

Summary

The dynamics of changes in thyroid hormones and thyrotropic hormone during pregnancy in the domestic rabbit (*Oryctolagus cuniculus f. domesticus*) have not been thoroughly investigated. To date, no comprehensive studies are available on the dynamics of these hormonal changes and the relationship between free and bound thyroxine, triiodothyronine fractions and thyrotropic hormone fractions.

The aim of this study was to examine the role of thyroid and thyroid-stimulating hormone (TSH) throughout the course of pregnancy in domestic rabbits. We also determined a screening laboratory index associated with the degree of risk of pregnancy toxemia and deaths of pregnant females caused by thyroid dysfunction. Analyses of the results of thyroid hormone concentrations in two breeds of domestic rabbit (Termond White and Popielno White) and the 3 measurements of the studied parameters made it possible to trace the dynamics of changes in indicators of health and function of the thyroid gland during pregnancy. Furthermore, our observations were intended to evaluate the fertility of the female rabbits, the birth weight of the litter and the viability of the newborns.

The study was approved by the 2nd Local Ethical Committee on Animal Experiments (Resolution No. 146/2018, dated 12/04/2018). Twelve female domestic rabbits, belonging to two breeds, Popielno White and Termond White, were qualified for the study. The females were clinically examined three times and their health status was assessed based on a questionnaire prepared for this purpose. After the clinical examination, blood was collected from the rabbits for morphological, biochemical and hormonal tests. Test I was done 3 days before mating, Test II at 15 days of gestation, Test III at 28 days of gestation. After birth, an examination of the litter was performed within the first 24 hours of life evaluating the number of newborns, birth weight of the litter and survival rate of the born rabbits.

In the blood morphological examination, the following were evaluated: leukocyte count (WBC), leukocyte fraction distribution, erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width – coefficient of variation (RDW-CV), RBC distribution width – standard deviation (RDW-SD), mean platelet volume (MPV), and platelet count (PLT). In the biochemical examination aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) activity were assessed, and concentrations of glucose, urea, creatinine, total cholesterol, total protein, albumin, globulin, calcium and phosphorus were evaluated. The concentrations of

thyroid stimulating hormone (TSH), total thyroxine (tT4), free thyroxine (fT4), total triiodothyronine (tT3), free triiodothyronine (fT3) were also examined.

The results were analyzed using the Statistica 13.3 (ANOVA). Arithmetic mean (X), standard deviation (\pm SD) and median (M) were determined. Data were analyzed using Friedman's test followed by appropriate post hoc testing using Statistica (13.3) at $\alpha=0.05$ level of significance. Spearman's rank correlation coefficient (r) was used to assess the strength of the correlation and the magnitude of significant correlations was assessed using the Guilford scale.

All the rabbits in the study were healthy and scored excellent on clinical examination. The morphological and biochemical test results obtained were within the reference values provided for the species. The blood morphological examination of the Termond White rabbits showed a significant decrease in the values of MCH and MPV, and a decrease in the values of WBC, RBC, RDW-CV, PLT in Test III. Popielno White rabbits showed significant decreases in WBC, RBC, HGB and MCH values in Test III.

In the study of baseline biochemical parameters, Termond White breed showed a significant decrease in AP activity and serum globulin, creatinine, urea, phosphorus and cholesterol levels in Test III, and a significant increase in serum AST activity on day 28 of gestation. In Popielno White rabbits, there was a significant decrease in albumin concentration in Test III.

Hormonal test results were analyzed by race (n=6) as well as combined (n=12). In Termond White race, TSH concentration significantly increased in Test II (11.29 ± 6.25 ng/ml) and then significantly decreased in Test III (6.18 ± 2.00 ng/ml). On the other hand, in Popielno White TSH showed an increasing tendency during pregnancy, although no significant differences were found between the Tests (I - 4.53 ± 1.57 ng/ml, II - 5.98 ± 2.10 ng/ml, III - 7.83 ± 2.48 ng/ml). An analysis of TSH concentration results obtained from all females (n=12) showed a significant increase in concentration in Test II (8.63 ± 5.24 ng/ml) compared to Test I (4.63 ± 1.22 ng/ml). Each breed showed a significant decrease in fT3 concentration in the test performed at the end of pregnancy (Termond White breed - 6.97 ± 1.97 pmol/l, Popielno White breed - 6.63 ± 1.83 pmol/l) compared to the test performed at mid-pregnancy (Termond White breed - 9.95 ± 0.61 pmol/l, Popielno White breed - 10.10 ± 1.58 pmol/l). Statistical analysis of changes in fT3 concentrations for all females showed significant differences among the three Tests, with concentrations increasing significantly between Tests I and II (8.75 ± 1.47 pmol/l, 10.03 ± 1.16 pmol/l, respectively) and then decreasing significantly to a mean value of 6.80 ± 1.82 pmol/l on Day 28. The other thyroid hormones tested i.e., tT4, fT4, tT3 in Termond

White and Popielno White together, as well as without the race division, showed little fluctuations and no statistically significant differences were found between the Tests.

The correlation analysis of the studied hormones in all females (n=12) indicated a high positive correlation in Test I i.e., before pregnancy, between tT3 and fT3 (r=0.720, p=0.008). At the end of pregnancy, in Test III, a high positive correlation was obtained for the hormone pairs tT4 and tT3 (r=0.601, p=0.039) and also a high positive correlation between tT3 and fT3 (r=0.601, p=0.039).

The obtained results of correlation analysis for hormones: TSH, tT4, fT4, tT3 and fT3 and number of offspring in all females (n=12) indicate the presence of high positive correlation between the number of offspring and tT4 before pregnancy (r=0.657, p=0.020). At later stages of pregnancy, a high and very high negative correlation was found between fT3 and the number of offspring (Test II – r= – 0.697, p=0.012, Test III – r= – 0.826, p=0.001).

The obtained results allow us to make the following conclusions:

1. Of the thyroid hormones studied in female domestic rabbits, fT3 is the most sensitive during pregnancy. Its serum concentration fluctuates significantly, in contrast to serum concentrations of tT4, fT4 and tT3, which are stable. In addition, the strong positive correlation between fT3 and fT4 concentrations in serum during pregnancy and negative correlation between serum fT3 levels in female rabbits and the number of offspring obtained during pregnancy confirms this thesis.
2. Determination of serum fT3 at day 15 of gestation can be a screening laboratory indicator to assess thyroid function and the risk of pregnancy toxemia in rabbits.
3. Blood morphological parameters such as WBC, RBC, MCH along with fT3 concentration can be used as components of a screening panel to assess the general health status of pregnant female domestic rabbits with indication for performance.
4. TSH levels in pregnant female rabbits are stable and not useful for the assessment of thyroid function and the risk of pregnancy toxemia in rabbits.