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# NEUROENDOCRINE-IMMUNE RELATIOSHIPS IN RATS FEMALES

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## Abstract

**Background.** Previously, in line with the concept of the neuroendocrine-immune complex, we analyzed the relationships between the parameters of the autonomic nervous and endocrine systems, on the one hand, and the parameters of immunity, on the other hand, in male rats. The purpose of this study is to analyze such interactions in female rats. **Material and methods.** In 60 females of rats, parameters of HRV, blood levels of hormones and electrolytes as well as parameters of leukocytogram, immunocytogram, thymocytogram and splenocytogram were determined. The coefficients of canonical correlation R between neuroendocrine parameters, on the one hand, and parameters of immunity, on the other hand, were calculated. **Results.** The following values of R for neuroendocrine parameters were found. Sympathetic tone: 0,702; Vagal tone: 0,756; Moda HRV: 0,896; the thickness of the Fascicular zone of adrenal cortex: 0,727; Glomerular zone: 0,650; Reticular zone: 0,442; plasma level of Corticosterone: 0,601; Testosterone: 0,753; Triiodo-thyronine: 0,544; Thyroxine: 0,441; Mineralocorticoid activity: 0,474; Calcitonine activity: 0,580; Parathyrine activity: 0,551. **Conclusion**. The results obtained by us complement and specify the concept of a triune neuroendocrine-immune complex.
**Keywords**: HRV, adaptation hormones, immunity, relationships, female rats.

## INRODUCTION

Previously, in line with the concept of the neuroendocrine-immune complex [4,5,7-9,11-13,22-30], we analyzed the relationships between the parameters of the autonomic nervous and endocrine systems, on the one hand, and the parameters of immunity, on the other hand, in male rats [16-21,32]. The purpose of this study is to analyze such interactions in female rats.

## MATERIAL AND METHODS

The experiment is at 60 white female rats Wistar line weighing 230-300 g. Of these 10 animals not subjected to any influences and 50 within 7 days subjected to moderate stress by daily 30-minute immobilization. The day after the completion of stressing in rats of both groups took samples of peripheral blood (through a cut tail) to analyze leukocytogram. An hour under light ether anesthesia for 15-20 sec recorded ECG in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [1]. Then the rats were placed in individual chambers with perforated bottom to collect daily urine, in which determined the concentration of calcium (by reaction with arsenazo III) and phospate (by phosphate-molibdate method). The next day, the animals were decapitated, for the purpose of collecting blood, in plasma which was determined concentration of adaptive hormones corticosterone, testosterone, thyroxine and triiodothyronine (by ELISA) as well as of calcium, phospate, sodium and potassium (by flamming photometry). In the same portion of the blood immunological parameters were determined by tests I and II levels of WHO as described in the handbook [14] and the previously developed algorithm [3,22,24]. On the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by phagocytic index, microbial (phagocytic) count and index of killing regarding museum culture Staphylococcus aureus (ATCC N 25423 F49) [3,6], with the calculation of derivative indices: microbial capacity (number of microbas that are able to absorb phagocytes contained in 1 L of blood) and bactericidal capacity (number of microbas that are able to neutralize neutrophils or monocytes contained in 1 L of blood) [22,24]. Among the parameters immunogram determined the relative amount of blood population of T-cells by spontaneous rosette test with sheep erythrocytes by M Jondal et al. [10], their theophylline resistant (T-helpers) and theophylline sensitive (T-cytolytic) subpopulations (by test sensitivity rosette to theophylline by S Limatibul et al. [15]), the population of B-lymphocytes by test complementary rosette of sheep erythrocytes by C Bianco [2]. Natural killers identified as big containing granules lymphocytes. After a blood sample was removed spleen, thymus and adrenal glands and weighed them. Since the spleen and thymus did smears for counting splenocytogram and thymocytogram [3]. For the latter, as well as to leukocytogram and immunocytogram we calculated entropy [31]. In sections of the adrenal glands was measured under a microscope the thickness of glomerular, fascicular, reticular and medullar zones [13,24].

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## RESULTS AND DISCUSION

First, a correlation matrix was created (Table 1). For a sample with n=60, the critical value |r| at $p<0,05$ ($t>2,00$): 0,25; at $p<0,01$ ($t>2,66$): 0,33; at $p<0,001$ ($t>3,46$): 0,42.

**Table 1. Coefficients of correlation between neuro-endocrine and immune parameters of female rats**

| **Variables** | DX | AMo | Mo | Cort | Fasc | MCA | Glom | Test | Ret | Adr | T3 | T4 | PTA | CTA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spleen Mass | | | | | ,22 | | | | | ,23 | | | -,31 | |
| Spleen Mass Ind | | | | | ,28 | | | | | | | | -,34 | |
| Lymphoblastes S | ,24 | -,27 | ,30 | | -,46 | | | | | | | | ,37 | ,31 |
| Plasmocytes S | | -,25 | | | -,41 | -,30 | -,32 | | | | | ,25 | | |
| Reticulocytes S | | | | | | -,22 | | | | | | | | |
| Fibroblastes S | | | | | | | | | | | | ,21 | -,28 | |
| Macrophages S | -,41 | ,68 | -,64 | | ,55 | | | ,32 | ,30 | | | | -,35 | -,26 |
| Eosinophils S | | | | | | | | | | | | -,20 | | |
| Thymus Mass | | | | -,23 | ,22 | | ,30 | | | | | | | |
| Thymus Mass Ind | | | | | ,24 | | ,24 | | | | | | | |
| Lymphocytes T | ,25 | -,31 | ,27 | | -,32 | | | -,20 | | | | | | |
| Reticulocytes T | | | | | | | | | | -,25 | | | | |
| Epitheliocytes T | | ,27 | -,29 | | ,52 | | | | | | | -,21 | | -,30 |
| Endotheliocytes T | | | | | ,27 | | | | | | | | | |
| Plasmocytes T | | | | | | | | | | | | | | ,31 |
| Macrophages T | | | | | | | | ,31 | | ,27 | | | | |
| EntropyThymoCG | -,23 | ,29 | -,25 | | ,25 | | | ,23 | | | | | | |
| Lymphocytes B | | | | | | | -,28 | | | | ,31 | | | |
| Stub Neutrophil B | | | | | | | | -,28 | | | -,38 | | | |
| Segment Neutr B | | | | | | | | | | | -,26 | | | |
| Eosinophils Blood | ,48 | | ,51 | -,32 | | | | | | | | | | |
| Basophils Blood | ,24 | | | | ,28 | ,22 | | | | | | | | |
| Monocytes Blood | | | | | | ,26 | | | | | | | | |
| Entropy LeukoCG | | | | | | | ,27 | | | | -,20 | | | |
| Killing Ind Neutrs | | | | ,21 | | | | | | | | | | |
| Phagoc Ind Neutr | | | | -,23 | | | | -,37 | | | | -,21 | | ,19 |
| Microbas Count N | ,33 | | ,31 | | | | | -,59 | | | | | | |
| Bacterocid Cap N | | | | | | | ,42 | -,40 | | | | | | |
| Phagoc Ind Mon | | -,29 | | | | | | | -,24 | | | | | |
| Microb Count M | | | | | | | | ,32 | | | -,24 | | | |
| Th-Lymphocytes | | | | | | | | -,30 | -,25 | | | | | ,28 |
| Tc-Lymphocytes | | | | -,23 | | | | | | | | | | |
| B-Lymphocytes | | | | | | | ,33 | | -,26 | | -,23 | | ,31 | ,27 |
| 0-Lymphocytes | | | | | | | | ,37 | ,34 | | | | -,25 | -,40 |
| NK-Lymphocytes | | | | ,24 | | | | | | | | | | |
| Entropy ImmuCG | ,31 | | | -,22 | | | | | | -,25 | | | | |

At the next stage, for each neuro-endocrine factor, regressive models with step-by-step exclusion were constructed, and at the final stage, a canonical correlation analysis of the connections between neuro-endocrine and immune constellations was performed.

The closest connection was detected between the sympathetic tone and the content of the macrophages in the spleen (Fig. 1). In addition, the sympathetic tone significantly affects the content of the lymphoblasts and endothelial cells in the thymus, so that the measurement of this immune cell constellation reaches 47% (Table 2 and Figure 2).

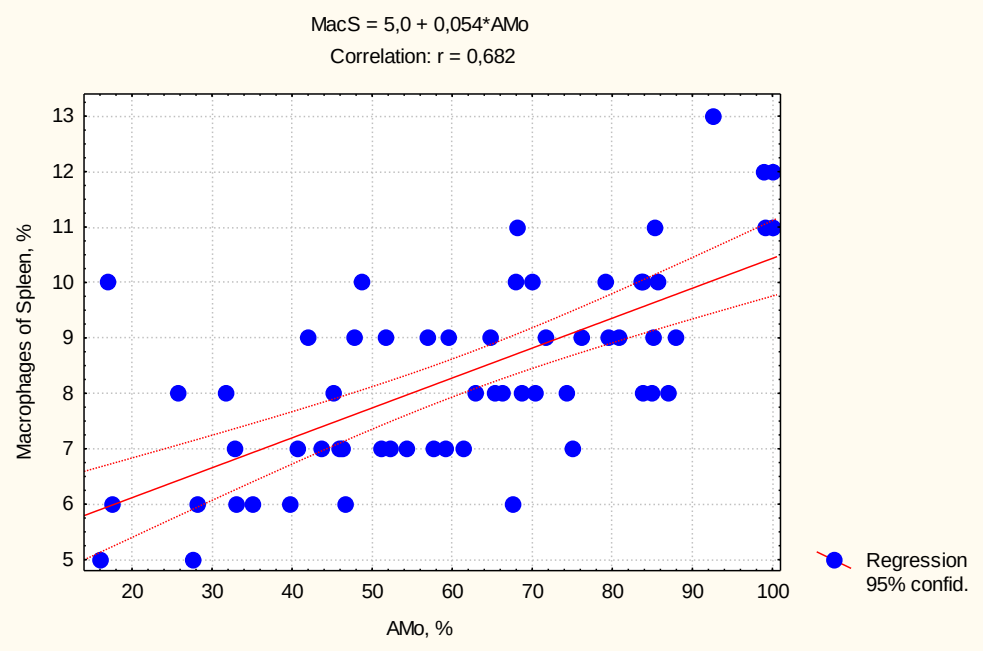



**Fig. 1. Relationship between the sympathetic tone (axis X) and the content in the spleen macrophages (axis Y)**

**Table 2. Regression model of multiple correlation of sympathetic tone (AMo) with indicators of immunity of female rats**

R=0,702; $R^2$=0,493; Adjusted $R^2$=0,466; $F_{(3,6)}$=18; $\chi^2_{(3)}$=38; $p<10^{-7}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(56)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 77,2 | 62,9 | 1,23 | ,225 |
| Macrophages S | 0,68 | ,696 | ,110 | 8,79 | 1,39 | 6,33 | $10^{-6}$ |
| Lymphocytes T | -0,31 | -,161 | ,100 | -1,40 | ,87 | -1,61 | ,114 |
| Lymphoblastes S | -0,27 | ,113 | ,111 | 1,94 | 1,91 | 1,02 | ,313 |

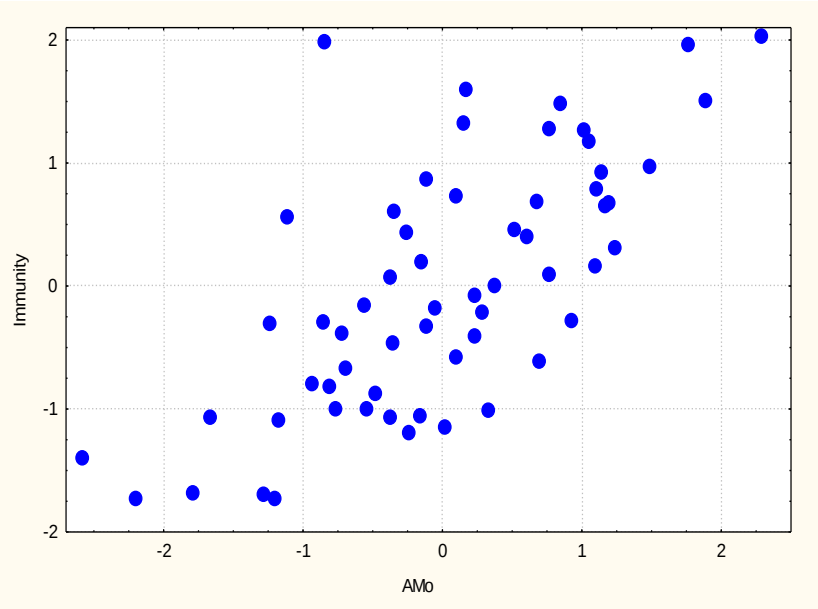



**Fig. 2. Canonical correlation between sympathetic tone (axis X) and immunity parameters of female rats (axis Y)**

The vagal tone as an antagonist of the sympathetic is related to the enumerated immunocytes in the opposite and weaker, but substantially correlates with the content of eosinophils and basophils in the blood, as well as with the entropy of the immunocytogram, which determines this immune constellation by 53% (Table 3, Fig. 3).

**Table 3. Regression model of multiple correlation of parasympathetic tone (DX) with indicators of immunity of female rats**

R=0,756; $R^2$=0,571; Adjusted $R^2$=0,531; $F_{(5,5)}$=14; $\chi^2_{(5)}$=47; $p<10^{-6}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(54)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | -438 | 235 | -1,86 | ,068 |
| Macrophages S | -0,41 | -,481 | ,095 | -11,94 | 2,36 | -5,05 | $10^{-5}$ |
| Eosinophils Blood | 0,48 | ,470 | ,096 | 10,66 | 2,17 | 4,92 | $10^{-5}$ |
| Lymphocytes T | 0,25 | ,188 | ,094 | 3,22 | 1,60 | 2,01 | ,050 |
| Basophils Blood | 0,24 | ,213 | ,098 | 18,80 | 8,66 | 2,17 | ,034 |
| Entropy ImmunoCG | 0,31 | ,148 | ,093 | ,672 | ,424 | 1,58 | ,119 |

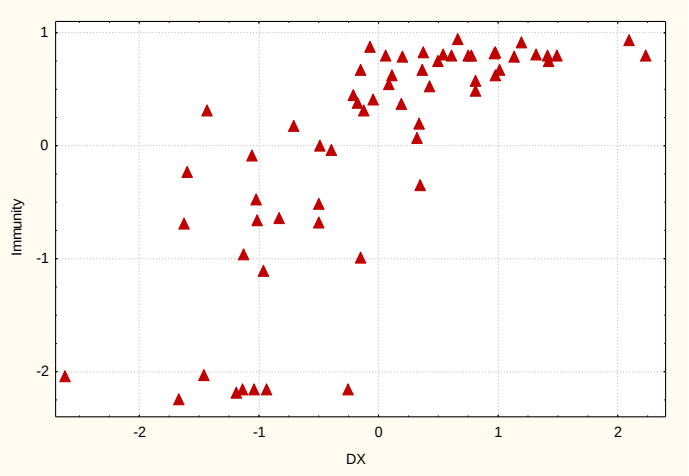



**Fig. 3. Canonical correlation between parasympathetic tone (axis X) and immunity parameters of female rats (axis Y)**

The Moda of HRV, on the one hand, closely correlates with the vagus (r=0,84) and sympathetic (r=-0,84) tone, and on the other hand, inversely associated with the macrophages of the spleen and directly with eosinophils of blood and thymus lymphocytes, determinating them by 79% (Table 4 and Figure 4).

**Table 4. Regression model of the multivariate correlation of Moda HRV with indicators of immunity of female rats**

R=0,896; $R^2$=0,803; Adjusted $R^2$=0,792; $F_{(3,6)}$=76; $\chi^2_{(3)}$=92; $p<10^{-6}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(56)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 106 | 34,7 | 3,05 | ,003 |
| Macrophages S | -0,64 | -,718 | ,062 | -8,04 | ,69 | -11,5 | $10^{-6}$ |
| Lymphocytes T | 0,27 | ,099 | ,061 | ,76 | ,47 | 1,61 | ,113 |
| Eosinophils B | 0,51 | ,626 | ,060 | 6,41 | ,62 | 10,4 | $10^{-6}$ |

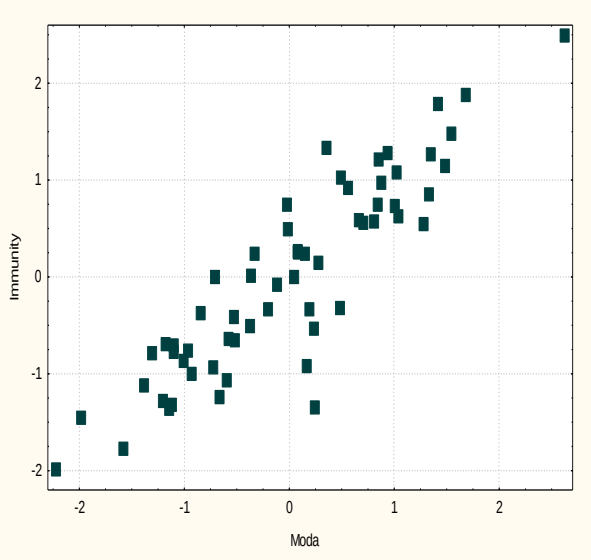
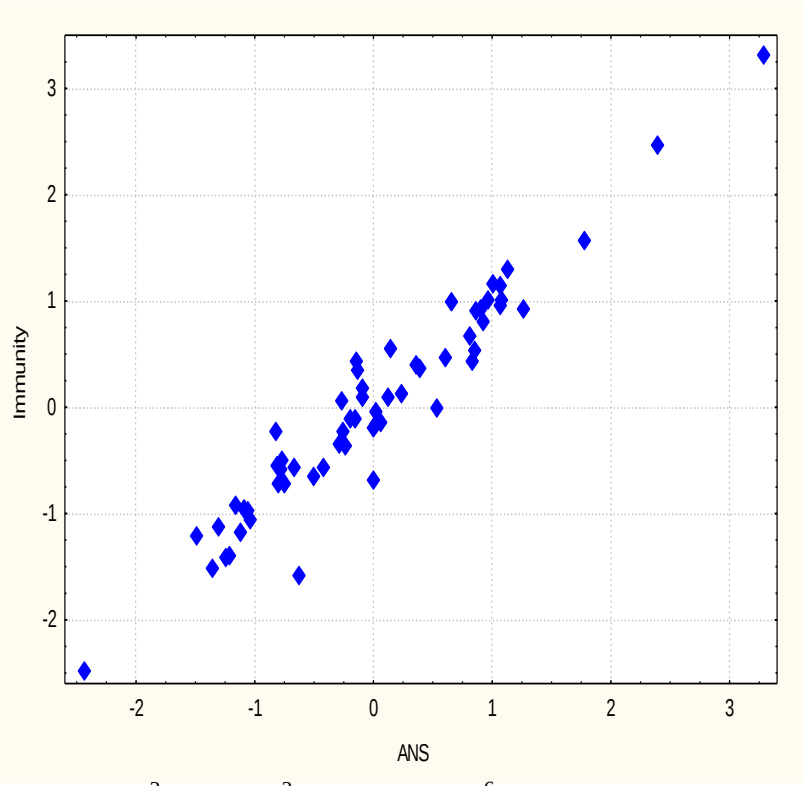



**Fig. 4. Canonical correlation between the Moda HRV (X axis) and the immune parameters of female rats (Y axis)**

The canonical correlation analysis shows that three parameters of vegetative regulation determine the mentioned 6 immune parameters on 93% (Table 5, Figure 5).

**Table 5. The factor structure of neuro-immune relationships in female rats**

| **Right set** | R |
|---|---|
| Moda HRV | **,89** |
| MxDMn | **,74** |
| Amplitude of Moda | **-,48** |
| **Left set** | R |
| Eosinophils of Blood | **,79** |
| Entropy Immunocytogram | **,28** |
| Lymphoblastes of Spleen | **,25** |
| Basophils of Blood | ,21 |
| Lymphocytes of Thymus | ,18 |
| Macrophages of Spleen | **-,46** |




R=0,964; $R^2$=0,929; $\chi^2_{(18)}$=189; $p<10^{-6}$

**Fig. 5. Canonical correlation between parameters of autonomous nervous system (X axis) and immunity of female rats (axis Y)**

The thickness of the fascicular zone of the adrenal cortex as a marker of their permanent glucocorticoid activity positively correlates with the mass of the spleen and the content of macrophages in it, as well as with the mass of the thymus and the content of epithelial cells and endothelial cells in it, while the negative with the content of lymphocytes in it. The measurement of determination of this immune constellation is 45,5% (Table 6 and Figure 6).

**Table 6. Regressive Model of Multiple Correlation of the thickness of the fascicular zone of adrenal cortex with indicators of immunity of female rats**

R=0,727; $R^2$=0,529; Adjusted $R^2$=0,455; $F_{(8,5)}$=7,2; $\chi^2_{(8)}$=41; $p<10^{-5}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(51)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | -523 | 350 | -1,49 | ,142 |
| Macrophages S | 0,55 | ,383 | ,112 | 16,8 | 4,9 | 3,42 | ,001 |
| Epitheliocytes T | 0,52 | ,532 | ,144 | 19,9 | 5,4 | 3,71 | ,001 |
| Spleen Mass Ind | 0,28 | 1,673 | ,847 | 196 | 99 | 1,97 | ,054 |
| Spleen Mass | 0,22 | -1,638 | ,834 | -,8 | ,4 | -1,97 | ,055 |
| Endotheliocytes T | 0,27 | ,256 | ,114 | 21,4 | 9,6 | 2,24 | ,029 |
| Thymus Mass Ind | 0,24 | -1,803 | ,982 | -1723 | 939 | -1,84 | ,072 |
| Thymus Mass | 0,22 | 1,683 | ,887 | 7,1 | 3,7 | 1,90 | ,063 |
| Lymphocytes T | -0,32 | ,253 | ,148 | 7,7 | 4,5 | 1,72 | ,092 |

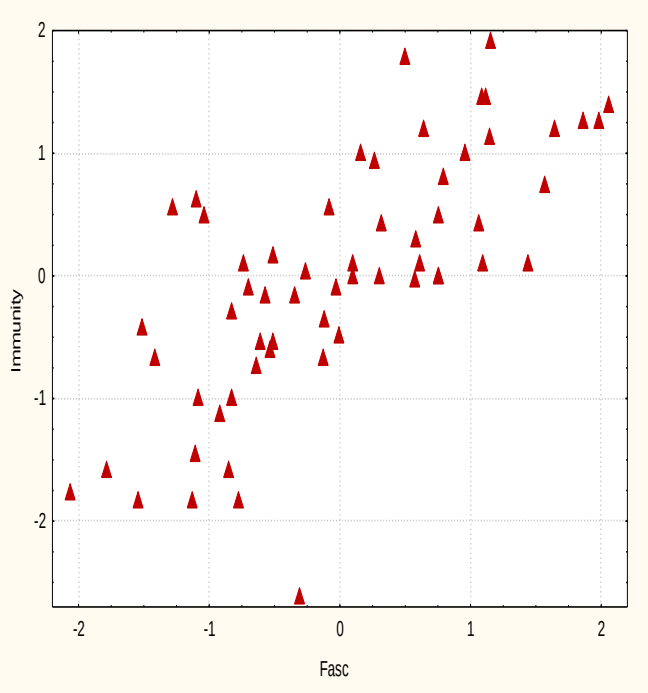



**Fig. 6. Canonical correlation between thickness of the fascicular zone of adrenal cortex (X axis) and immunity of female rats (axis Y)**

The level of corticosterone plasma as a marker of situational glucocorticoid activity correlates with the thickness of the fascicular zone of the adrenal cortex very weakly (r=-0,19), and is associated with another constellation of immune parameters: negative - with the content of eosinophils and T-killers in the blood, activity the phagocytosis of its microphages, as well as the entropy of the immunocytogram and the thymus mass, while positive - with the content of natural killers in the blood and the completeness of phagocytosis of its microphages. Corticosterone determines the above constellation of immune parameters by 27,5% (Table 7 and Figure 7).

**Table 7. Regressive Model of Multiple Correlation of the corticosterone with indicators of immunity of female rats**

$R=0,601$; $R^2=0,361$; Adjusted $R^2=0,275$; $F_{(7,5)}=4,2$; $\chi^2_{(7)}=24$; $p<10^{-3}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(52)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 1621 | 1032 | 1,57 | ,122 |
| Thymus Mass | -0,23 | -,134 | ,123 | -1,27 | 1,17 | -1,09 | ,282 |
| Eosinophils Blood | -0,32 | -,354 | ,118 | -31,8 | 10,6 | -2,99 | ,004 |
| Killing Ind Neutrophils | 0,21 | ,170 | ,118 | 4,62 | 3,22 | 1,44 | ,157 |
| Phagocytose Ind Neutroph | -0,23 | -,123 | ,117 | -5,72 | 5,46 | -1,05 | ,299 |
| T-Cytolytic Lymphocytes | -0,23 | -,216 | ,123 | -12,3 | 7,0 | -1,75 | ,086 |
| NK-Lymphocytes | 0,24 | ,318 | ,117 | 26,0 | 9,54 | 2,72 | ,009 |
| Entropy Immunocytogram | -0,22 | -,122 | ,121 | -220 | 218 | -1,01 | ,317 |

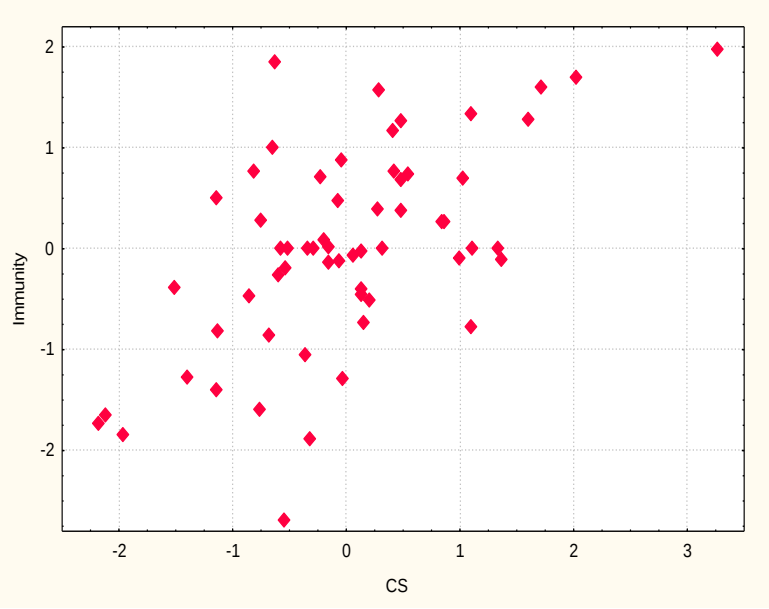
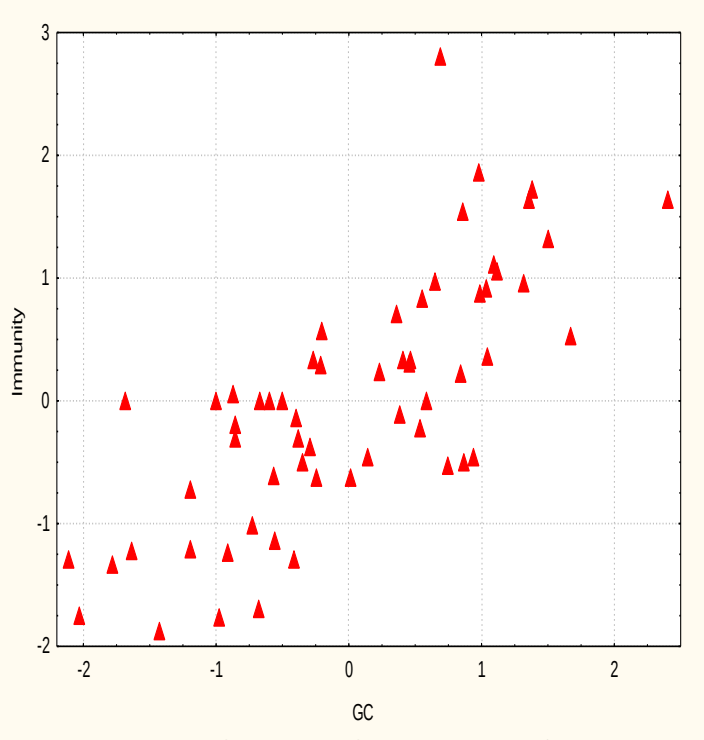



**Fig. 7. Canonical correlation between plasma corticosterone (X axis) and immunity of female rats (axis Y)**

Both parameters of glucocorticoid activity, taken together, determine the same constellation of immune parameters more strongly: by 57,5% (Table 8 and Figure 8).

**Table 8. Factor structure of glucocorticoid-immune relationships of female rats**

| **Right set** | R |
|---|---|
| Fasciculary Zone Adrenal Cortex | **,98** |
| Corticosterone of Plasma | **-,39** |
| **Left set** | R |
| Macrophages of Spleen | **,71** |
| Epitheliocytes of Thymus | **,68** |
| Endotheliocytes of Thymus | **,37** |
| Thymus Mass | **,34** |
| Thymus Mass Index | **,34** |
| Spleen Mass Index | **,31** |
| Spleen Mass | **,25** |
| Eosinophils of Blood | **,25** |
| Entropy of Immunocytogram | **,25** |
| Lymphocytes of Thymus | **-,43** |
| Killing Index Neutrophils of Blood | **-,24** |
| T-Cytotoxic Lymphocytes of Blood | ,04 |
| Phagocytose Index Neutrophils of Blood | ,03 |
| NK-Lymphocytes of Blood | -,02 |




$R=0,758$; $R^2=0,575$; $\chi^2_{(28)}=66$; $p<10^{-4}$

**Fig. 8. Canonical correlation between the parameters of glucocorticoid activity (X axis) and immunity of female rats (axis Y)**

The thickness of the glomerular zone of the adrenal cortex as a marker of their permanent mineralocorticoid activity positively correlates with the bactericidal ability of blood neutrophils and the content of B-lymphocytes, the mass of thymus and the content in the spleen of the macrophages, while the negative - with the contents of plasmocytes in it, determinating this immune constellation by 36% (Table 9).

Situational mineralocorticoid activity, estimated by the Na/K-ratio of plasma, is, at first, completely unconnected with the permanent activity (r=0,06), and secondly, slightly positively correlated with the content of monocytes and basophils in the blood while negative with contents in the spleen of plasmacytes and reticulocytes, so that the measure of determination of these immune parameters reaches only 17% (Table 10).

**Table 9. Regressive Model of Multiple Correlation of the thickness of the glomerular zone of adrenal cortex with indicators of immunity of female rats**
R=0,650; $R^2$=0,422; Adjusted $R^2$=0,357; $F_{(6,5)}$=6,5; $p<10^{-4}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(53)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 65,8 | 33,4 | 1,97 | ,054 |
| Bacterocidal Capacity N | 0,42 | ,342 | ,108 | 2,23 | ,71 | 3,17 | ,003 |
| B-Lymphocytes | 0,33 | ,244 | ,111 | 2,86 | 1,30 | 2,20 | ,032 |
| Macrophages S | 0,31 | ,200 | ,117 | 4,07 | 2,37 | 1,72 | ,092 |
| Thymus Mass | 0,30 | ,606 | ,315 | 1,19 | ,62 | 1,92 | ,060 |
| Thymus Mass Index | 0,24 | -,449 | ,311 | -197 | 138 | -1,44 | ,155 |
| Plasmocytes S | -0,32 | -,204 | ,117 | -5,80 | 3,32 | -1,75 | ,087 |

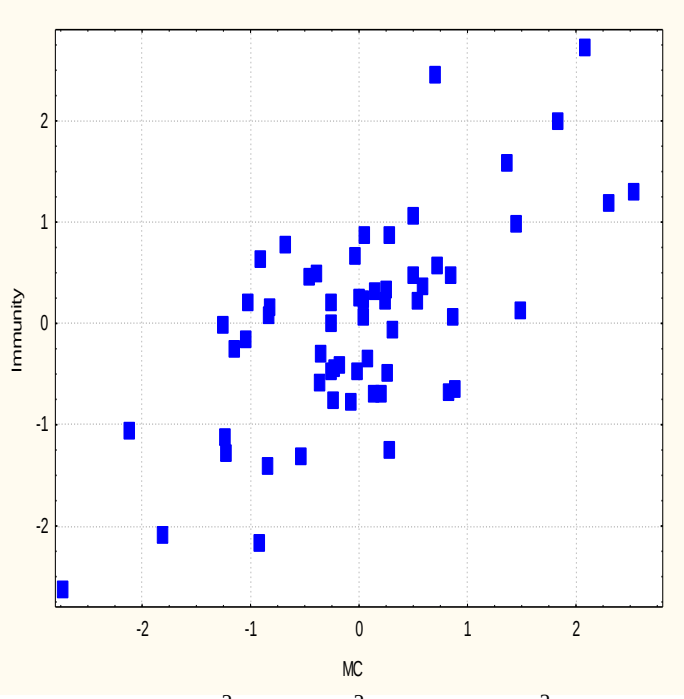
**Table 10. Regression model of multiple correlation of mineralocorticoid activity as $(Nap/Kp)^{0,5}$ ratio with indicators of immunity of female rats**
R=0,474; $R^2$=0,225; Adjusted $R^2$=0,168; $F_{(4,6)}$=4,0; p=0,007

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(55)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 7,8 | 0,8 | 9,27 | $10^{-6}$ |
| Plasmocytes S | -0,30 | -,316 | ,129 | -,173 | ,071 | -2,44 | ,018 |
| Reticulocytes S | -0,22 | -,285 | ,125 | -,112 | ,049 | -2,28 | ,027 |
| Monocytes Blood | 0,26 | ,145 | ,124 | ,044 | ,037 | 1,18 | ,244 |
| Basophils Blood | 0,22 | ,132 | ,125 | ,184 | ,174 | 1,06 | ,294 |

Taken together, both parameters of mineralocorticoid activity determine the same constellation of immune parameters more strongly - by 46% (Table 11 and Figure 9).

**Table 11. Factor structure of mineralocorticoid-immune relationships of female rats**

| **Right set** | R |
|---|---|
| Glomerulary Zone Adrenal Cortex | **-,96** |
| Mineralocorticoide Activity as $(Nap/Kp)^{0,5}$ | **-,33** |
| **Left set** | R |
| Bacterocidal Capacity of Neutrophils | **-,51** |
| B-Lymphocytes of Blood | **-,50** |
| Thymus Mass | **-,50** |
| Thymus Mass Index | **-,43** |
| Macrophages of Spleen | **-,45** |
| Monocytes of Blood | **-,24** |
| Basophils of Blood | -,07 |
| Plasmocytes of Spleen | **,56** |
| Reticulocytes of Spleen | ,18 |




$R=0,680$; $R^2=0,462$; $\chi^2_{(18)}=48$; $p<10^{-3}$

**Fig. 9. Canonical correlation between the parameters of mineralocorticoid activity (X axis) and the immunity of female rats (Y axis)**

The level of testosterone in plasma negatively correlates with the intensity of phagocytosis of blood microphages and the content of rodenuclear neutrophils in it and lymphocytes in thymocytogram, while positively - with its entropy, the content of macrophages in the spleen and 0-lymphocytes in the blood, which determines the constellation of these immune parameters by 52% (Table 12).

**Table 12. Regression model of the multiple correlation of testosterone with the parameters of immunity of female rats**

$R=0,753$; $R^2=0,567$; Adjusted $R^2=0,518$; $F_{(6,5)}=11,6$; $p<10^{-5}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(53)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | -44,2 | 28,9 | -1,53 | ,132 |
| Macrophages S | 0,32 | ,170 | ,097 | ,19 | ,11 | 1,76 | ,084 |
| Lymphocytes T | -0,20 | ,542 | ,335 | ,43 | ,26 | 1,62 | ,111 |
| Entropy Thymocytogram | 0,23 | ,703 | ,332 | ,050 | ,024 | 2,12 | ,039 |
| Stub Neutrophils Blood | -0,28 | -,157 | ,093 | -,28 | ,17 | -1,70 | ,095 |
| Microbas Count Neutroph | -0,59 | -,504 | ,094 | -,76 | ,14 | -5,38 | $10^{-5}$ |
| 0-Lymphocytes | 0,37 | ,314 | ,093 | ,09 | ,03 | 3,38 | ,001 |

The thickness of the reticular zone of the adrenal cortex as a marker of their permanent androgenic activity, correlating with plasma testosterone (r=0,61), correlates only with two relevant immune parameters (macrophages of the spleen and blood 0-lymphocytes), as well as with the activity of phagocytosis in the blood macrophages. As a result, the measure of determination of this immune constellation is only 15% (Table 13).

**Table 13. Regression model of multiple correlation of reticular zone of adrenal cortex with immunity of female rats**

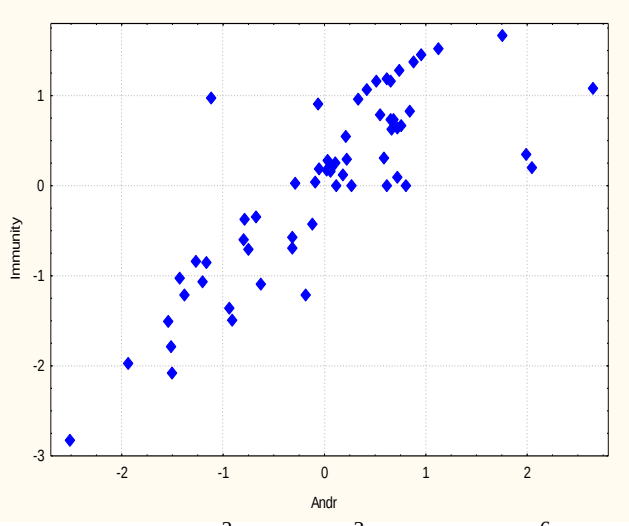
R=0,442; $R^2$=0,196; Adjusted $R^2$=0,152; $F_{(3,6)}$=4,5; p=0,006

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(56)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 29,3 | 9,3 | 3,15 | ,003 |
| 0-Lymphocytes | 0,34 | ,299 | ,122 | ,43 | ,18 | 2,46 | ,017 |
| Macrophages S | 0,30 | ,198 | ,129 | 1,15 | ,75 | 1,54 | ,130 |
| Phagoc Ind Mon | 0,24 | -,146 | ,128 | -1,78 | 1,55 | -1,14 | ,257 |

Taken together, both parameters of androgenic activity determine the same constellation of immune parameters more strongly - by 66% (Table 13 and Figure 10).

**Table 13. The factor structure of androgen-immune relationships in female rats**

| **Right set** | R |
|---|---|
| Testosterone of Plasma | **-,89** |
| Reticulary Zone Adrenal Cortex | -,18 |
| **Left set** | R |
| Microbas Count of Neutrophils | **,86** |
| Stub Neutrophils of Blood | **,52** |
| T-helper Lymphocytes of Blood | **,28** |
| Lymphocytes of Thymus | **,22** |
| Phagocytose Index of Monocytes | ,04 |
| 0-Lymphocytes of Blood | **-,32** |
| Macrophages of Spleen | **-,27** |
| Entropy of Thymocytogram | **-,26** |




R=0,815; $R^2$=0,664; $\chi^2_{(16)}$=74; $p<10^{-6}$

**Fig. 10. Canonical correlation between parameters of androgen function (X axis) and immunity (Y axis) of female rats**

The total mass of adrenal glands, unlike the thickness of its morpho-functional compartments, is weakly related to immune parameters, but the canonical correlation with their constellation is statistically significant (Table 14).

**Table 14. Regression model of multiple correlation of adrenal mass with indicators of immunity of female rats**
R=0,535; $R^2$=0,286; Adjusted $R^2$=0,234; $F_{(4,6)}$=5,5; $p<10^{-3}$

| | | Beta | St. Err.of Beta | B | St. Err. of B | $t_{(55)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 223 | 69,6 | 3,20 | ,002 |
| Macrophages T | 0,27 | ,357 | ,116 | 3,90 | 1,27 | 3,07 | ,003 |
| Spleen Mass | 0,23 | ,248 | ,116 | ,02 | ,01 | 2,13 | ,037 |
| Reticulocytes T | -0,25 | -,255 | ,114 | -2,63 | 1,17 | -2,23 | ,030 |
| Entropy Immunocytogram | -0,25 | -,279 | ,115 | -35,4 | 14,6 | -2,42 | ,019 |

Calcitonin activity (CTA), calculated by the formula: $CTA=(Cau•Pu)/(Cap•Pp)^{0.25}$, correlates negatively with the content of 0-Lymphocytes in the blood and epithelial cells in the thymus, while positively with the content of plasmacytes in it, as well as lymphoblasts in the spleen and T-killers in the blood. This immune constellation is determined by calcitonin activity by 25% (Table 15 and Figure 11).

**Table 15. Regression model of multiple correlation of calcitonin activity with indicators of immunity of female rats**
R=0,580; $R^2$=0,336; Adjusted $R^2$=0,246; $F_{(7,5)}$=3,8; $\chi^2_{(7)}$=22; p=0,002

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(52)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 2,47 | 1,57 | 1,57 | ,124 |
| 0-Lymphocytes | -0,40 | -,434 | ,154 | -,036 | ,013 | -2,81 | ,007 |
| Epitheliocytes T | -0,30 | -,188 | ,140 | -,053 | ,040 | -1,34 | ,186 |
| Plasmocytes T | 0,31 | ,272 | ,121 | ,195 | ,087 | 2,24 | ,029 |
| Lymphoblastes S | 0,31 | ,195 | ,145 | ,088 | ,065 | 1,35 | ,183 |
| T-Cytolytic Lymph | 0,20 | -,195 | ,159 | -,037 | ,030 | -1,22 | ,227 |
| Phagoc Ind Neutr | 0,19 | ,158 | ,115 | ,025 | ,018 | 1,37 | ,176 |
| Plasmocytes S | 0,19 | -,153 | ,147 | -,071 | ,068 | -1,04 | ,302 |

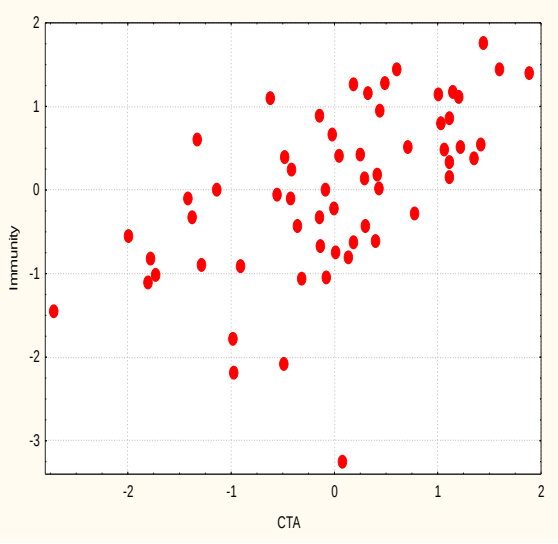



**Fig. 11. Canonical correlation between calcitonin activity (X axis) and immunity parameters of female rats (axis Y)**

Parathyrin activity (PTA), calculated by the formula: $PTA=(Cap•Pu)/(Cau•Pp)^{0,25}$, negatively correlates with the mass of the spleen and the content of macrophages, fibroblasts and eosinophils in it, while positively with the content in blood B –lymphocytes. This immune constellation is determined by paratyrin activity by 24% (Table 16 and Figure 12).

**Table 16. Regression model of multiple correlation of parathyrin activity with immunity parameters of female rats**

$R=0,551$; $R^2=0,304$; Adjusted $R^2=0,239$; $F_{(5,5)}=4,7$; $\chi^2_{(5)}=20$; $p<10^{-3}$

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(54)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 4,34 | ,67 | 6,45 | $10^{-6}$ |
| Spleen Mass Ind | -0,34 | -,141 | ,127 | -,130 | ,117 | -1,11 | ,272 |
| Macrophages S | -0,35 | -,247 | ,123 | -,085 | ,042 | -2,01 | ,049 |
| Fibroblastes S | -0,28 | -,190 | ,119 | -,068 | ,043 | -1,59 | ,117 |
| Eosinophils S | -0,23 | -,205 | ,115 | -,151 | ,085 | -1,78 | ,081 |
| B-Lymphocytes | 0,31 | ,214 | ,117 | ,042 | ,023 | 1,82 | ,074 |

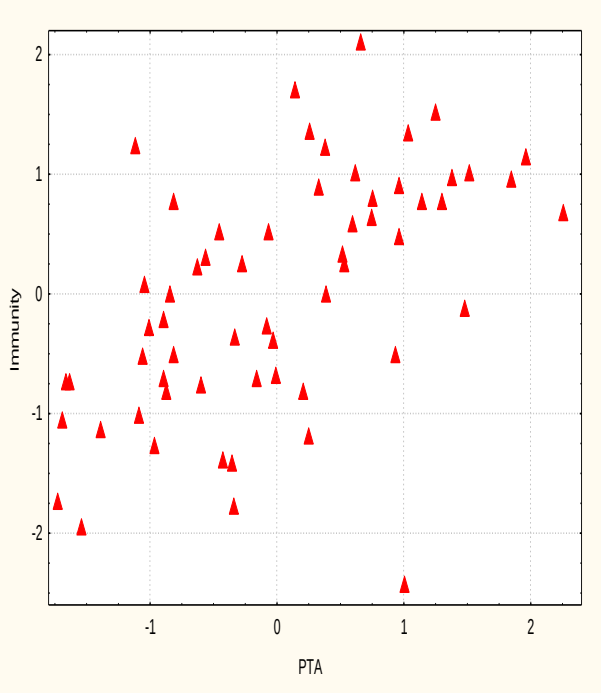



**Fig. 12. Canonical correlation between parathyrin activity (X axis) and immunity parameters of female rats (axis Y)**

The level of triiodothyronine in the blood positively correlates with the content of the common lymphocytes in the blood, while negative to the population of B-lymphocytes, as well as rod-and segmental neutrophils. Together with the intensity of phagocytosis of Staph. aureus by Monocytes, such a constellation of immune parameters is determined by triiodothyronine at 22% (Table 17).

The plasma level of thyroxin, first, is inversely related to the level of triiodothyronine (r=-0,68), and secondly, it correlates with another constellation of immune parameters, which determines them only 13,5% (Table 18).

**Table 17. Regression model of multiple correlation of triiodothyronine with immune parameters of female rats**

R=0,544; $R^2$=0,296; Adjusted $R^2$=0,216; $F_{(6,5)}$=3,7; p=0,004

| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(53)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | -5,82 | 3,61 | -1,62 | ,112 |
| Stub Neutrophiles | -0,38 | -,356 | ,171 | -,127 | ,061 | -2,09 | ,042 |
| Segmented Neutrophiles | -0,26 | ,847 | ,396 | ,054 | ,025 | 2,14 | ,037 |
| Microbas Count Monoc | -0,24 | -,189 | ,121 | -,041 | ,026 | -1,56 | ,126 |
| B-Lymphocytes | -0,23 | -,256 | ,119 | -,033 | ,015 | -2,16 | ,035 |
| Entropy Leukocytogram | -0,20 | ,792 | ,319 | 1,001 | ,404 | 2,48 | ,016 |
| Pan-Lymphocytes Blood | 0,31 | 1,412 | ,579 | ,076 | ,031 | 2,44 | ,018 |

**Table 18. Regression model of multiple correlation of thyroxine with indicators of immunity of female rats**

R=0,441; $R^2$=0,194; Adjusted $R^2$=0,135; $F_{(4,6)}$=3,3; p=0,017

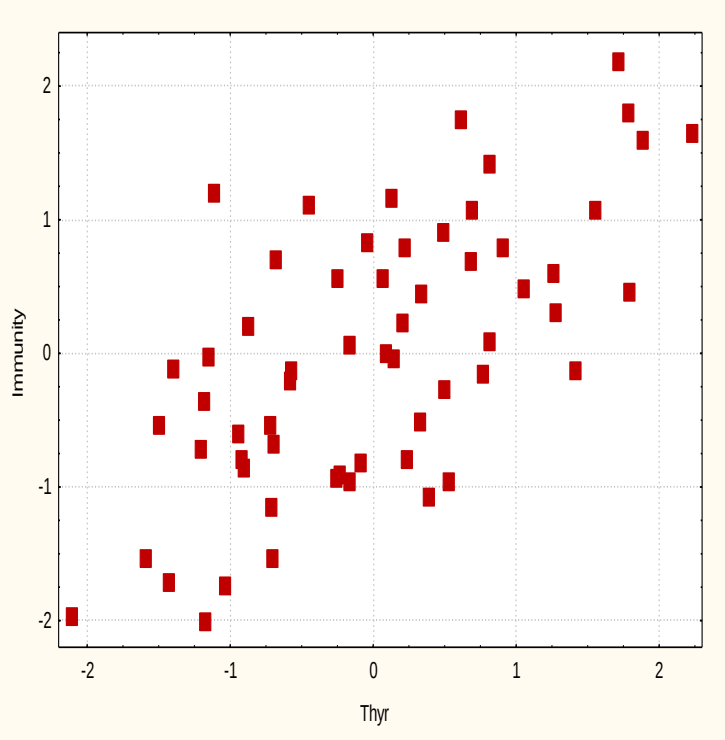
| | | Beta | St. Err. of Beta | B | St. Err. of B | $t_{(55)}$ | p-level |
|---|---|---|---|---|---|---|---|
| | r | | Intercpt | 92,8 | 33,4 | 2,78 | ,007 |
| Plasmocytes S | 0,25 | ,290 | ,126 | 3,13 | 1,36 | 2,30 | ,025 |
| Fibroblastes S | 0,21 | ,244 | ,125 | 1,98 | 1,01 | 1,95 | ,056 |
| Eosinophils S | -0,20 | -,139 | ,124 | -2,30 | 2,04 | -1,13 | ,265 |
| Phagocytose Ind Neutr | -0,21 | -,203 | ,122 | -,75 | ,45 | -1,67 | ,100 |

The canonical correlation between the two parameters of the thyroid function, on the one hand, and immunity parameters, on the other hand, was significantly stronger than with respect to individual thyroid hormones. It is noteworthy that the factor load on the thyroid canonical root from the side of triiodothyronine is four times that of thyroxine, which coincides with the ratio of their physiological activity (4:1) [cyt. by: 13]. Accordingly, the immune canonical root receives major load from the indices associated with triiodothyronine (Table 19).

**Table 19. Factor structure of thyroid-immune relationships in female rats**

| **Right set** | R |
|---|---|
| Triiodo-thyronine | **-,63** |
| Thyroxin | **-,14** |
| **Left set** | R |
| Stub Neutrophiles of Blood | **,63** |
| B-Lymphocytes of Blood | **,63** |
| Segmented Neutrophiles of Blood | **,46** |
| Entropy of Leukocytogram | **,51** |
| Eosinophils of Spleen | **,28** |
| Microbas Count of Monocytes | ,14 |
| Phagocytose Index of Neutrophils | ,10 |
| Pan-Lymphocytes of Blood | **-,57** |
| Plasmocytes of Spleen | -,14 |
| Fibroblastes of Spleen | -,10 |

In general, thyroid activity determines immune parameters by 44% (Fig. 13).




R=0,664; $R^2$=0,440; $\chi^2_{(20)}$=45; p=0,001

**Fig. 13. Canonical correlation between thyroid function (axis X) and immunity parameters of female rats (Y axis)**

## CONCLUSION

So, as in the previous experiment with males, in females it was found that each of our registered neuroendocrine factors more or less closely correlates positively or negatively with those or other immune parameters of the thymus, spleen and blood, which testifies to their interaction within the framework triple neuro-endocrine-immune complex. At the same time, a number of features have been identified that will become the subject of the next article.

## CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the National Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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