Influence of environmental conditions and technology of cultivation on the morphological structure of the testes of bull calves of three breeds in beef production

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Abstract. One of the main indicators of the successful adaptation of an organism to environmental conditions is a good reproductive ability of gobies. After all, the fertilizing ability of sperm is the most important quality indicator. Different breeds of animals are not equally resistant to environmental influences. In some, the changes are relatively superficial, in others, they lead to a significant change in the physiological functions of the body. Scientific research was carried out in the SPK PZ "Druzhba" Apanasenkovsky district, which is one of the driest regions of the Stavropol Territory, with a sharply continental climate. The histology, immunohistochemistry and morphometry of the testes (6 animals of each breed) of 18-month-old bulls of the Hereford, Kazakh white-headed and Kalmyk cattle breeds were influenced by climatic factors. The results of the study showed that 18-month-old bulls of all three studied breeds of cattle have full-fledged spermatogenesis. Structural elements of the connective tissue part of the wall of convoluted tubules demonstrated morphological equivalents of the integrity of the hematotesticular barrier. The proportion of tubules with pronounced destruction of spermatogenic epithelium was 2–3 percent. The diameter of the convoluted seminal tubules in animals of all breeds was more than 200 microns. The population of Leydig cells was characterized by a variety of cell forms, the predominant being rounded and polygonal cells with light nuclei and well-developed cytoplasm. The morphology of Leydig cells indicated their ability to actively synthesize sex hormones. A comparative analysis of the testes of gobies of the three studied breeds did not reveal any pronounced breed differences in the structure of the male sex glands of these animals. This indicates that the morphofunctional characteristic of the testes is a stable and conservative indicator, which changes little in the course of selection work.

1. Introduction
Animals are considered acclimatized if they have actively adapted to life in new conditions and give viable offspring. Among the natural and climatic factors, air temperature, humidity, atmospheric pressure, vegetation, terrain and physical properties of the soil are of prime importance. Mostly, these factors act in the form of a complex, but some in certain conditions become dominant.

The adaptation of imported beef cattle breeds to the dry steppe climate of the Stavropol Territory, which is unusual for them, is of great biological importance, since under these conditions all functions must be balanced so that the metabolism in the body proceeds without tension of reserve mechanisms.
The observations of scientists show that animals of a warm climate are easier to acclimatize in a cold zone than animals brought from a cold climate to a warm one. It is also known that adult animals adapt to a new climate worse than young animals, since the formed organism of an adult animal is difficult to rebuild. Young growth, reared in new conditions, adapts better and more easily and changes its heredity in accordance with living conditions. But in any case, animals, finding themselves in new climatic conditions of existence, enter into certain interactions with the environment. In this case, a special role is played by those factors that have a direct impact on the animal's body. In order to judge with full justification the degree of significance of these factors on the adaptation process (and, if necessary, smooth out their unfavorable influence), it is necessary to know exactly and take into account both the old conditions for animals, familiar to them, and new ones - in places of acclimatization.

When animals of a particular breed move from one climatic zone to another, under the influence of a new environment for them, changes in breed properties occur. How deep these changes will be depends on the characteristics of the animals themselves and the conditions in which they fall.

In the last decade, in many regions of the Russian Federation, including in the Stavropol Territory, due to prolonged anthropogenic impact, an ecologically crisis situation has developed, characterized by the presence of anomalies of various origins, for example, sharp changes in temperature, air humidity and atmospheric pressure. It is believed that the level of sexual behavior of animals is determined by environmental factors. It has been proven that the qualitative and quantitative characteristics of semen are subject to seasonal fluctuations. Deviations in the morphological and functional state of the reproductive organs of cattle located in different natural and climatic zones of the sharply continental climate lead to a decrease in the reproductive capacity of animals. Under the influence of temperature and changes in the hormonal background, the percentage of sperm with normal morphology is disturbed, and atmospheric pressure and high air humidity significantly affect the indicators of sperm production in breeding bulls. The imbalance of weather and climate is harmful to the health of farm animals.

Although the Hereford, Kazakh white-headed and Kalmyk breeds of cattle are among the most common breeds raised in a harsh continental climate in the Southeast of Russia, the Volga region and the Urals, it should be noted that the Hereford breed was developed in Great Britain, and the Kalmyk and Kazakh white-headed - in Russia (Kalmyk - in the 17th century, and Kazakh white-headed - in the middle of the XX). A number of works are devoted to studies of various aspects of the morphology and physiology of reproduction of these animals [1, 2]. The impact of natural and climatic conditions on the patterns of the morphofunctional organization of the testes of these animals has not been sufficiently studied and need further in-depth research, supplement and clarification.

2. Purpose of research
The purpose of the study is the impact of climate on the morphology of the testes of gobbies of the Hereford, Kazakh white-headed and Kalmyk cattle breeds.

3. Materials and methods
The object of the study was the testes of foreign cattle (Hereford) and domestic breeds (Kazakh white-headed and Kalmyk), grown in the Stavropol territory in the period of 2018–2019. During the growing process, the weight of bulls after birth at the age of 3, 8, 12, 15 and 18 months was determined. Samples of testes for research were taken from 18-month-old animals in October 2019 during the slaughter of bulls at a meat processing plant in the village of Divnoye, Stavropol territory. For histological studies, the material was fixed in a 12 % aqueous solution of formalin, dehydrated in ethanol of increasing concentration, and poured into paraffin. Paraffin sections 5–7 micrometers thick, made on a rotary microtome, were stained with review histological (Mayer's hematoxylin and eosin), histochemical (SHIK-reaction) and immunohistochemical methods (for detecting ki67 and P53). For these purposes, we used monoclonal mouse antibodies to protein P53, a clone of DO-7 in 1:100 dilution, monoclonal mouse antibodies to Ki-67 in 1:600 dilution, a clone of Mm1 which is the same
as MB67 (Diagnostic BioSystems) and an imaging system (BioGenex, USA). Using histological sections, morphometry of the structural elements of the testes was performed (the diameter of the convoluted seminal tubules, the proportion of convoluted seminal tubules and interstitial tissue, the diameter of the nuclei of Leydig cells and their number on the conventional unit of the testis section area. This is on the area of a square with a side of 35 microns). There was also the content of Leydig cells with different sizes of nuclei, the ratio of stromal and endocrine components in the interstitial organ). The obtained digital indicators were subjected to statistical processing using the program "Statistica-8".

4. Results and Discussion

The rate of increase in the mass of animals indicates a good rate of weight gain in bulls of all the studied breeds. The slaughter weight was 480.60±20.94 kg for Hereford bulls, 460.90±11.72 kg – for Kazakh white-headed bulls, and 437.90±12.51 kg – for Kalmyk bulls (table 1).

| Breeds           | Age in months |
|------------------|---------------|
|                  | Newborns      | 8              | 12             | 15             | 18             |
| Gereford         | 30.4±1.72     | 229.2±2.35     | 329.8±2.67     | 423.3±3.98     | 488.4±1.98     |
| Kazakh white-headed | 24.3±1.94     | 216.5±0.77     | 317.1±3.51     | 406.7±3.34     | 471.7±1.82     |
| Kalmuck          | 21.9±1.69     | 209.6±1.89     | 309.7±3.12     | 393.6±4.29     | 460.5±1.28     |

The analysis of histological preparations showed that the bulls of all three studied breeds of cattle have full-fledged spermatogenesis in their testes.

In the convoluted seminal tubules, all cells characteristic of the stages of spermatogenesis are detected (spermatogonies, spermatocytes of the first and second order, spermatids and spermatozoa), mitotic division of spermatogonies and meiotic divisions of spermatocytes are observed. Pathological forms of spermatogenic cells in most of the convoluted seminiferous tubules were isolated. The proportion of convoluted seminal tubules with pronounced destruction (disorganization of the spermatogenic epithelium, the presence of multinucleated cells, a large number of pathologically altered cells of the spermatogenic epithelium, violation of the integrity of the hematotesticular barrier) in animals of the studied animal breeds was insignificant and varied within 2–3 percent, which corresponded to the morphofunctional characteristic of the normal testis [1–3]. The diameter of the convoluted seminal tubules exceeded 200 micrometers (table 2), which is characteristic of sexually mature individuals of these animals. The significant content of collagen fibrils and CHIC-positive mucopolysaccharides in the connective tissue part of the wall of the convoluted seminal tubules is noteworthy. Cellular and non-cellular elements of the connective tissue part of the wall of the convoluted seminal tubules demonstrate structural equivalents of the integrity of the hematotesticular barrier.

The population of Leydig cells in the bulls of the studied breeds was characterized by a variety of cell forms. The main number of Leydig cells on the sections had a rounded or polygonal shape, in a small number there were also spindle-shaped and process-shaped cells. The rounded cells had a diameter of 10–13 microns. The length of oval and polygonal cells ranged from 10–14 microns, and the width was 8–10 microns. These cell forms were characterized by medium-sized and large nuclei and a well-developed cytoplasm. In the nuclei, euchromatin was distributed evenly over the entire area, and the content of heterochromatin was variable. Analysis of the volume of Leydig cell nuclei showed that in the testes of bulls of all the studied breeds, cells with average volumes of nuclei (from 50 to 100 mm$^3$) predominated. For example, in the testes of Kazakh white-headed bulls, their content was 68.4±3.2 %. And the content of cells with large nuclei (over 100 mm$^3$) was 28.5±2.2 %. We did not find any breed differences in the characteristics of the Leydig cell population among the studied
breeds. Morphofunctional characteristics of interstitial tissue of Leydig cells in the testes of all studied animals indicate their ability to active steroidogenesis.

Comparison of the structural and functional characteristics of the convoluted seminal tubules and Leydig cells of the testes indicates a good reproductive potential of the studied animals of three breeds.

The area of interstitial tissue in the testes of bulls of the studied breeds was significant (table 2), which is a characteristic feature of the male gonads of these animals. According to this indicator, significant differences were found among the studied breeds.

| Breed of parameter | Hereford | Kazakh white-headed | Kalmyk |
|--------------------|----------|---------------------|--------|
| Diameter of convoluted seed tubules (microns) | 204.3±2.9 | 201.3±3.2 | 205.3±2.6 |
| Percentage of interstitial tissue (%) | 15.0±0.54 | 16.2±0.62 | 16.4±0.41 |
| Average volume of Leydig cell nuclei (mm³) | 73.8±2.54 | 75.1±3.39 | 79.3±4.12 |
| Number of Leydig cells per unit area of interstitial tissue | 16.2±0.58 | 15.2±0.63 | 14.6±0.46 |
| Percentage of stromal components in the testis (%) | 7.2±0.29 | 8.2±0.30 | 8.1±0.25 |
| Proportion of Leydig cells in the testis (%) | 7.8±0.27 | 8.0±0.30 | 8.3±0.29 |

Thus, the content of interstitial tissue in the testes of animals of the Hereford breed was lower than that of the Kazakh white-headed and Kalmyk breeds, while there were no differences in this indicator between the bulls of the Kazakh white-headed and Kalmyk breeds. Similar differences were found in the content of stromal components of connective tissue in the testis (table 2). Comparative analysis showed that there were no significant differences between the animals of the studied breeds in terms of such indicators as the diameter of the convoluted seminal tubules and the content of Leydig cells (table 2). Similar data was provided by other researchers [4–10] when studying the testes of bulls of other breeds of cattle. Despite the identified some breed features of the histophysiology of the testes, the data obtained and their comparison with the literature indicate the absence of pronounced breed specificity of the testes for the main characteristics of the organ (the diameter of the convoluted seminal tubules, the state of the spermatogenic epithelium, morphofunctional characteristics of the population of interstitial endocrinocytes) in these animals.

The expression of p53 protein in the convoluted seminal tubules and interstitial tissue was low, while the expression of this protein in the convoluted seminal tubules was more pronounced in comparison with the interstitial tissue of the testis. Ki67 expression was detected in spermatogenic epithelium in spermatogonia and spermatocytes, in interstitial tissue-in fibroblastic differon cells. Ki67 protein expression was not detected in Leydig cells. Analysis of the results of detection of ki67 expression confirms the available literature data that the population of Leydig cells belongs to stable cell populations, and the recovery of the number of Leydig cells can occur due to their differentiation from low-differentiated precursors of mesenchymal origin (low-differentiated fibroblastic differon cells).

5. Conclusion
Regardless of the influence of natural and climatic conditions, the results obtained indicate that the main parameters of the testes (the diameter of the convoluted seminiferous tubules, the state of spermatogenesis, the content of Leydig cells) in the studied animals of the Hereford, Kazakh white-headed and Kalmyk breeds do not have significant differences, therefore, the structure of reproductive glands are primarily determined by hereditary factors that determine the morphogenesis of the gonads. This indicates that the morphological and functional characteristics of the testes is a stable and conservative indicator that changes little under the conditions of the dry steppe zone of the Stavropol Territory.
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