

**MORPHOFUNCTIONAL DESCRIPTION OF THE ENDOCRINE AND
GERMINATIVE STRUCTURES OF THE TESTES OF MARMOTS
(*Marmota bobak* M.) ACCORDING TO SEASONAL CHANGES IN
REPRODUCTIVE ACTIVITY**

**МОРФОФУНКЦИОНАЛЬНАЯ ХАРАКТЕРИСТИКА ЭНДОКРИННЫХ
И ГЕРМИНАТИВНЫХ СТРУКТУР СЕМЕННИКОВ СУРКОВ
(*Marmota bobak* M.) В СВЯЗИ С СЕЗОННЫМИ ИЗМЕНЕНИЯМИ ИХ
РЕПРОДУКТИВНОЙ АКТИВНОСТИ**

**DESCRIPTION MORPHO-FONCTIONNELLE DES STRUCTURES ENDOCRINES ET
GERMINATIVES DES TESTICULES DE MARMOTTES (*Marmota bobak* M.) EN
FONCTION DES VARIATIONS SAISONNIÈRES DE L'ACTIVITÉ REPRODUCTRICE**

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Abstract

By means of light and electron microscopy the dynamics of structural and functional changes of pubertal bobacs testicles during seasonal fluctuations of its reproductive activity (from the beginning of April to August) were investigated. It was revealed that only a half of males number took part in the reproduction. A phenomenon of the activization of testicles germinative and endocrine structures before hibernation was demonstrated. The activization of androgen-producing structures (Leydig' cells) in testicles precedes the spermatogenesis activation in convoluted seminiferous tubules. The biological significance of this phenomenon is the preparation of males reproductive organs to mating process which begins after winter hibernation, before the marmots appear from burrows.

Key-words: Bobacs, testicles, convoluted seminiferous tubules, Leydig' cells.

Резюме

С использованием методов световой и электронной микроскопии исследована динамика структурных и функциональных изменений семенников половозрелых сурков в процессе сезонных изменений их репродуктивной активности (с начала апреля по август). Обнаружено, что в сезон наблюдений только половина самцов принимала участие в размножении. Выявлен феномен активизации эндокринных и герминативных структур семенников перед началом гибернации. Явление активизации андрогенпродуцирующих структур в семенниках (клеток Лейдига) предшествует активизации сперматогенеза в извитых семенных канальцах. Биологическое значение этого феномена заключается в подготовке репродуктивных органов самцов к спариванию, которое у сурков начинается сразу после завершения зимней спячки, до выхода животных на поверхность.

Ключевые слова: сурок-байбак, семенники, извитые семенные канальцы, клетки Лейдига.

Résumé

Les modifications structurelles et fonctionnelles des testicules de marmottes bobacs adultes au cours des saisons (début avril-Août) a été étudié en microscopie photonique et électronique. La moitié seulement des mâles adultes prennent part à la reproduction. L'activation des structures endocrines et germinatives des testicules précèdent l'hibernation. L'activation des structures productrices d'androgènes (cellules de Leydig) des testicules précède la spermatogenèses dans les tubes séminifères. L'importance biologique de ce phénomène est de préparer les organes reproducteurs des mâles au processus d'accouplement qui débute à la fin de l'hibernation, mais avant que les marmottes n'émergent de leurs terriers.

Mots-clés : Marmota bobak, tubes séminifères, cellules de Leydig.

Terrestrial rodents from the squirrel family are an unique model for histophysiological investigations of seasonal changes in the reproductive system. The unique features are in the fixed rhythm of the reproductive processes and in the following deep suppression of the germinative structures. In addition, we need to point out that some aspects of marmot reproduction remains controversial (Bibikov 1989, Mashkin 1996, Rymalov 1996, Rudi 1995, Spivakova 1988, Shevljuk 1995).

Thus, a comparative morphofunctional investigation of the testes of *Marmota bobak* during seasonal changes in reproductive activity was the aim of this work. Testes of 66 marmots ranging in age from two to seven years were the object of our investigation. Bobacs were from populations inhabiting the ecologically favorable regions of the Orenburg province. Material was taken during the period of Spring-Summer activity (Table 1). We used histological, histochemical, and morphometrical methods and electron microscopy.

Table 1.

Morphometric indices of body and testicular mass of *M. bobak* Müll.
during spring-summer seasons. All values are means $\pm$ SE.

Time of capture and breeding	Number of animals	Mean mass of animals (kg)	Mean mass of testes (g)	Diameter of seminal convoluted tubules (mkm)
Early April (breeding)	6	4.68 $\pm$ 0.37	3.41 $\pm$ 0.25	154.7 $\pm$ 5.3
Early April (nonbreeding)	6	3.71 $\pm$ 0.30*	1.13 $\pm$ 0.15*	73.2 $\pm$ 6.9*
May (reproductive pause)	9	4.85 $\pm$ 0.35	1.65 $\pm$ 0.26*	60.2 $\pm$ 5.4*
June (reproductive pause)	7	5.98 $\pm$ 0.47*	1.27 $\pm$ 0.18	58.0 $\pm$ 2.8
July (reproductive pause)	20	6.14 $\pm$ 0.39	1.77 $\pm$ 0.21*	91.5 $\pm$ 3.2*
August (reproductive pause)	18	6.75 $\pm$ 0.32	2.41 $\pm$ 0.27*	106.4 $\pm$ 7.7*

* Differences between stages are significant at $p < 0.05$.

Morphometry included light-optical preparations and electronogram investigations. In the light-optical cuts, the percent correlation between the area of interstitial tissue and seminal convoluted tubules; linear and volumetric indices of the endocrinocytes, enumeration of endocrinocytes and their nuclei and nucleoles; and the nucleus/cytoplasm ratio were determined. From precise cross-cuts diameters of the seminal convoluted tubules were measured.

From electronograms quantative indices of cytoplasmatic structures such as the comparative area of the smooth endoplasmatic reticulum (SER), mitochondria and lipid drops, their linear dimensions and their amount in the unit of electronogram area were measured. The results were analyzed by statistic computer programs.

Our investigation revealed large differences between the morphofunctional condition of the testes in marmots, which were caught in early April (when this animal first emerges above ground after hibernation). None of them had a peak of spermatogenesis activation. Based on the morphology of the gonads, 12 mature bobacs were divided into two groups. The first one contained six animals whose testes had a minimal area of interstitial tissue (Table 2). The average diameter of the seminal convoluted tubules was 154.7 $\pm$ 5.3 mkm; it does not differ from the index at the mating period. Besides, existed some significant at the beginning of the extinction of spermatogenesis.

Interstitial endocrinocytes were active and cells with developed cytoplasm and large nuclei prevailed in the cell population (volume of nuclei was large and ranged from 102.5 $\pm$ 6.1 mkm³ to 198.4 $\pm$ 8.6 mkm³). Most large endocrinocytes were around the tubules, where either meioses or cessation of spermatogenesis was observed.

The second group of animals had a low mass of gonads (Table 1), with spermatogenesis commonly absent. Comparative area of interstitial tissue was significantly increased, but the diameter of the seminal convoluted tubules was approximately two times less than that of the first group (Tables 1, 2). Meioses were extremely rare. But morphometric analysis of the interstitial endocrinocytes did not reveal any difference between the groups. Finding large and hypertrophic Leydig cells around the tubules with a minimal diameter was especially paradoxical.

The evidence indicates that not all of the 12 marmots were in breeding condition at the time of capture in early April. It reconfirms the theory that mating of the marmots occurs in the burrows before emergence.

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Table 2.

Morphometric indices of interstitial tissue of testes of *M. bobak* Müll.
 during spring-summer seasons. All values are means $\pm$ SE.

Time of capture and breeding	Number of animals	Interstitial tissue area in %	Count of endocrinocytes at the unit area	Nuclei volume of the interstitial endocrinocytes, mkm^3	Count of mature, functionally active endocrinocytes in %
Early April (breeding)	6	19.6 $\pm$ 2.8	9.3 $\pm$ 0.3	137.8 $\pm$ 6.5	64.2 $\pm$ 2.9
Early April (nonbreeding)	6	46.5 $\pm$ 5.7*	11.2 $\pm$ 0.5*	82.4 $\pm$ 7.9*	50.3 $\pm$ 4.1*
May (reproductive pause)	9	67.6 $\pm$ 3.6*	8.9 $\pm$ 0.5*	48.4 $\pm$ 3.2*	40.9 $\pm$ 6*
June (reproductive pause)	7	49.9 $\pm$ 2.4*	7.1 $\pm$ 0.3*	61.6 $\pm$ 4.2*	55.8 $\pm$ 2.9*
July (reproductive pause)	2	41.4 $\pm$ 2.3*	14.0 $\pm$ 0.5*	61.6 $\pm$ 2.5	56.0 $\pm$ 3.8
August (reproductive pause)	18	41.4 $\pm$ 3.3	8.4 $\pm$ 0.5*	98.3 $\pm$ 4.5*	56.0 $\pm$ 2.6

* Differences between stages are significant at $p < 0.05$.

Another interesting significant characteristic of interstitial tissue of the marmot testes is the low content of connective tissue stromal components, which was observed in both the reproductive condition and the reproductive pause.

Why does the interstitial endocrinocytes have such high secretory activity under the different conditions of the tubules? Maybe it is important for another regulatory pathway in the marmot organism.

The population of endocrinocytes of marmot testes is characterized by heteromorphology and it contains roundish, oval, fusiform, and polygonal cells. Nuclei were more often oval or roundish. The maximal count of Leydig cells was in early April (Table 2). During reproductive pause, the number of the endocrinocytes is reduced with the lowest value in May-June (Table 2). In the June-July period the index was increased due to differentiation of endocrinocytes from precursors. Interstitial area was increased from late April-June, but in July-August it decreased.

Agranular (smooth) endoplasmatic reticulum, the main structure in steroid synthesis, is represented by the vesicular structures distributed among the cytoplasmic area. The degree of its development changed minimally during the Spring-Summer seasons. Most development of the smooth endoplasmatic reticulum coincides with maximal cell dimensions. The testicular endocrinocytes often contain electronic dense lipid drops, which are larger than the same structures in gophers. Around most all Leydig cells is a delicate moderately osmiophilic structure, the basal membrane.

A high quantity of pigment inclusions in the cytoplasm is one of the typical peculiarities of the Leydig cells of the marmot testes. The amount of pigment varies within wide limits. Sometimes the level of pigment inclusions is so high that the color of the testes varies from olive to brown. Another item of significance is the extensive presence of argentaffine cells.

From our data, before immergence into hibernation, due to morphofunctional reconstructions, the mass of the testes increased about three times. The area of interstitial tissue is reduced following the increase in the area of the convoluted tubules. In May-June, the area of the convoluted tubules varied from 15.1 $\pm$ 2.3% to 30.8 $\pm$ 3.6%, and with the beginning of hibernation, the area becomes about half of the cuts area. The increase is associated with a sharp increase in tubular diameter from 58.0 $\pm$ 2.8 mkm in June to 106.4 $\pm$ 4.4 mkm in early August. With the activation of spermatogenesis, spermatogenic epithelium becomes polylayering. However, mature spermatozoa are not found, which means that spermatogenesis does not occur until the spermatocytogenesis stage and spermiogenesis occurs later, before the reproductive season.

The dimensions of the endocrinocytes and their nuclei in the hibernation period sharply increase from a nuclear volume of 31.2 $\pm$ 2.1 mkm^3 in June to 98.3 $\pm$ 3.7 mkm^3 in early August (same as the April indices).

In July-August, mature and functionally active cells prevailed in the endocrinocyte population. They had developing cytoplasm and large nuclei with a rich abundance of the euchromatin structures. Most of the cytoplasm was occupied by the smooth endoplasmatic reticulum; also there were many mitochondria and lipid inclusions. Activating changes in Leydig cells are earlier and more striking than activation of spermatogenesis.

According to the data of other authors (Novales et al. 1978), the "hibernating gland" of the forest marmots contains much androgen hormone during hibernation. The phenomenon is unique, because usually androgens are

