Archives of Razi Institute, Vol. 76, No. 3 (2021) 649-657

DOI: 10.22092/ari.2021.355354.1676

Original Article

The Endocrine Function of Testes in 12- and 18-Month-Old Boars of Different Breeds

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Received 16 July 2021; Accepted 31 July 2021
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Abstract

Testes have several primary functions, such as male gametes production (spermatozoa) and secretion of several endocrine factors, including the production of steroid and protein hormones which facilitate elements in the healthy reproductive function of mammals. The potential of an animal functional endocrine reservoir could be an interesting finding in animal reproduction management. Therefore, the current study aimed to analyze the functional endocrine reserves of testes in 12- and 18-month-old boars of four different boar breeds, namely Large White, Landrace, Duroc, and Tempo, as the animal model in this research (n=10). To determine the functional endocrine reserves of the testes at 12 and 18 months of age, boars received injections of human chorionic gonadotropin (hCG) 3 times every 72 hours. Testosterone was measured in blood samples (10 mL) taken from the jugular vein before the administration of hCG as well as 2, 12, 24, 48, and 72 hours after administration of each of three hCG injections. The index of endocrine activity of the testes was determined by the formula: $I_{ta} = T_1 - T_0 / T_0$, where I_{ta} is the index of endocrine activity of the testes, T_0 is the testosterone concentration before the administration of hCG, and T_1 is the maximum testosterone concentration after the third administration of hCG. The effects of the hCG injection on boar testes at different age periods indicated that the testes of Large White and Duroc boars have higher potential endocrine reserves compared with the Landrace and Tempo breeds.

Keywords: boars, breeds, Large White, Landrace, Duroc, Tempo, testosterone, chorionic gonadotropin

La Fonction Endocrinienne des Testicules chez les Sangliers de 12 et 18 Mois de Différentes Races

Résumé: Les testicules ont plusieurs fonctions principales, telles que la production de gamètes mâles (spermatozoïdes) et la sécrétion de plusieurs facteurs endocriniens, notamment la production d'hormones stéroïdiennes et protéiques qui facilitent les éléments de la fonction reproductive saine des mammifères. Le potentiel d'un réservoir endocrinien fonctionnel animal pourrait être une découverte intéressante dans la gestion de la reproduction animale. Par conséquent, la présente étude visait à analyser les réserves endocriniennes fonctionnelles des testicules chez des Sangliers de 12 et 18 mois de quatre races différentes de Sangliers, à savoir Large White, Landrace, Duroc et Tempo, comme modèle animal dans cette recherche (n=10). Pour déterminer les réserves endocriniennes fonctionnelles des testicules à 12 et 18 mois, des Sangliers ont reçu des injections de gonadotrophine chorionique humaine (hCG) 3 fois toutes les 72 heures. La testostérone a été mesurée dans des échantillons de sang (10 mL) prélevés dans la veine jugulaire avant l'administration d'hCG ainsi que 2, 12, 24

48 et 72 heures après l'administration de chacune des trois injections d'hCG. L'indice d'activité endocrinienne des testicules a été déterminé par la formule: $I_{ta} = T_1 – T_0 / T_0$, où I_{ta} est l'indice d'activité endocrinienne des testicules, T_0 est la concentration de testostérone avant l'administration d'hCG, et T_1 est la concentration maximale de testostérone après la troisième administration d'hCG. Les effets de l'injection d'hCG sur les testicules des Sangliers à différentes périodes d'âge ont indiqué que les testicules des Sangliers Large White et Duroc ont des réserves endocriniennes potentielles plus élevées que celles des races Landrace et Tempo.
Mots-clés: Sangliers, Races, Large White, Landrace, Duroc, Tempo, Testostérone, Gonadotrophine Chorionique

1. Introduction

Testosterone is a steroid hormone secreted from the Leydig cells of the testes in males, adrenals, and ovaries. The dihydro derivative of testosterone exerts a potent anabolic action responsible for the post pubescent growth rate and subsequent muscle and bone tissue maintenance of adult males. The blood level of hormones is extremely insufficient for a complete and objective assessment of the functional state of the endocrine gland, as the concentration of hormones in the blood is influenced by various factors, both internal and external. These pivotal factors include: animal breed, physiological state of the body, feeding and nutritional status, time of day, and in seasonal breeders the season of the year, etc. (1-3).

These factors make it difficult to assess the genetic potential for the functional state of endocrine glands such as testes. Therefore, classical endocrinology uses the “stress" method to assess the potential reserves of the endocrine gland. This method is based on the stimulation of endocrine gland function and allows setting its limits of functional activity. The central link in the endocrine system in males is the testes, which synthesize the main male hormone, i.e. testosterone (4-7), which affects the development of secondary sexual characteristics, the formation of muscle mass and bone tissue, and affects behavioral responses (8, 9). Studies in cattle have shown that the male testes respond to stimulation by human chorionic gonadotropin (hCG) (10-12). Steroid measurements on the testis and peripheral blood of boars has been done previously. In a series of previously published studies, it was shown that relatively high levels of androgen were present during the post-natal period, decreasing during early puberty before increasing again between late puberty and maturity in boars. Testosterone determination in the peripheral blood of adult boars showed a concentration of >2 mg/ml. No such studies have been conducted on boars.

2. Material and Methods

2.1. Animals and Experimental Conditions

All chemicals used in this study were purchased from Sigma Aldrich unless otherwise stated. The testosterone ELISA kit was purchased from Abnova (Catalog Number KA2349) for measuring testosterone in blood serum samples. The boars were housed at 25-27 °C and allowed ad libitum access to food and water. Four different boar breeds, Large White, Landrace, Duroc, and Tempo, were assigned to the experimental groups (n=10). Each group consisted of 10 head of each breed similar in age (12 and 18 months of age).

2.2. Blood Sampling, hCG Injection, and Testosterone Measurement

A blood sample (10 mL) was aspirated from the jugular vein of each animal by inserting a 21-gauge hypodermic needle attached to a 10-ml Venoject (BD Life Sciences, Cockeysville, MD, USA) through the surface veins in the ear. To separate serum for hormone measurements, the blood samples were allowed to clot at 4 ℃, and serum was obtained by centrifuging at 2500×g for 10 min. Serums were kept at -20 ℃ until the day of hormone measurements, as repeated thawing and freezing should be avoided. Hormones were assayed with the boar testosterone ELISA kit as previously

described by Tang et al. (2). A summary of the assay and step-by-step protocol is described as follows: 1) Before use and assay procedures, all reagents and chemicals were allowed to reach room temperature (18-25 °C); 2) 50 µL of standards, samples, and controls were pipetted into appropriate wells; 3) 100 µL of testosterone enzyme conjugate solution was added to each well (except those sets), shaken well for 1-2 minutes, and incubated at 37 °C for 1 hour; 4) The contents of the wells were discarded, and the plates were washed 5 times with Wash Solution (250-300 µL per well). Plates were inverted and tapped firmly against absorbent paper to remove any residual moisture; 5) 100 µL TMB color was added into each well (including the blanks) according to pipetting order; 6) Plates were incubated for 20 minutes at room temperature; 7) The reaction was stopped by adding 50 µL of stopping solution to wells in the same sequence that the substrate solution was added and gently mixed; 8) Absorbance at 450 nm was read with a microwell reader.

To determine the functional endocrine reserves of the testes of different boar breeds at the age of 12 and 18 months, the animals received injections of 200 IU of hCG 3 times every 72 hours given as a single IM injection. To measure testosterone, blood was taken before the administration of hCG as well as 2, 12, 24, 48, and 72 hours after each administration of hCG. The index of endocrine activity of the testes was determined using the formula: $I_{ta} = T_1 – T_0 / T_0$, where I_{ta} is the index of endocrine activity of the testes, T_0 is the testosterone concentration before the administration of CG, and T_1 is the maximum testosterone concentration after the third administration of hCG. The results were biometrically processed.

2.3. Data Analysis

The recorded data was analyzed based on the guidelines provided by the Abnova Company. In brief: 1) The mean absorbance values (A) for each set of reference standards, controls, samples and blanks were calculated; 2) The values for blanks were subtracted from those for standards, control, and unknown samples; 3) B/B0 values were calculated by dividing each value by the value for the zero-standard; 4) For the standards, a graph was plotted on semi-log graph paper with B/B0% values on the ordinate and testosterone concentrations (pg/mL) on the abscissa; 5) Using the graph, testosterone concentrations for the unknown samples were read; 6) For values above and below the readable range, procedures were repeated using the appropriate dilution. The sensitivity of the assay was 25 pg/mL.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was carried out using SPSS 10.0 software (SPSS Inc., Chicago, Illinois, USA) in a completely randomized design. Duncan's multiple range test and Student's t-test were used to compare mean values of individual treatments when the F-value was significant ($p < 0.05$).

3. Results and Discussion

The results of testosterone measurements in 12-month-old boars in all breeds showed that the basal level of testosterone before hCG ranged from 7.0 to 7.8 nmol/L (Figure 1).

Two hours after the injection of hCG, the testosterone concentration in all experimental groups began to gradually increase. By 12 hours after the first administration of hCG, testosterone levels had increased by 0.2 and 0.3 nmol/l in Large White and Tempo boars, and in Landrace and Duroc boars, respectively. After 24 hours, the testosterone concentration in Large White boars was 8.2±0.6 nmol/l, in Landrace was 7.9±0.6 nmol/l, in Duroc was 7.5±0.5 nmol/l, and in Tempo was 7.7±0.5 nmol/l. 48 h after the administration of hCG, the hormone level continued to increase in all experimental groups. The hormone concentration during this period was 8.4±0.6 nmol/l in the Large White breed, 8.0±0.7 nmol/l in the Landrace breed, 7.9±0.6 nmol/l in Duroc, and 7.8±0.6 nmol/l in Tempo. After a period of 72 h post-hCG administration, the hormone levels in all breeds of boars remained steady at the level seen at 48 h after the

first administration of hCG. 72 h after the first round of hCG injection, the boars were subjected to the second round of endocrine functional stress by the hCG injection (Figure 2).

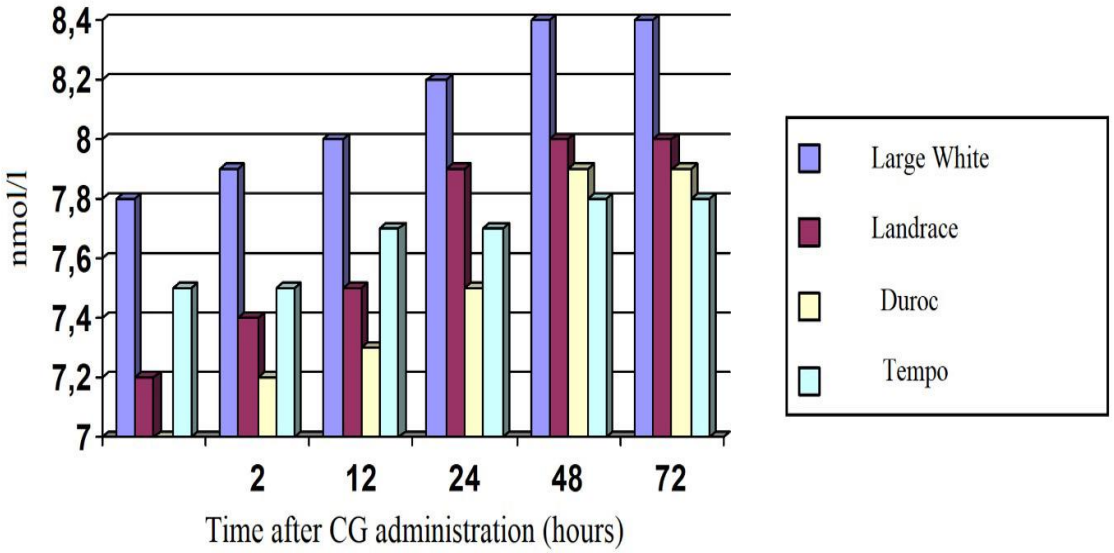

Figure 1. Testosterone levels in the blood of 12-month-old boars following the 1st CG administration

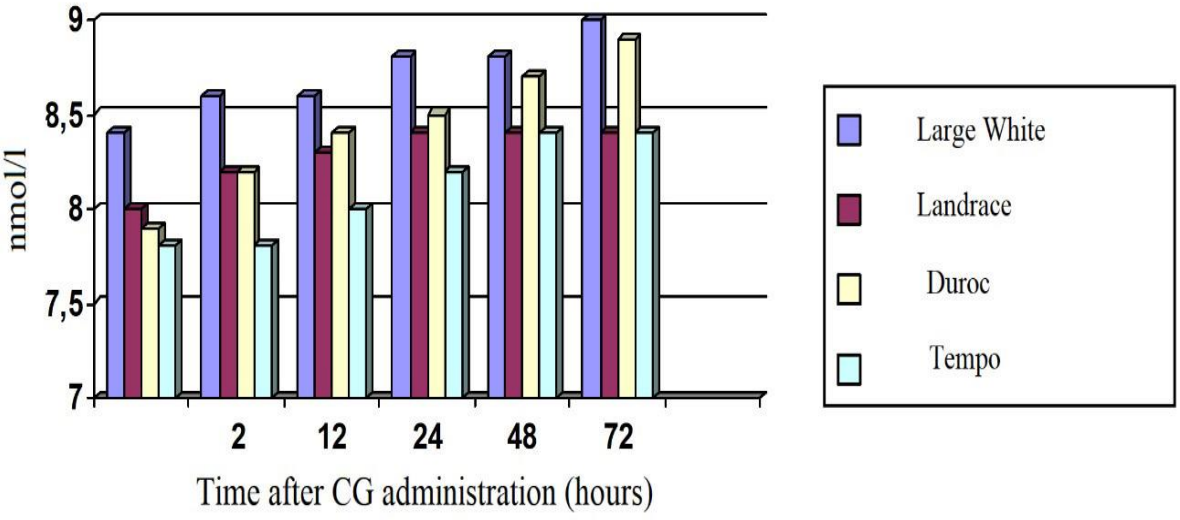

Figure 2. Testosterone levels in the blood of 12-month-old boars following the 2nd hCG injection (given 72 h after the first round of hCG administration)

Data from the current study revealed that the potential of endocrine function reserves in the boars' testes was not satisfactory after the first administration of hCG, as evidenced by the data recorded after the 2nd administration of hCG. In Large White boars, the testosterone concentrations changed after 2, 12, 48, and 72 hours to 8.6±0.7, 8.8±0.7, 9.0±0.8, and 9.1±0.7 nmol/l, respectively. In the Landrace breed, levels changed to 8.2±0.6, 8.3±0.5, 8.4±0.7, 8.4±0.5, and 8.4±0.5 nmol/l, respectively. In the Duroc breed, these changes were 8.2±0.6, 8.4±0.7, 8.5±0.6, 8.7±0.6, and 8.9±0.7 nmol/l, respectively. In the Tempo breed, they were 7.8±0.6, 8.0±0.6, 8.2±0.7, 8.4±0.7, and 8.4±0.7 nmol/l, respectively. These changes in testosterone concentration indicate incomplete endocrine function of the testes after the second administration of hCG. These findings are in contrast with previously published works (8, 9).

The third administration of hCG was performed 72 hours after the 2nd hCG administration (Figure 3).

The results showed that after the 3rd administration of hCG, the concentration of testosterone in the blood continued to increase in all experimental groups. However, the testosterone level in the serum samples did not peak in the same way among different boar breeds. In Large White boars, the maximum level of testosterone (10.8±0.8 nmol/l) occurred 24 h after the 3rd administration of hCG. In Landrace boars, the maximum testosterone concentration (8.6±0.7 nmol/L) occurred 2 h following the administration of hCG. The maximum testosterone level in Duroc boars was 9.5±0.7 nmol/l after 24 h, and in Tempo boars the hormone reached its maximum (8.7±0.6 nmol/l) 12 h after the administration of hCG. In general, the concentration of testosterone after three stimulations of hCG in the Large White breed of boars increased by 38.5%, by 19.4% in the Landrace breed, by 35.7% in the Duroc breed, and in the Tempo breed by 16%. The activity index of the testes (I_{ta}) in Large White boars was 0.39, in Landrace was 0.20, in Duroc was 0.36, and in Tempo was 0.16. The 18-month-old boars were subjected to similar functional stresses on their testes. These findings are in agreement with previously published studies (2, 4, 5, 7)

The concentration of testosterone in the blood samples obtained from the 18-month-old boars before hCG administration was 7.6±0.9 in the Large White, 7.4 ± 0.7 in Landrace, 7.0 ± 0.6 in Duroc, and 7.6 ± 0.6 nmol/L in Tempo boars. Two hours after functional stress with hCG, the testosterone concentration increased slightly to7.8 ± 0.5 in Large White, 7.6 ± 0.6 in Landrace, and 7.8 ± 0.6 nmol/L in Tempo boars (Figure 4).

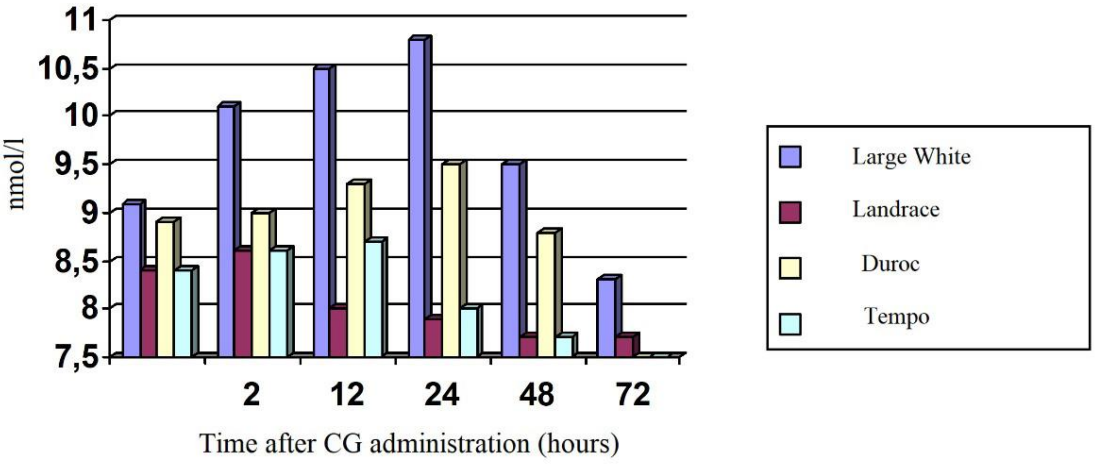

Figure 3. Testosterone levels in the blood of 12-month-old boars after the 3rd CG administration

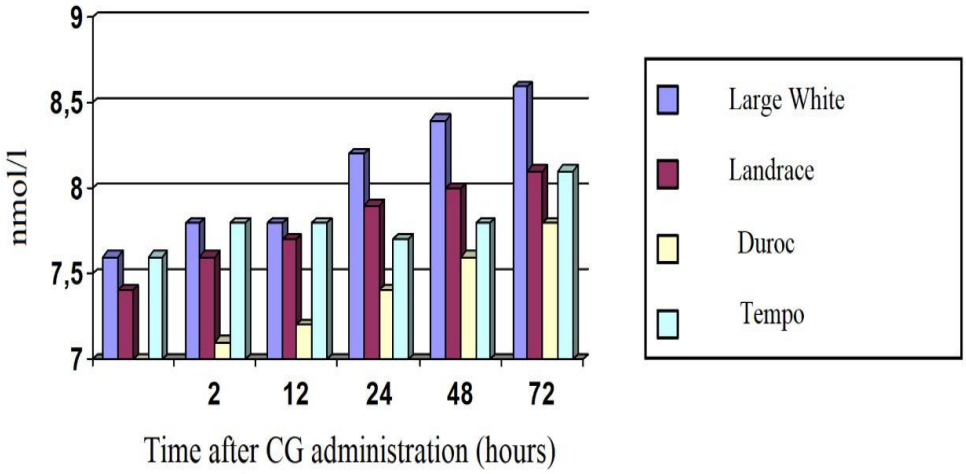

Figure 4. Testosterone levels in the blood of 18-month-old boars after the 1st CG administration

During first 2 and 12 h after the administration of hCG, the hormone level continued to increase in the Landrace and Duroc breeds by 0.1 nmol/l, reaching 7.7 ± 0.6 and 7.2 ± 0.6 nmol/l, respectively, while in the Large White breed, the hormone concentration did not change and remained at the same level. Following a period of 24 h after the introduction of hCG, the testosterone concentration in Large White boars increased compared with the data recorded after 12 hours by 0.4 nmol/l and by 0.9 nmol/l in Landrace and Duroc boars. In Tempo boars, the hormone concentration did not change but remained at the same level of 7.8 ± 0.6 nmol/L as well as 48 h after the administration of hCG. In the compared breeds of boars, the testosterone concentration continued to increase and reached 8.4 ± 0.7 nmol/L in Large White, 8.0 ± 0.7 nmol/l in Landrace, and 7.6 ± 0.6 nmol/l in Duroc boars. 72 h after the administration of hCG, the hormone in all experimental boars increased: in Large White – up to 8.6 ± 0.6 nmol/L, in Landrace – up to 8.1 ± 0.5 nmol/L, in Duroc – up to 7.8 ± 0.6 nmol/L, and in Tempo – up to 8.1 ± 0.6 nmol/L. According to the scheme of the functional stresses on the testes, after taking blood from experimental boars, the second administration of hCG was carried out 72 hours after the first administration of CG (Figure 5).

After a period of 24 h following hCG injection, the testosterone level in Landrace boars did not change but remained at 8.1 ± 0.7 nmol/L. In other boar breeds, the hormone concentration increased during the same period. In Large White and Tempo, the testosterone level increased by 0.1 nmol/l, and in Duroc by 0.2 nmol/l. 12 h after the functional stress, the testosterone concentration increased in all breeds of boars to 8.9 ± 0.7 in Large White, 8.2 ± 0.7 in Landrace, 8.3 ± 0.8 in Duroc, and 8.3 ± 0.7 nmol/l in Tempo. These concentrations remained at the same level after 24 hours. The hormone concentration was not altered in the Landrace breed (8.2 ± 0.7 nmol/L). In Large White, testosterone increased up to 9.0 ± 0.7 nmol/l. In Duroc, it significantly increased by 0.5 nmol/l and reached 8.8 ± 0.8 nmol/L. 48 hours after the second administration of hCG, the hormone concentration in Large White boars increased to 9.2 ± 0.7, to 8.3 ± 0.5 in Landrace, to 9.0 ± 0.7 in Duroc, and to 8.4 ± 0.5 nmol/L in Tempo. After 72 h, the testosterone concentration remained unchanged in Tempo and Landrace boars (8.4 ± 0.6 nmol/l and 8.3 ± 0.5 nmol/l, respectively). Large White and Duroc had an increase of 0.1 nmol/L. Thus, in general, the increase in Large White after the second administration of hCG was 8.1%; in Landrace, it was low and reached 2.5%; in Duroc it reached 16.6%, and in Tempo it reached 3.7%. This increase in manure

indicates partial activation of the potential reserves of the testes after the second stress, i.e. hCG injection. For a more precise evaluation, the boars were subjected to a third functional stress with hCG (Figure 6). Kotula-Balak et al. (5) had the same findings in the manure of Mishlan boars.

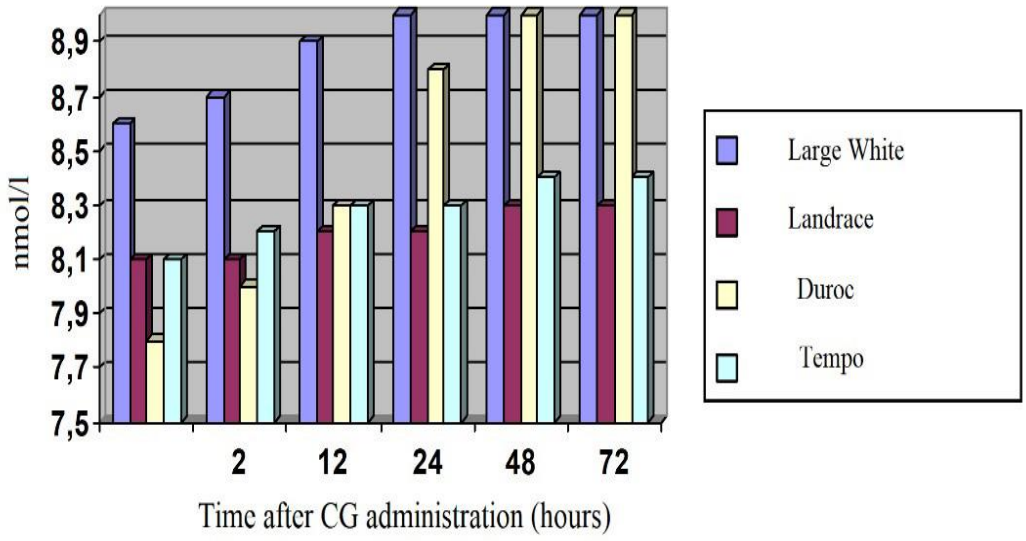

Figure 5. Testosterone levels in the blood of 18-month-old boars after the 2nd hCG administration

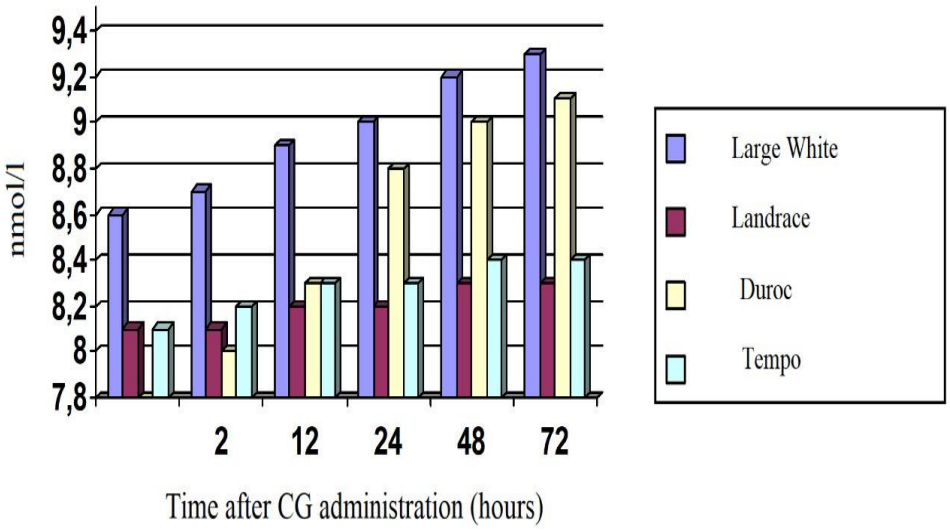

Figure 6. Testosterone levels in the blood of 18-month-old boars after the 3rd CG administration

Two hours after the third administration of hCG, the testosterone level increased in all experimental groups. In Large White boars, the testosterone level increased by 1.2 nmol/l, amounting to 10.5 ± 0.8 nmol/l. In Landrace, it increased by 0.2 nmol/l to 8.5 ± 0.7 nmol/l. In Duroc boars, the level increased by 0.3 nmol/l to 9.4 ± 0.8 nmol/l. In Tempo, the testosterone level increased by 0.1 nmol/l and amounted to 8.5 ± 0.5 nmol/l. In Large White boars, an increase in the testosterone concentration was also observed later. After 12 hours, the testosterone concentration increased up to 10.9 ± 0.8 nmol/l and continued to increase for 24 hours. During this period, the concentration of the hormone was the highest after three administrations of CG and amounted to 11.0 ± 0.9 nmol/l. The increase from the basal to the maximum level after the 3rd administration was 44.7%. In Landrace, the testosterone level reached its maximum 2 hours after the third administration of CG and amounted to 8.5 ± 0.7 nmol/l. The increase was 14.8%. Subsequently, after 12, 24, 48, and 72 hours, the testosterone concentration gradually decreased, reaching 7.7 ± 0.5 nmol/l after 72 hours. In Duroc boars, the concentration of the hormone reached its maximum 24 hours after the third administration of CG and amounted to 9.8 ± 0.8 nmol/l. From the basal level, testosterone increased 40%. Subsequently, the level of the hormone after 48 and 72 hours decreased up to 7.3 ± 0.4 nmol/l. In Tempo boars, the maximum testosterone level was observed 12 hours after the 3rd administration of CG and amounted to 8.6 ± 0.6 nmol/l. In general, the increase in testosterone after the 3rd administration of CG was 13.1%. Subsequently, after 24, 48, and 72 hours, the concentration of the hormone gradually increased to 7.9 ± 0.5 nmol/l. Calculation of the testicular activity indices (I_{ta}) also indicated that, as at the early age periods of 6 and 12 months, I_{ta} was high in Large White boars, amounting to 0.41, and in Duroc amounting to 0.40. In Landrace and Tempo, this index was much lower, amounting to 0.18 and 0.15, respectively.

Overall, the primary aim of the current study was to investigate the functional endocrine reserves of testes in 12- and 18-month-old boars of different breeds. To fulfill that aim, this survey was performed in boars of the Large White, Landrace, Duroc, and Tempo breeds. Based on the results, it is revealed that the Large White and Duroc boars have higher potential endocrine reserves of testes compared to Landrace and Tempo boars.

Authors' Contribution

Study concept and design: V. I. E.
Acquisition of data: A. V. T.
Analysis and interpretation of data: A. A. M.
Drafting of the manuscript: O. S. D.
Critical revision of the manuscript for important intellectual content: A. V. G.
Statistical analysis: I. F. V.
Administrative, technical, and material support: V. I. E.

Ethics

All the procedures and animal handling were approved by the Animal Ethics Committee at the I.I. Ivanov Kursk State Agricultural Academy, Russia, under the project number of 2021-5478965-23.

Conflict of Interest

The authors declare that they have no conflict of interest.

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