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AUTOIMMUNITY AND THE ENDOGENOUS HORMONE PROFILES OF BULL SIRES

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Abstract

Morpho-functional changes in reproductive organs of bulls due to the influence of continental climate with cold winters and hot summers can repress reproductive function (I.M. Donnik et al., 2005). It requires a deep knowledge of immunobiological, hormonal and biochemical mechanisms regulating the reproductive function in animals, with regard to their adaptability to various environmental and farming conditions (I.M. Donnik et al., 2015). Here, we compared for the first time the autoimmune state of domestic- and foreign-bred Holstein bull sires aged 3 to 9 years (n = 101) which were housed in two ecologically different regions, the Moscow Province (Head Center for Reproduction of Farm Animals) and the Sverdlov Province (Uralplemcenter, Regional Information and Selection Center). Autoimmunity detected by sperm immobilization test was unidentified in 69 % and 83 % of bull sires from the Sverdlov Region and the Moscow Province, respectively. The percentages of bulls with a high titer (> 1:8) of sperm immobilization comprised 3 % and 8 % in the Sverdlov Region and the Moscow Region, respectively. At the titers of approximately 1:4, the autoimmune anti-sperm state of the animals was reversible and easily restored following recommended technologies during two cycles of spermatogenesis. A comparatively high titer was recorded during more intense exploitation of bulls. The Holstein bulls of different origin also differed in susceptibility to developing the autoimmune anti-sperm response. The sires of the Canadian selection were more susceptible, while the lowest-level autoimmunity was characteristic of the Holland bulls. Endogenous hormone levels correlated with the autoimmunity: in case of the titer elevation, the endogenous hormone concentrations decreased, but unequally, i.e. by 38 % and 46 % for testosterone and estradiol, respectively. The variations in correlation between testosterone and estradiol could reach 49 %. The percentage of animals with the titers of 1:4 to 1:8 was higher among the young bull sires aged 28 months, while the anti-sperm antibodies were not revealed or autoimmunity was weakly expressed in the mature bulls aged 35 months, with the titers of 0 to 1:2. Therefore, the young bulls at the start of their intensive use are more susceptible to various impacts as compared to the pubertal sires.

Keywords: autoimmunity, bull sires, testosterone, estradiol, thyroxin

Reproductive function is closely related to animal metabolism, resistance, and adaptiveness [1]. Deviations in morphofunctional state of the reproductive organs in bovine animals located in different natural zones may result in sexual disorders and deterioration of reproductive ability [2]. Thus, in the context of extreme continental climate the ovarian function in females is reduced with noted lack of primary and secondary follicles in atresia phase. In the diary breeding context, up to 95 % of the population genetic progress is attributed to the influence of servicing bulls. To ensure qualitative production of sperm it

is required to adhere to feeding, maintenance and sperm collection technologies with simultaneous systematic multifactor analysis of the head count, including biochemical, hormonal, spermatologic, histological, and immunological methods (identification of circulating autoimmune antibodies to cell structure antigens of testicular apparatus) [3, 4].

The unfavorable properties such as increased sensitivity to stresses, and pathologic response to unfavorable environmental effects are identified in high productive animals of pure-bred lines. They are susceptible even to insignificant deviations from the diet and maintenance regime, which is expressed by metabolic disorder affecting their immune status. It also results in reduction of the productive, reproductive properties, advance cull, and, consequently, in large economic damage [5].

Pathogenesis of autoimmune diseases is very complicated and relates to interoperation of many endogenous and exogenous factors [6]. Anti-sperm antibodies are able to disturb spermatogenesis and may cause pathospermia, may prevent penetration of spermatozoids through the cervical mucus and ovum fertilation [7-10]. Autoimmune response against spermatozoids may be caused by mechanical trauma, overheating, infections of the reproductive tract, cryptorchidism, prostatitis [7, 11-14], and genetic predisposition [15]. Production of anti-sperm antibodies is deemed to be one of the male infertility reasons [13, 16-18], but may be usually found even in fertile males [18, 19].

Real reasons for deterioration of the qualitative sperm characteristics could not be always foreseen. Any unbalance in the diet by nutritional value (deficit or excess) or by any component has negative effect on all spermatogenesis stages: division of seminiferous epithelium, formation and maturing of spermatozoids, biochemical content of secretion of accessory sex organs, and integrity of blood-testis barrier [20]. Physiological state and resistance ability of the livestock in the industrial intensive animal breeding are under effect of a number of technological stresses [21]. Unfavorable ecologic factors (technogenic environmental pollution, natural and climate conditions of highlands) also change the biochemical state in animals. Unfavorable ecologic zones are considered to be the regions close to industrial enterprises, atomic, aluminum, and metallurgical plants-polluting water and fed for animals by toxic elements [22]. Natural resistance, humoral and cell immunity are weakened under the effect of negative factors, accompanied by pollution by heavy metals, secondary immunodeficits, and deterioration of reproductive ability [23].

Autoimmunity, as a consequence of secondary immunedeficit, may serve one of the main reasons of reproductive malfunction in agricultural animals. Depending on the duration of factor and its nature, autoimmune state may have temporary, and reversible or long-lasting and non-reversible effects which significantly affect the reproductive ability in males. Negative correlation was established between the presence of autoimmune antibodies to sperm in blood of servicing bulls and fertilization effectiveness. Sperm concentration in ejaculates and quantity of semen suitable for cryo-conservation is validly reduced in bulls with autoimmune anti-sperm antibodies. Systematic and timely identification of the sires with autoimmune antibodies to sperm is practically important in breeding enterprises. Normative value of immune status accounting for the main ecologic factors should be developed at studying of the populations of practically healthy animals [24-26].

For a long time, reproductive and immune systems in males were studied independently. However, the interest in their interoperation, especially in autoimmunity effect on infertility or reduction of the male fertility, constantly grows [13, 27]. Autoimmune process in testicular apparatus in males is considered to

be an etiologic factor of testicular failure, resulting in reduction of the hormonal function of gonad [28]. Admittedly, valid ($P < 0.001$) reduction in volume and mass of both testis were found in animals suffering from autoimmune orchitis, and testosterone content was significantly reduced in animals suffering from autoimmune hypogonadism. Relationship between the immune and hormonal systems (disorders in one of them leads to failure of the other one) is a known fact. As exemplified by highly productive cows, it was testified that simultaneous correction of immune, hormonal, and biochemical state in animals has more appreciable effect [29].

Information on effect of anti-sperm autoimmune antibodies on fertilization is contradictory. There is no common understanding of the role of humoral immunity upon reproduction and anti-sperm antibodies in development of infertility [7, 8, 30]. Practically, no clinical studies of the autoimmune process in interstitial Leydig's testicular cells producing the main androgene – testosterone were conducted [28]. Since there is no clear diagnostics algorithm of autoimmune hypogonadism in males and females, it challenges the real assessment of its spread and correction ability. Presence of autoimmune antibodies to steroid producing cells of other endocrine organs and nonorganospecific antibodies may serve as a supplementary diagnostic criterion of such pathology. Development of the methods for assessment of the influence of anti-sperm antibodies on fertility of spermatozoids and male infertility has an undisputable clinical importance [31].

Here, we have for the first time conducted a comprehensive immunobiological and hormonal monitoring of producing bulls in the conditions of Moscow and Sverdlovsk regions, accounting for the country of origin, location, and use mode, age, interbreed differences (animal paints), hormonal status in terms of testosterone and estradiol, as well as concentration in blood serum of cholesterine, the precursor of such hormones. Relation of autoimmune titer with age, origin, and geographical location of the animals, as well as the number of endogenous hormones that grew up with decreasing of the autoimmunity titer has been established. Share of the aforesaid autoimmune animals is found among young bulls, whereas autoimmune state is not found or is poorly manifested in majority of mature species.

Purpose of this research was assessment of autoimmune antibody titer and hormonal profile in servicing bulls at adaptation to different conditions of care and use.

Techniques. The studies in November 2016 involved superior servicing bull sires of the dmestic and foreign selection aged 3-9 years ($n = 101$) in two regions, Moscow (JSC Head Center for Reproduction of Farm Animals, $n = 64$) and Sverdlovsk (OJSC Uralplemcentr, $n = 37$). Blood was collected from the jugular vein in sterile vials. Blood serum was separated, and samples were inactivated at temperature of 56 °C in thermostat during 30 minutes and were kept at temperature of below –18 °C prior to their use. Sperm was individually collected on artificial vagina and was immediately used during studies after assessment.

Titer of autoimmune antibodies to spermatozoids was determined by sperm immobilization test (SIT) in the presence of complement (I.I. Sokolovskaya et al., 1990) of Guinea pig. The semen used in SIR has spermatozoid mobility scoring ≥ 0.7 with concentration of > 0.8 billion/ml preliminary diluted with 0.9 % NaCl solution up to concentration of 300 million/ml. For SIR, we used 32-well plates (MiniMed LLC, Russia) with 0.1 ml of 1 % NaCl solution added to each well. The first upper well served as the control. A 0.1 ml aliquot of undiluted blood serum of the studied male was added to the next well in vertical row, and 0.1 ml of 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of titrated serum were added to other wells of the vertical row. Then, 0.1 ml of 1:10 diluted Guinea pig

blood serum and 0.1 ml of diluted semen aliquots were added to each well. The plates were shaken delicately, covered with glass cup and half-dumped in water-bath (37 °C). Results were recorded 2 hours after formation of precipitates. The last blood serum dilution providing sperm immobilization was considered the sperm-immobilizing antibody titer of the bull (the autoimmunity titer). Reaction was conducted in three analytical replicates.

Endogenous blood testosterone and estradiol concentration was assayed by ELISA test with the use of a Uniplan AFG-01 device (CJSC Pikon, Russia), testosterone reagent kit Immuno-FA-TS, and estradiol reagent kit Immuni-FA-E (Russia). Analysis kit (Spinreac, Spain) and a ChemWell® 2902 automated analyzer (Awareness Technology, Inc., USA) were used to measure blood cholesterol concentration.

Mean (*M*) and standard error of the mean (±SEM) was calculated using Microsoft Office (MS Excel) software. Validity of differences were assessed by Student *t*-test, differences were statistically significant at $P < 0.05$.

Results. When comparing bulls' immune status, we accounted for animal location, climate zone, and ecologic factors, age, breed, and the mode of exploitation. Sperm immobilization test we used is based on binding autoimmune anti-sperm antibodies circulating in the blood with spermatozoids via their surface antigens in the presence of Guinea pig complement (Table 1).

1. Blood anti-sperm antibody titers in servicing bulls of different origin at two breeding enterprises (November 2016)

Enterprise, region	Bull number (%)	Total (share, %)	SIT titer$^+$ (total and share)							
			0	1:2	1:4	1:8	1:16	*M*±SEM		
								0-1:2	1:4-1:8	1:16
OJSC Uralplemcentr (Sverdlov Region)	37 (100)	35 (94.59)	17 (48.57)	7 (20.00)	10 (28.57)	1 (2.86)	0 0	24 (68.57±7.85)	11 (31.43±7.85)	0 0
AO GCV (Moscow)	64 (100)	61 (95.31)	34 (53.12)	19 (29.68)	3 (4.69)	4 (6.25)	1 (1.56)	53 (82.81±4.72)	7 (10.94±3.90)	1 (1.56±1.55)
Differences compared to the values for OJSC Uralplemcentr								+14.24	–20.49*	±1.56

N o t e. AO GCV — OJSC Head Center for Reproduction of Farm Animals. SIT — sperm immobilization test.
* Differences between animals of OJSC Uralplemcentr and OJSC Head Center for Reproduction of Farm Animals are statistically significant at $P < 0.05$.

2. Blood anti-sperm antibody titers in Holstein Black and White bulls depending on their age ($n = 35$, OJSC Uralplemcentr, Sverdlov Region, November 2016)

Bull age, months	*n*	SIT titer$^+$						
		total					portion of the total amount, % (*M*±SEM)	
		0	1:2	1:4	1:8	1:16	0-1:2	1:4-1:8
16-24	9	4	2	3	0	0	66.67±15.71	33.33±15.71
25-36	11	3	2	6	0	0	45.45±15.01	54.55±15.01
37-48	11	8	0	2	1	0	72.73±13.43	27.27±13.43
Over 48	4	2	2	0	0	0	100	0

N o t e s. SIT — sperm immobilization test.

The obtained results testify that the animals of OJSC Head Center for Reproduction of Farm Animals are superior, as 82.8 % of livestock (53 out of 61 bulls) lack autoimmune antibodies or have the lowest titers (0-1:2). That is, the autoimmune status of the bull sires is normal. In OJSC Uralplemcentr this parameter is 14 % lower and the number of bulls with relatively high SIT titers (1:4-1:8) is significantly higher compared to the animals of OJSC Head Center for Reproduction of Farm Animals ($P < 0.05$). At that, 10 of 11 bulls from OJSC Uralplemcentr had relatively low titers of anti-sperm antibodies — 1:4. Such titers may result from physiological disorders of technological origin, e.g. at sperm collection, and/or because of improper diet, mainly at lack of carotene. According to our previous studies, such autoimmune state can be easily corrected

during one or two cycles of spermatogenesis.

The age of bulls from OJSC Uralplemcentr significantly affected their autoimmunity (Table 2). Similar results were also obtained in bulls from JSC Head Center for Reproduction of Farm Animals (n = 15, data not shown).

Comparison of autoimmunity titers in Holstein bulls of different selection at two breeding enterprises (Tables 3) revealed the influence of the origin and place of use of the animals on their immune status.

3. Blood anti-sperm antibody titers in Holstein bulls of different origin at two breeding enterprises (November 2016)

Country of origin	n	SIT titer $^{+}$							
		total					portion of the total amount, % (M±SEM)		
		0	1:2	1:4	1:8	1:16	0-1:2	1:4-1:8	1:16
OJSC Uralplemcentr (Sverdlov Region)									
Netherlands	11	5	3	3	0	0	72.73±13.43	27.27±13.43	0
Denmark	10	8	–	1	1	0	80.00±12.65	20.00±12.65	0
Russia	5	1	2	2	0	0	60.00±21.91	40.00±21.91	0
JSC Head Center for Reproduction of Farm Animals (Moscow Region)									
Canada	30	5	11	5	7	2	53,33	40,00	6.67±4.03

N o t e. SIT — sperm immobilization test. Dash means absence of data.

SIT titers of 0-1:2 were characteristic of most tested Danish bulls. About half of Canadian bulls also had such titers, and autoimmune shifts were typical of a significant part of the population, i.e. in 40 % that approximately is 2 times higher than in Danish bulls. A total of 6.7 % of the Canadian bulls showed the highest SIT titer (1:16) indicating serious immune disorders. These bulls were 4-5 year-old animals which have been exploited more intensively. Apparently, their autoimmune disorder is caused by improper diets during active use leading to a decline in carotene and vitamin A blood level below the norm. Improper semen collection may also cause small traumas of sexual apparatus that is one of the main etiological factors of bulls' autoimmunity.

To study the dependence of immune status on the intrabreed differences, we compared SIT titers in the Black Pied and Red Pied Canadian Holsteins (Table 4). A total of 42.86 % Black Pied bulls had rather high titers, whereas among the Red Pied bulls this parameter was approximately 2 times lower. Nevertheless, final conclusions about the discovered phenomenon are not possible until more animals will be involved in the studying.

4. Blood anti-sperm antibody titers in Black Pied and Red Pied Holstein bulls (JSC Head Center for Reproduction of Farm Animals, Moscow Region, November 2016)

Suit	n	SIT titer^{+}							
		total					portion of the total amount, (M±SEM)		
		0	1:2	1:4	1:8	1:16	0-1:2	1:4-1:8	1:16
Black Pied	19	1	8	3	6	1	42.86±0.80	42.86±10.80	4.76±4.65
Red Pied	11	4	3	2	1	1	53.85±15.03	23.08±11.69	7.62±7.34

N o t e. SIT — sperm immobilization test.

5. Blood endogenous hormones (μmol/l) **in Holstein bulls depending on anti-sperm antibody titers** (M±SEM, JSC Head Center for Reproduction of Farm Animals, Moscow Region, November 2016)

SIT titer^{+}	n	Thyroxine	Estradiol	Testosterone
0-1:2	22	66.50±9.64	0.087±0.050	16.81±5.33
1:4-1:16	18	66.22±10.16	0.087±0.080	15.13±5.55

N o t e. SIT — sperm immobilization test.

In studying endogenous hormone production in a relationship with autoimmunity, we conditionally divided the Canadian Holstein 24-40 month-old bulls from JSC Head Center for Reproduction of Farm Animals into two groups,

with SIT titer of 0-1:2 (conventional lack of autoimmunity) and more than 1:4 (different degree of autoimmunity) (Table 5). The results show trend towards 10 % reduction in testosterone concentration with an increase in anti-sperm antibody titers. The thyroxine content shows no reliable differences. Among the animals of JSC Uralplemcentr a decrease in blood testosterone concentration with an increase in SIT titers is even more pronounced (by 38 %) (Table 6). Cholesterol content also increased (by 14 %) within the physiological limits (see Table 6).

6. Blood endogenous hormones and cholesterol, their precursor, in Holstein bulls depending on anti-sperm antibody titers (*M*±SEM, JSC Uralplemcentr, Sverdlov Region, November 2016)

SIT titer+	*n*	Testosterone, μmol/l	Estradiol, μmol/l	Cholesterol, μmol/l	Testosterone/estradiol	Age, months
Total	34	17.5±3.1	0.44±0.21	3.50±0.10	5.2±1.6	35.1±3.1
of which:						
0-1:2	23	20.0±3.8	0.45±0.24	3.27±0.20	6.3±2.3	38.1±4.1
1:4-1:8	11	12.4±4.9	0.32±0.44	3.72±0.20	3.2±1.1	28.0±4.6
difference		–37.9%	–46 %	+13.7 %	–48.9 %	–10.0

N o t e. SIT — sperm immobilization test.

The data we obtained in different regions correlate (see Tables 5, 6) that is indicative of the relationship between the autoimmune status and androgenous hormones.

Thus, alteration of anti-sperm antibody titers depends on the bulls' age. High titers (from 1:4 to 1:8) are characteristic of younger animals aged 28 months, while low titers (0-1:2) are typical for bulls aged 35 months. Accordingly, young bulls at the beginning of intensive use are more subjected to different affecting factors, whereas mature animals are more sustainable. Testosterone/estradiol ratio of the bulls aged 38 months is 6.3 at higher autoimmune titers. In these bulls testosterone/estradiol ratio is approximately 2 times higher compared to that of the animals aged 28 months. We did not detect any significant differences in the content of cholesterol, the precursor of most sexual hormones, between the bulls of different age. More animals with relatively high autoimmunity are among the bulls of Russian and Canadian selection. The studied Red Pied Holstein bulls are more subjected to different changes or damages of testicular apparatus than Black Pied sires (7.62 and 4.76 %, respectively, the titer of 1:16). Anti-sperm titers are significantly higher in Holstein bulls from JSC Uralplemcentr. In our view, this is caused by adverse technogenic agents in the regions. However, the increase of titer (1:4) is reversible and can be easily normalized for 1-2 cycles of spermatogenesis in the absence of the etiologic factor. Sperm immobilization test, together with measurement of blood concentration of endogenous hormones may be helpful tools for diagnostic of autoimmune disorders in male reproductive system.

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