AGRICULTURAL BIOLOGY,
2018, V. 53, № 4, pp. 743-752
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.2:636.082:577.17

doi: 10.15389/agrobiology.2018.4.743eng
doi: 10.15389/agrobiology.2018.4.743rus

ENDOGENOUS HORMONE LEVEL IN BULL SIRES IN RELATION TO AGE, AUTOIMMUNE STATUS, AND PRODUCTION PERFORMANCE OF MATERNAL ANCESTORS

A.I. ABILOV[1], O.S. MITYASHOVA[1], S.V. MYMRIN[2], A.A. GUDILINA[2], E.A. PYZHOVA[3], N.A. KOMBAROVA[1, 4], G.N. LEVINA[1]

[1]*Ernst Federal Science Center for Animal Husbandry,* Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail ahmed.abilov@mail.ru (✉ corresponding author), mityashova_o@mail.ru, gnlevina@yandex.ru;
[2]*Uralplemcentr OAO*, 21 km, Siberian tract, Ekaterinburg, 620913 Russia, e-mail uralplem@mail.ru;
[3]*Russian Academy of Livestock Management*, 9, ul. Akademicheskaya, pos. Bykovo, Podolsk Region, Moscow Province, 142143 Russia, e-mail elena@ramj.ru;
[4]*Head Center for Reproduction of Farm Animals AO*, 3, ul. Tsentralnaya, pos. Bykovo, Podolsk Region, Moscow Province, 142143 Russia, e-mail komnina@list.ru
ORCID:
Abilov A.I. orcid.org/0000-0001-6236-8634
Mityashova O.S. orcid.org/0000-0002-0401-5088
Mymrin S.V. orcid.org/0000-0001-5173-984X
Gudilina A.A. orcid.org/0000-0002-7962-5315
Pyzhova E.A. orcid.org/0000-0001-5719-3075
Kombarova N.A. orcid.org/0000-0003-3861-4465
Levina G.N. orcid.org/0000-0002-7978-6696
The authors declare no conflict of interests
Received April 11, 2018

Abstract

The role of sex steroid hormones, their physiological functions in the bull sires, and mechanism of action are not still completely elucidated. This paper is the first report of a large-scale survey that we carried out among bull sires under the ecological conditions of Ural region to estimate production of endogenous hormones estradiol and testosterone, and their precursor cholesterol, as depending on the bull age, origin (Denmark, Netherlands, Russia, the USA, France, and Germany), the milk performance of their maternal ancestors, and the titers of anti-sperm antibodies. The relationship between the endogenous hormones and the studied parameters is ascertained, and it is found that the synthesis of steroid sex hormones is sustainable even at low level of blood cholesterol. Our objective was to assay concentration of blood hormones and the titers of anti-sperm antibodies in 56 bull sires including 49 Black-and-White Holsteins and 7 Black-and-White animals of different origin aged from 24 to 91 months which are exploited in the Ural Regional Information and Breeding Center (UralPlemCenter, Sverdlovsk Province, 2016-2017). The blood serum hormones were measured by ELISA with testosterone Immuna-FA-TC and Immuna-FA-Estradiol kits (Immunotech, Russia). The ratios of testosterone to estradiol were calculated. The cholesterol level was assayed with a ChemWell 2902 automated analyzer (Awareness Technology, Inc., USA). The autoantibody titers were detected in the sperm immobilization test with blood auto serum of the bull sires and guinea pig serum complement. The obtained results were processed depending on the country of origin, the bull age, and the retrospective data on milk productivity of the bulls' mothers (M), the mothers' mothers (MM), and the fathers' mothers (FM). The highest (30.7 nmol/l) and the lowest (13.4 nmol/l and 10.7 nmol/l) concentrations of testosterone had the Danish, Holland and French bull sires, respectively. It is found that the testosterone level was rising to five-year age and eventually reached 26.9 nmol/l vs. 9.6 nmol/l in two-year old animals. The blood concentrations of testosterone and estradiol inversely correlated. The testosterone to estradiol ratio significantly varied ($P < 0.001$). The lowest testosterone concentration (15.4 nmol/l) was in hyperestrogenization, and the indicator value reached 25.1 nmol/l in the animals with low estrogens. The testosterone to estradiol ratio (T/E) increased with the age, from 0.4 at 28 months of age up to 19.3 at 50 months, by a statistically significant value ($P < 0.001$). No significant relationship was revealed between the endogenous hormone concentrations in the bulls and the milk performance of their maternal ancestors, except some effect of the father's mothers with milk yield of 12000 to 16000 kg. An increase in the sperm autoantibody titer was accompanied by the decreases in the serum testosterone and estradiol levels by 37.9 % and 4.6 %, respectively. The cholesterol level increased by 13.7 %. Changes between the groups were within the normative range. Therefore, the concentration of the endogenous hormones (testosterone and estradiol) depends on the sire age and origin. The test for anti-sperm antibodies during clinical andrological examination is diagnostically important to indirectly characterize the hormonal status of sires.

Keywords: bull sires, testosterone, estradiol, cholesterol, anti-sperm antibodies

The chemical composition of endogenous testosterone and androsterone hormones is similar to ovarian hormones estrogen and progesterone [1]. The bodies of males produce both androgenic hormones and estrogenic hormones [2]. The androgenic hormones ensure gender differentiation and determine the function of male reproductive organs (ovaries, testicles and prostate) [3]. The synthesis of steroid hormones and spermatogenesis in testicles is controlled by gonadotropic hormones and testosterone [4]. In turn, spermatogenesis is controlled by peptide hormones and steroid hormones: the follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estradiol [5]. About 2-3% of androgens are biologically active and circulate freely, the rest remain in blood plasma and form a complex with testosterone-estradiol-binding globulin (TEBG) [6, 7]. Hyperestrogenism and hyperthyrosis result in decline of the free testosterone function and increase of testosterone-estradiol-binding globulin content [8]. The reduced testosterone levels in blood plasma with increased levels of luteinizing hormone and follicle-stimulating hormone can result in primary and secondary testicular failure [9, 10] and changes mobility of sperm cells in ejaculate. The involvement of testosterone in activation of mitosis protein synthesis, formation of androgen-dependant enzymes, structural changes of chromatin in sperm cells, amplification of DNA and RNA synthesis [4, 11] has been proven. Testosterone affects libido, endurance, improves muscle capacity and oxygen capacity of blood [12].

Male estrogens are represented by estrone, estriol and 17β-estradiol; 20% of endogenous estrogens are synthesized directly in testicles, 80% are synthesized in peripheral tissues on account of aromatization with predominant formation of 17β-estradiol [13, 14]. The largest amount of estradiol in blood plasma is associated with testosterone-estradiol-binding globulin and albumins [7], where biologically active fraction constitutes 1-2%. The main purpose of functions of male estrogens is to enable the mechanism of negative feedback via gonadotropins (luteinizing hormones and follicle-stimulating hormones) and to regulate testosterone synthesis in testicles [13, 14]. In most cases, the increased levels of estradiol are caused by various functional disorders of estradiol metabolism. The estrogen receptors (α and β are coded with *ESR1* and *ESR2* genes) are 44 % homological and contain domains characteristic for intracellular receptors. The estrogen receptors of α-type are located in adenohypophysis, testicles, liver, kidneys, bones and brain, the receptors of β-types are located in bones, cartilages, gastrointestinal tract, thyroid gland, prostate, skin and bladder, and estrogen effect manifests itself via them [15]. The luteinizing hormone causes proliferation of seminiferous tubules and stimulates the initial stages of spermatogenesis [16], and progestin is crucial for meiosis initiation [17]. The impact of estrogens on spermatogenesis via hypothalamus is known [8]. The impact of estradiol on behavior, qualitative and quantitative characteristics of semen of bull sires [18] has been identified. The maximum amount of ejaculate was registered at minimal levels of estradiol, and the lower were the levels on the day of semen extraction, the better were the results of cow insemination [19].

In males, the volumes of testosterone and estradiol, as well as balance of their free fractions strictly correlate. The balance of sex hormones is upset in case of overweight: the content of estrogens increases [20] resulting in reduced sperm concentration [21]. Fat tissue increases [23] in case of physiological activity increase of aromatase enzyme [14, 22] converting testosterone into estrogen (estradiol) during normal ageing of males. In case of insufficient body weight the quality of semen also deteriorates [24].

It has been determined that for Holstein cattle the levels of testosterone

in blood plasma positively correlate with the quantity of sperm cells carrying Y-chromosome [25]. In Simmental bulls a positive correlation between semen concentration in ejaculate and testosterone levels has been observed, and negative correlation has been observed between semen pH and blood plasma testosterone depending on season [26, 27]. It has been mentioned that for bulls at the age of 2-4 months to 2 years the levels of blood plasma testosterone are statistically significantly different, and for older animals this parameter tends to increase [28]. We have shown the dependency of endogenous testosterone levels in the blood of bull sires on age and season [29]. It is presumed that seasonal fluctuations of testosterone are related with seasonal factors (pesticides, exogenous estrogenic hormones, physical and other phenomena) that affect the survivability of sperm cells detrimentally by producing structural and genetic changes and damaging chromatin integrity [30].

Modern industrial countries tend to accumulate synthetic endocrine-active compounds in their biosphere (with ultimate estrogen and/or antiandrogenic activity) such as ecological estrogens (xenoestrogens) and antiandrogens capable of affecting the reproductive function, nervous, immune and endocrine systems of animals and humans [15, 31-33].

Similar to glucocorticoids, testosterone causes immunodepressive effect [34, 35]. There is a connection between endogenous hormone levels (thyroxine, estradiol, and testosterone) and presence of spermatic autoantibodies in blood plasma of bull sires [36]. In the context of autoimmune response development the new data about sex steroid levels in blood (androgens, gestagens, and estrogens) and spermatogenesis will help better understand the physiological and pathophysiological function of so-called female sex hormones in a male body, whose mechanism is still unclear.

In the course of a wide-scale survey of bull sires first performed by us in the environmental conditions of the Sverdlovsk region (the region with dominating ferrous metal industry, nonferrous metal industry and ore dressing plants) we identified a correlation between the titres of spermatic autoantibodies with estradiol and testosterone levels in blood plasma, and correlation between the levels of estradiol, testosterone and cholesterol in blood, as well as age and origins of animals (Denmark, the Netherlands, Russia, USA, France, Germany) and milk productivity of their maternal ancestors. It has been observed that sex steroid hormones were synthesized in bulls even with low cholesterol levels in blood.

Our goal was to conduct qualitative evaluation of endogenous serum hormones and antibodies regarding the antibodies and spermatic antigens in bulls depending on age, origins and productivity of maternal ancestors.

Techbiques. The sampling included 56 bull sires of different origins at the age of 2-9 years (49 black-and-white Holstein animals, 7 black-and-white animals) (OJSC Uralplemcentre, Sverdlovsk region, 2016-2017). Animal breeding and maintenance conditions complied with the national technological requirements for freezing and use of semen of bull sires.

The blood was taken from jugular vein (December of 2016). After separation of blood plasma from formed elements the levels of endogenous hormones were identified using the enzyme-linked immunosorbent assay method twice using laboratory reagents (CJSC Immunotech, Russia): for testosterone Immuna-FA-TC, for estradiol Immuna-FA-estradiol. The testosterone and estradiol ratio was calculated. The cholesterol levels were evaluated using an automatic analyzer Chem Well 2902 (Awareness Technology, Inc., USA).

The ratio of autoantibody titres and own sperm cells was evaluated in a sperm immobilization test [36].

The obtained results were compared by taking into consideration the

country of origin and age of producers (24-91 months), as well as data about milk productivity of maternal ancestors, i.e. the mothers of bulls (M), mothers of mothers (MM) and mothers of fathers (MF).

Microsoft Excel was used for statistical processing of data. The tables display mean (*M*) and errors in mean (±SEM) values. The statistical significance of differences was evaluated according to Student *t*- criterion with statistical significance at $p < 0.05$, $p < 0.01$, $p < 0.001$.

Results. The highest and lowest testosterone levels (30.7 and 13.4 nmol/l) at sampling size $n > 6$ were registered for producers of the Danish and Dutch selection respectively (table 1). Testosterone levels directly depended on age because by the time of the survey the age of producers of Danish selection was 3.3 years, whereas bulls imported from the Netherlands were only 2 years of age. The lowest estradiol levels were characteristic for American selection bulls (3383.3 pmol/l), whereas for other animals this parameter was in the range of 4052-4956 pmol/l. The bulls of different origin did not differ significantly in terms of cholesterol content.

1. The levels of endogenous hormones and cholesterol in blood plasma of bull sires of different origins (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Origin	*n*	Breed	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units
Denmark	11	H	30.7±7.7*	4052.0±477.6	3.2±0.4	4.0±1.3
the Netherlands	11	H	13.4±5.2	4786.4±341.7	3.1±0.3	2.8±1.0
Russia	13	H	15.7±4.4	4254.3±510.1	3.5±0.1	4.4±1.4
Russia	7	BW	27.4±10.1	4593.4±324.2	3.2±0.6	6.9±2.8
USA	6	H	21.9±8.0	3383.3±822.7	2.9±0.2	5.4±1.7
France	3	H	10.7±6.4	4166.9±1031.8	2.8±0.4	4.3±3.4
Germany	4	H	15.7±6.6	4956.5±444.0	4.1±0.3	2.2±1.4
(France+ Germany)	(3 + 4)	H	13.5±4.4	4618.1±483.5	3.6±0.3	3.6±1.6
Total	55					

N o t e. H — black-and-white Holstein breed, BW— black-and-white breed. T/E — quantitative testosterone to estradiol ratio.
* Differences with a group of bulls from Russia ($n = 13$) are statistically significant at $p < 0.05$.

2. The levels of endogenous hormones and cholesterol in blood plasma of bull sires of black-and-white Holstein breed and black-and-white breed depending on age (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Age group, months	*n*	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units
До 24	13	9.6±2.9	5044.2±147.1	3.0±0.3	1.7±0.5
25-36	13	19.7±5.8	4559.5±321.6	3.4±0.3	4.8±1.6
37-60	23	26.9±4.7*	3955.4±384.2**	3.6±0.1*	5.4±1.1*
61 и старше	7	16.0±7.5	3426.5±549.2*	2.6±0.4*	5.7±2.4

N o t e. T/E — quantitative testosterone to estradiol ratio.
*, ** Differences with a group below 24 months are statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

The analyzed bulls were divided into four age groups: below 2 years of age, 2-3 years of age, 4-5 years of age and above 5 years of age. The most numerous group ($n = 23$) was the group of animals from 3 to 5 years of age. The breeding farms primarily use producers of this age. Testosterone levels in bulls was dynamically increasing until the age of 5 (table 2). Below 2 years of age this parameter was 9.6 nmol/l, by 25-36 months it increased, at the age of 37-60 months it reached its maximum level (26.9 nmol/l). After the age of 7-8 years it was declining (down to 16.0 nmol/l), most probably due to commencement of physiological aging. Alternatively, the levels of estradiol below the age of 2 years were the highest, and in animals older than 5 years of age this parameter declined by 32 %. The levels of cholesterol in all groups corresponded to the lower limit of normal, and after the age of 5 it did not reach the normal level. The animal origin feeds served as source of cholesterol, and, consequently, a corresponding adjustment of the ratios of bull sires is required: cholesterol is the precursor of more

than 40 hormones and its deficit can result in a lack of sex steroids.

The bull sires at the age below 24 months and at the age of 37-60 months displayed reliably ($p < 0.01$) differing levels of testosterone and estradiol in blood plasma. These parameters were statistically significant ($p < 0.05$) for young (below 2 years of age) bull sires and bull sires at the beginning of physiological aging (above 5 years of age). In terms of cholesterol levels, the 37-60 month group, 61 month group and older ($p < 0.05$) groups differed reliably, and in terms of testosterone and estradiol levels ($p < 0.01$) the groups below 24 months and groups of 37-60 months of age differed reliably.

We conditionally divided the animals by their testosterone levels into three groups: with low (below 10.0 nmol/l), average (10.1-30.0 nmol/l) and high (30.0 and above nmol/l) levels of this hormone. The testosterone levels reversely depended on the levels of estradiol in blood plasma. Depending on testosterone levels, the testosterone and estradiol ratio increased by a statistically reliable value ($p < 0.001$). The levels of cholesterol in all groups remained completely identical and within limits of normal (Table 3).

3. The levels of estradiol and cholesterol in blood plasma of bulls sires of black-and-white Holstein breed and black-and-white breed depending on testosterone levels (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Groups by testosterone levels, nmol/l	*n*	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units
Below 10.0	28	4.8±0.5	4688.1±207.5	3.0±0.3	1.1±0.1
10.1-30.00	15	22.2±1.5*	3837.7±470.5	3.2±0.3	6.9±0.9*
30 and above	13	50.6±5.5*	3998.1±435.4	3.3±0.3	18.9±4.9*

N o t e. T/E — quantitative testosterone to estradiol ratio.
* Differences with a group with testosterone levels below 10.0 nmol/l are statistically significant $p < 0.001$.

The bull sires were divided into four groups by estradiol levels in blood plasma: from 3000 pmol/l with a difference of 1000 pmol/l (Table 4). The testosterone levels changed in reverse proportion in groups with estradiol levels from 3001 to 5,001 pmol/l and higher (the difference from the group with estradiol levels below 3000 pmol/l are statistically significant at $p < 0.001$). No significant difference was observed in cholesterol level dynamics.

4. The levels of testosterone and cholesterol in blood plasma of bulls sires of black-and-white Holstein breed and black-and-white breed depending on estradiol levels (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Groups by estradiol levels, pmol/l	*n*	Estradiol (actual), pmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units
Below 3000	8	1887.3±293.0	25.1±10.0	2.8±0.4	18.8±8.8
3001-4000	9	3588.6±104.7*	30.8±7.3	3.3±0.4	8.8±2.4
4001-5000	11	4495.4±72.8*	16.9±6.6	3.7±0.3	2.8±1.0
5001 and above	28	5401.2±45.8*	15.4±2.9	3.3±0.2	3.3±0.7

N o t e. T/E — quantitative testosterone to estradiol ratio.
* Differences with a group with estradiol levels below 3000 pmol/l are statistically significant at $p < 0.001$.

5. The levels of endogenous hormones and cholesterol in blood plasma of bull of black-and-white Holstein breed and black-and-white breed depending on testosterone to estradiol levels (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Groups by T/E ratio, units	*n*	T/E (actual), units	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l
Below 1.0	10	0.4±0.1	2.3±0.4	4604.3±492.0	3.3±0.4
1.1-5.0	24	2.1±0.3*	9.2±1.4*	4673.3±247.0	3.1±0.2
5.1-10.0	9	6.3±0.3*	25.7±2.1*	4299.7±449.9	3.6±0.1
10.1 and above	13	19.3±4.9*	43.3±6.8*	3069.8±493.7	3.1±0.4

N o t e. T/E — quantitative testosterone to estradiol ratio.
* $p < 0.001$ as compared to the group with T/E ratio below 1.0 units.

In order to identify the impact of testosterone and estradiol ratio and as-

sociation of this parameter with the levels of hormones analyzed, bull sires were divided into groups with a variance of 5 T/E units (Table 5). Among T/E groups the actual value of this ratio differed by a statistically significant value ($p < 0.001$). As T/E ratio increased, so did testosterone levels in blood plasma. The levels of cholesterol did not depend on T/E and remained at the levels of 3.1-3.6 mol/l. Furthermore, the T/E ratio increased depending on age. For instance, at the age of 28 months the actual T/E ratio constituted 0.4 units, and at the age of 50 months and more the ratio was 19.3 units.

Knowing that cholesterol acts as precursor of sex hormones in the bodies of bull sires, we analyzed the connection between testosterone and estradiol levels and cholesterol levels (Table 6). In 41 animals cholesterol levels remained within limits of normal and on the average was 3.8 mmol/l, in 14 bull sires this parameter was lower than normal (2.5 mol/l) with average age in both groups of 36 months. The levels of testosterone and estradiol were within physiological range. Apparently, the synthesis of sex hormones is prioritized and is enabled even with low cholesterol levels.

6. The levels of endogenous hormones in blood plasma of bull of black-and-white Holstein breed and black-and-white breed depending on cholesterol levels (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017

Groups by cholesterol levels, mol/l	*n*	Cholesterol (actual), mol/l	Testosterone, nmol/l	Estradiol, pmol/l	T/E, units
Normal	41	3,8±0,1	22,6±3,2	4382,3±220,6	5,3±0,8
Below normal	14	2,5±0,2*	25,2±9,5	4025,3±463,4	2,7±1,1
Above normal	1	5,6	2,6	4780,3	0,5

N o t e. T/E — quantitative testosterone to estradiol ratio.
* Differences with the parameters in a group with normal cholesterol levels are statistically significant at $p < 0.05$. In a group with reliable reduction of cholesterol levels below normal the T/E difference with the group that had normal cholesterol levels was close to significant.

7. The levels of endogenous hormones and cholesterol in blood plasma of bull of black-and-white Holstein breed and black-and-white breed depending on milk productivity of maternal ancestors (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Groups by productivity, kg	*n*	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units
Productivity of mothers (M)					
Below 12000	21	20.8±5.1	4687.2±204.0	3.6±0.2	4.6±1.2
12001-14000	22	18.9±4.2	4108.9±325.2	3.4±0.1	3.4±2.8
14001-16000	13	23.9±5.3	4537.1±381.2	3.5±0.1	8.7±3.9
Productivity of fathers' mothers (MF)					
Below 12000	6	9.5±2.8	4405.4±480.1	3.2±0.3	2.3±0.8
12001-14000	27	24.8±4.0**	4580.3±230.1	3.4±0.2	7.4±2.0**
14001-16000	16	22.1±5.7*	4096.6±349.9	3.4±0.2	7.2±3.8
16001 and above	5	28.3±11.1	4423.5±890.9	3.7±0.2	8.1±2.9
Productivity of mothers' mothers (MM)					
Below 12000	23	17.8±4.1	4338.6±256.1	3.6±0.1	5.1±1.2
12001-14000	11	27.4±8.1	4114.7±454.1	3.5±0.2	10.3±5.4
14001-16000	5	24.8±2.8	4417.2±551.3	2.7±0.3	6.0±2.8
16001 and above	8	18.2±5.8	4720.7±406.9	3.8±0.2	4.2±1.5

N o t e. T/E — quantitative testosterone to estradiol ratio. M, MM and MF — milk productivity of mothers of bull sires, mothers of mothers and mothers of fathers, respectively.
*, ** Differences with the group in terms of productivity of mothers of fathers 12000 kg are statistically significant at $p < 0.05$ and $p < 0.001$, respectively.

The milk productivity of maternal ancestors can affect sex hormone levels of bull sires; therefore, we analyzed the dependency of hormone levels on productivity of bull sire maternal ancestors (M), mothers of mothers (MM) and mothers of fathers (MF). The milk productivity M and MM did not have a significant impact on endogenous hormones of bill sires, whereas MF reliably affected ($p < 0.05$ and $p < 0.01$) this parameter. No significant differences were

identified in terms of estradiol and cholesterol levels (Table 7).

A sufficient amount of information has been accumulated about mutual regulation of endocrine and immune systems, because it is known that testosterone suppresses the immune response (34, 35). Keeping this in mind, we have studied the dependency between endogenous hormone levels and autoimmune response of bill sires to own sperm cells (Table 8). As sperm immobilization test titers increased, testosterone levels in blood plasma declined by 37.9%, estradiol levels in blood plasma declined by 4.6%, and T/E ratio declined by 48.9%. At the same time, we registered increase of cholesterol levels by 13.7%, which, however, remained within normal ranges. We have obtained similar results in previous studies where we identified the connection between the immune system function with endogenous hormones [36].

8. The levels of endogenous hormones and cholesterol in blood plasma of bull of black-and-white Holstein breed and black-and-white breed depending on autoimmunity titre in sperm immobilization tests (*M*±*m*, OJSC Uralplemcenter, Sverdlovsk region, 2016-2017)

Titer in sperm immobilization tests	Testosterone, nmol/l	Estradiol, pmol/l	Cholesterol, mol/l	T/E, units	Age, months
Total for sampling (n = 34)	17,5±3,1	4452,2±212,3	3,5±0,1	5,217±1,6	35,1±3,1
Including:					
in a group with the titer 0-1:2 (n = 23)	20,0±3,8	4528,9±242,6	3,270±0,2	6,338±2,3	38,1±4,1
in a group with the titer 1:4-1:8 2 (n = 11)	12,4±4,9	4319,1±438,1	3,718±0,2	3,236±1,1	28,0±4,6

N o t e. T/E — quantitative testosterone to estradiol ratio.

The results obtained in this study correlate with our previous data and medical studies of other authors. For instance, autoimmune orchitis is characterized with increased levels of autoantibodies compared with the steroid-producing testicular cells, reliable reduction of total and free testosterone in blood and disruption of spermatogenesis, which manifests itself in significant reduction of sperm cell levels and percentage of progressively mobile and morphologically normal forms of sperm cells in ejaculate [37].

We have analyzed the levels of female estradiol hormone in blood plasma of bulls in the conditions of the Sverdlovsk region and its connection with the other parameters. In similar works [38, 39] producers were examined at the age of 16-35 months. We believe that the data received at the age below 3 years are insufficient for valid conclusions because during this period the physiological development is not complete. Therefore, we compared the analyzed parameters in all age groups of bull sires, from 12 to 91 months old (from young to adult and senior). As the immune method we used a specific sperm immobilization test, whereas in the aforementioned publications the authors evaluated the levels of globulin and neutrophil phagocytic activity.

To summarize, testosterone levels in blood plasma of surveyed bull sires depended on origins and age ($p < 0.05$), and estradiol and cholesterol levels depended on age ($p < 0.05$). The qualitative testosterone and estradiol ratio (T/E) increases proportional to age by a reliable value. 25% animals displayed cholesterol levels (2.5 mol/l) at the lower limit of normal; however, testosterone and estradiol levels did not differ significantly from those in groups with normal cholesterol levels, which can be indicative of priority of synthesis of sex steroid hormones. The impact of milk productivity of mothers of fathers on testosterone synthesis and T/E ($p < 0.05$, $p < 0.01$) value has been observed. It has been established that upon increase of titres of spermatic autoantibodies the synthesis of endogenous steroid hormones is reduced: testosterone levels dropped by 37.9 %, estradiol levels dropped by 4.6 % and T/E value dropped by 48.9%. These data validate the necessity of monitoring spermatic autoantibodies, which can act as indirect markers of endogenous hormones levels in blood plasma in bull sires.

REFERENCES

1. Dluzen D.E. Estrogen, testosterone, and gender differences. *Endocrine*, 2005, 27(3): 259-267 (doi: 10.1385/ENDO:27:3:259).
2. Hess R.A. Estrogen in the adult male reproductive tract: a review. *Reprod. Biol. Endocrinol.*, 2003, 1: 52 (doi: 10.1186/1477-7827-1-52).
3. Köhn F.M. Testosterone and body functions. *Aging Male*, 2006, 9(4): 183-188 (doi: 10.1080/13685530601060396).
4. Walker W.H. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis*, 2011, 1(2): 116-120 (doi: 10.4161/spmg.1.2.16956).
5. Kumanov P., Nandipati K., Tomova A., Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertil. Steril.*, 2006, 86(2): 332-338 (doi: 10.1016/j.fertnstert.2006.01.022).
6. Hammond G.L. Diverse roles for sex hormone-binding globulin in reproduction. *Biol. Reprod.*, 2011, 85(3): 431-441 (doi: 10.1095/biolreprod.111.092593).
7. Grasa M.D., Gulfo J., Camps N., Alcalá R., Monserrat L., Moreno-Navarrete J.M., Ortega F.J., Esteve M.3, Remesar X., Fernández-López J.A., Fernández-Real J.M., Alemany M. Modulation of SHBG binding to testosterone and estradiol by sex and morbid obesity. *Eur. J. Endocrinol.*, 2017, 176(4): 393-404 (doi: 10.1530/EJE-16-0834).
8. Rochira V., Madeo B., Diazzi C., Zirilli L., Daniele S., Carani C. Estrogens and male reproduction. Available https://www.ncbi.nlm.nih.gov/books/NBK278933/. Accessed August 11, 2018.
9. Nieschlag E., Nieschlag S. Testosterone deficiency: a historical perspective. *Asian J. Androl.*, 2014, 16(2): 161-168 (doi: 10.4103/1008-682X.122358).
10. McBride J.A., Carson C.C., Coward R.M. Diagnosis and management of testosterone deficiency. *Asian. J. Androl.*, 2015, 17(2): 177-186 (doi: 10.4103/1008-682X.143317).
11. Okuda Y., Fujisawa M., Matsumoto O., Kamidono S. Testosterone dependent regulation of the enzymes involved in DNA synthesis in the rat ventral prostate. *The Journal of Urology*, 1991, 145(1): 188-191 (doi: 10.1016/S0022-5347(17)38288-5).
12. Bain J. The many faces of testosterone. *Clin. Interv. Aging*, 2007, 2(4): 567-576.
13. Kalinchenko S.Yu., Tyuzikov I.A. *Prakticheskaya andrologiya* [Practical andrology]. Moscow, 2009 (in Russ.).
14. Tyuzikov I.A., Kalinchenko S.Yu., Vorslov L.O., Tishova Yu.A. *Andrologiya i genital'naya khirurgiya*, 2014, 4: 8-12 (in Russ.).
15. McLachlan J.A. Environment signaling: what embryos and evolution teach us about endocrine disrupting chemicals. *Endocr. Rev.*, 2001, 22(3): 319-341 (doi: 10.1210/edrv.22.3.0432).
16. Araujo A.B., Wittert G.A. Endocrinology of the aging male. *Best Pract. Res. Clin. Endocrinol. Metab.*, 2011, 25(2): 303-319 (doi: 10.1016/j.beem.2010.11.004).
17. Miura T., Higuchi M., Ozaki Y., Ohta T., Miura C. Progestin is an essential factor for the initiation of the meiosis in spermatogenetic cells of the eel. *PNAS USA*, 2006, 103(19): 7333-7338 (doi: 10.1073/pnas.0508419103).
18. Brockett C.C., Presicce G.A., Foote R.H., Kaproth M.T., Rycroft H.E. Semen quality and behavior of Holstein bulls exposed to estradiol-treated bulls for mounts. *J. Dairy Sci.*, 1994, 77(1): 124-131.
19. Abilov A.I., Eskin G.V., Kombarova N.A. Blood estradiol level in bull sires influences sperm count and effectiveness of artificial insemination. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2016, 51(6): 830-836 (doi: 10.15389/agrobiology.2016.6.830eng).
20. Gates M.A., Mekary R.A., Chiu G.R. Ding E.L., Wittert G.A., Araujo A.B. Sex steroid hormone levels and body composition in men. *J. Clin. Endocr. Metab.*, 2013, 98(6): 2442-2450 (doi: 10.1210/jc.2012-2582).
21. Fejes I., Koloszár S., Závaczki Z., Daru J., Szöllösi J., Pál A. Effect of body weight on testosterone/estradiol ratio in oligozoospermic patients. *Arch. Androloigy,* 2006, 52(2): 97-102 (doi: 10.1080/01485010500315479).
22. Kadioglu P., Oral G., Sayitoglu M., Erensoy N., Senel B., Gazioglu N., Sav A., Cetin G., Ozbek U. Aromatase cytochrome P450 enzyme expression in human pituitary. *Pituitary*, 2008, 11(1): 29-35 (doi: 10.1007/s11102-007-0065-3).
23. Braunstein G.D. Gynecomastia. *New Engl. J. Med.*, 2007, 357(12): 1229-1237 (doi: 10.1056/NEJMcp070677).
24. Qin D.-D., Yuan W., Zhou W.-J., Cui Y.-Q., Wu J.-Q., Gao E.-S. Do reproductive hormones explain the association between body mass index and semen quality? *Asian J. Androl.,* 2007, 9(6): 827-834 (doi: 10.1111/j.1745-7262.2007.00268.x).
25. Heidary F., Kholghi M., Rostam Zade Y. Analysis of the effect of blood testosterone on sex chromosome ratio in Holstein cattle. Reproduction in domestic animals. *Proc. 18 Annual Conference of the European Society for Domestic animal reproduction (ESDAR)*. Helsinki, Finland, 2014, V. 49, Supplement 3: 71.
26. Chacur M.G.M., Mizusaki K.T., Gabriel Filho L.R.A., Oba E., Ramos A.A. Seasonal effects on semen and testosterone in Zebu and Taurine bulls. *Acta Scientiae Veterinariae*, 2013, 41: pub. 1110.

27. Javed M.T., Abrar K., Mumtaz A. Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal bulls and their relationship with other semen parameters. *Veterinary Archive*, 2000, 70(3): 141-149.
28. Kozumplik J. The level of plasma testosterone during the prenatal and postnatal period of development in bulls. *Acta Vet. Brno*, 1981, 50: 27-32 (doi: 10.2754/avb198150010027).
29. Amerkhanov Kh.A., Abilov A.I., Eskin G.V., Kombarova N.A., Turbina I.S., Fedorova E.V., Varenikov M.V., Gusev I.V. Concentration of testosterone and cholesterol in blood serum of servicing bulls depending on their type of productivity, age and the season. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2014, 2: 59-66 (doi: 10.15389/agrobiology.2014.2.59eng) (in Russ.).
30. Delbes G., Hales B.F., Robaire B. Toxicant and human sperm chromatin integrity. *Mol. Hum. Reprod.*, 2010, 16(1): 14-22.
31. Amstislavskii S.Ya., Eroshenko V.P. *Ontogenez*, 2000, 31: 165-177 (in Russ.).
32. Sharpe R.M., Skakkebaek N.E. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet*, 1993, 341(8857): 1392-1395 (doi: 10.1016/0140-6736(93)90953-E).
33. Davis D.L., Cottlieb M.B., Stampnitzky J.R. Reduced ratio of male to female births in several industrialised countries. A sentinel health indicator? *JAMA*, 1998, 279(13): 1018-1023 (doi: 10.1001/jama.279.13.1018).
34. Roberts M., Peters A. Is testosterone immunosuppressive in a condition-dependent manner? An experimental test in blue tits. *J. Exp. Biol.*, 2009, 212(Pt 12):1811-1818 (doi: 10.1242/jeb.031047).
35. Roberts M.L., Buchanan K.L., Evans M.R., Marin R.H., Satterlee D.G. The effects of testosterone on immune function in quail selected for divergent plasma corticosterone response. *J. Exp. Biol.*, 2009, 212(19): 3125-3131 (doi: 10.1242/jeb.030726).
36. Abilov A.I., Amerkhanov Kh.A., Eskin G.V., Fedorova E.V., Zhavoronkova N.V., Kombarova N.A., Varennikov M.V. *Zootekhniya*, 2013, 9: 25-28 (in Russ.).
37. Gzgzyan A.M. *Autoimmunnyi gipogonadizm (patogenez, diagnostika, printsipy lecheniya). Avtoreferat doktorskoi dissertatsii* [Autoimmune hypogonadism (pathogenesis, diagnosis, treatment principles). PhD Thesis]. St. Petersburg, 2008 (in Russ.).
38. Mymrin V.S., Khalturina L.V., Shkuratova I.A., Ryaposova M.V. *Molochnoe i myasnoe skotovodstvo*, 2012, 4: 7-9 (in Russ.).
39. Khalturina L.V. *Agrarnyi vestnik Urala*, 2012, 9(101): 22-21 (in Russ.).