st experimental group, number of the atretic bodies in the ovaries was 20,6% less than in the control group; number of the Graafian follicles was 18,5% more as compared with the control level; number of the follicles with two or more granulosa cells layers was 29,0% more. However, number of the follicles with a single granulosa cells layer was 4,2% less as compared with the control group.

Thus, based on the study results the following conclusions can be made:

– sesame seeds oil meal supplement causes gonadotropic effect: an increase of the testes and prostate gland mass ratio, a decrease of the pathological sperm cells in males, a decrease of the atretic bodies in the ovaries and an increase of the mature follicles.

– depending on the duration of the oil meal intake by the females, the average number of the pups per litter and the implantation sites per female increases;

– after sesame seeds oil meal supplement, overall embryonic mortality decreases, weight and cranio-caudal size of the fetuses increases, as well as the average weight of the placenta; probability of the hemorrhages in the meninges decreases, but the probability of local bleeding increases, possibly due to increased blood coagulation.

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