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BLOOD ESTRADIOL LEVEL IN BULL SIRES INFLUENCES SPERM COUNT AND EFFECTIVENESS OF ARTIFICIAL INSEMINATION

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Abstract

Wide use of artificial insemination necessitates a deeper understanding of how hormones and immune system influence on semen production in sires. With this regard, the role of follicle stimulating hormone and luteinizing hormone in spermatogenesis is under consideration. Blood testosterone and estradiol levels are related and depend on a testosterone-estradiol binding globulin function. We first examined seasonal effects of blood estradiol levels as a reproduction marker in Holstein bull sires and showed the relationship between the blood estradiol concertation, semen fertilizing ability, semen volume, and semen concentration which, in turn, impact on the results of artificial insemination. Estradiol level in Holstein sires aged 30±6 months (n = 18) was assayed using Immuno-FA-E ELISA kit and a Uniplan equipment (ZAO Pikon, Russia). The effectiveness of artificial insemination was tested in 214 cows. We showed that in the bulls the blood estradiol level varied significantly depending on a season ($P < 0.001$). In spring, the lowest (0.100 nmol/l) estradiol level detected in 78 % of the bulls was mostly characteristic, and only in 17 % of the bulls estradiol was beyond 0.200 nmol/l ($P < 0.001$). At autumn, blood estradiol concentration increased in 94 % bulls ($P < 0.001$). When estradiol level rises two times and more, a 31 % decrease in semen volume per ejaculation is observed (i.e. 3.4 ml vs 4.6 ml) which results in about 50 % decrease in semen dose number (112 vs 171). When low blood estradiol on the day of semen collecting, a 12-17 % success rate occurred in cows after a single insemination ($P < 0.05$), and 17-29 % heifers became pregnant to first insemination ($P < 0.001$). Thus the blood estradiol in bulls additionally indicates a fertilizing ability of the semen and can be used to improve effectiveness of artificial insemination technique.

Keywords: estradiol, bull sires, seasonal changes, semen indices, success rate of insemination

For effective breeding and artificial insemination of farm animals, the rational use of sires' sperm [1] should be employed, which requires, in turn, an in-depth research of the role of the hormonal and immune systems in the sperm production. Even V.K. Milovanov [2] indicated to a major importance of sex hormones for normal functioning of both reproductive organs and the whole body of sires. Sexual function of animals is known to be under neuroendocrine control. Hormones exert their effects on the metabolism and all physiological functions at very low concentrations (10^{-6}-10^{-12} mol/l) [3]. In all mammalians, spermatogenesis is modulated by peptide and steroid hormones, such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH), testosterone, estradiol, etc. [4]. Under the influence of LH, which is secreted by the pituitary gland after puberty, Leydig cells begin actively to synthesize testosterone which acts on Sertoli cells. Steroid hormones secreted by the testes are represented by androgens and progesterone. They can easily penetrate the cytoplasm and control the cell function, with the participation of specific high-molecular-weight protein receptors [3, 5-7].

Significant variations in the testosterone concentrations have been documented in animals of different origin. Thus, in the Sverdlovsk region, testos-

terone concentrations in sires imported from abroad were 1.64 times higher than those in domestic bulls (14.28±2.26 vs. 8.72±1.92 nmol/l) [8]. M. Anderson [9] reported a positive correlation between the testosterone concentrations in bulls and the pregnancy occurrence in cows. In addition, there was a high positive correlation between the sperm counts in the semen and the amounts of testosterone, as well as a negative correlation between the semen pH and testosterone blood concentrations [10]. Testosterone levels in the blood of bulls correlate with their age and breed (higher in beef breeds vs. milk breeds) as well as exogenous factors [11). A positive correlation has been identified between the concentrations of cholesterol and testosterone in bulls, where the latter increases when the former is raised [12].

Testosterone-estradiol binding globulin, involved in transport, regulatory and protective functions, plays a major role in the formation of the testosterone/estradiol complex. A certain amount of androgens is converted to DHT and estradiol. Estradiol is synthesized from testosterone by the enzyme aromatase [13, 14]. In mammalian males, the adrenal glands also produce estrogens, moreover, their positive impact on the quality of sperm is observed.

Estrogens influence the development of the genitals and the secondary sexual characteristics, fat metabolism (in particular, increase the plasma concentrations of phospholipids and β-lipoprotein, reduce the levels of cholesterol and α-lipoprotein), stimulate protein anabolism and the growth hormone production, and slow bone growth in mature adult animals. Under influence of estrogens, the reticuloendothelial system is stimulated, the body's resistance to infections increases, and tissue regeneration is enhanced [3]. LH induces the secretion of androgens in the testes, stimulates the development of interstitial tissue and the production of the male hormone testosterone, and, together with FSH, promotes the proliferation of the seminiferous tubules (the initial stages of spermatogenesis). In males and females, FSH promotes the development of sperm and egg cells, respectively [2, 3].

However, at high doses, estrogens can cause the opposite effect (up to necrotic phenomena in the kidneys and liver) [3]. With increases in the concentrations of estradiol over the upper limits, worsening of spermatologic parameters has been revealed [15]. The excess weight in sires may serve as an indirect signal of excessive amounts of estrogens and worsened quantitative and qualitative indicators of the sperm [16, 17].

A.I. Abilov et al. [11] revealed the dependence of the blood concentrations of estradiols in sires on exogenous factors. Furthermore, the quantitative indicators of the sperm production increased with decreasing amounts of estradiol. There is evidence on the relationship between the spermatozoa autoantibodies in sires and the estradiol concentrations [18].

With increasing productivity in cattle, hormonal processes, metabolic rate [19] and, as a consequence, the reproductive capacity are changed. Therefore, it is important to take into account the productive type of the animal when studying its hormonal status.

In view of the discussion on the role of estrogens in the sperm production, papers assessing the impact of phyto-, xenoestrogens and chemically synthesized estradiol on the metabolism and animal reproductive function are also of interest. Xenoestrogens demonstrate activity similar to that of the endogenous estrogens and mimic their properties, therefore, influencing the synthesis, secretion, transport, metabolism, binding and excretion of endogenous hormones involved in the regulation of homeostasis, reproduction and development [20]. Data are available for the negative impact of phytoestrogens on reproductive function. For instance, it has been shown that phytoestrogens may inhibit LH and

FSH synthesis in women [21] and sexual behavior in animals [22].

In this paper, we have first defined the blood estradiol concentrations in sires in connection to the quantity and quality of sperm production, depending on the season, and how the sire's estradiol status on the day of semen collection could affect the performance of artificial insemination.

The aim of the study was to evaluate the influence of serum endogenous estradiol on the sperm productivity in sires and the efficacy of the insemination using the collected sperm.

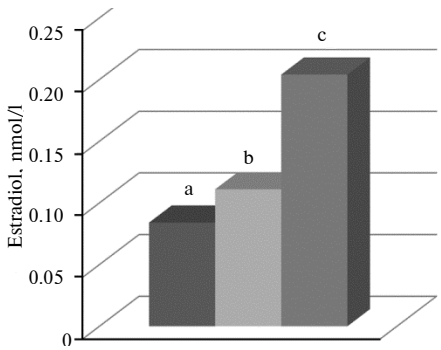
Technique. Eighteen Holstein sires aged 30±6 months were selected to enter the study (Head Center for Reproduction of Farm Animals, 2012-2014). The animals received a diet balanced according to the nutritional standards by L.K. Ernst All-Russian Research Institute of Animal Husbandry (VIZh), and were maintained under the conditions met the established requirements and standards [23].

Blood samples were collected from the jugular vein 1 hour after collecting semen. The blood serum was separated and stored at –18...–20 °C until testing. Serum concentrations of estradiol were determined by enzyme immunoassay (ELISA) using the Immuno-FA-E test kit and a Uniplan AFG-01 analyzer (JSC Picon, Russia).

Estrus in cows (the Klenovo-Chegodaevo experimental farm, New Moscow, 2012-2014) was determined twice, the optimal time for insemination was identified visually by the standing reflex, and by rectal examination, based on the follicle maturity. Animals were inseminated two times per estrus with a 10-12 hour interval. Insemination was considered productive in the absence of re-heat, and based on a rectal examination on days 45-60 after the last insemination.

The significance of differences between the compared options was evaluated by Student's *t*-test. The tables below summarize mean values (X) and mean deviations ($\pm x$).

Results. When evaluating endogenous blood estradiol concentrations in Holstein sires depending on the season, we revealed (Fig.) that in the spring (April 23, 2012) it averaged to 0.084±0.070 nmol/l, and in the autumn (September 5 and October 18, 2012) it significantly, almost 2.5-fold, increased and amounted up to 0.204±0.060 nmol/l ($P < 0.001$).



Seasonal blood estradiol concentrations in the Holstein sires: a — spring, b — summer, c — autumn (n = 18, the animals aged 30±6 months; $P < 0.001$; Moscow Province, 2012).

Based on these data, the sires were conventionally assigned to groups by the estradiol levels: low (0.100 nmol/l) for group I, mean (0.101-0.200 nmol/l) for group II, and high (> 0.200 nmol/l) for group III. The distribution of all the animals in groups according to the seasonal estradiol levels is presented in Table 1.

It appeared that most of sires (77.80 % on average in the sample) had minimum estradiol level in the spring (0.100 nmol/l), and only in 16.80 % sires on average the concentrations were significantly ($P < 0.001$) higher than the maximum value (0.200 nmol/l). In the autumn, a significant increase of the estradiol amount ($P < 0.001$) was recorded in 94.28 % sires.

The quantitative estimation of the sperm production in sires showed (Table 2) that with a significant ($P < 0.001$) increase in blood estradiol, as com-

pared to that in group I, the quantitative indices of sperm decreased and, as a consequence, there was a decrease in an output of qualitative semen doses per ejaculate, in a total number of qualitative doses (approximately by 50 %), and in the ejaculate semen volume (by 31 %). We suppose this is due to the fact that when the male experience hyperestrogenization, there is an increase in the level of testosterone-estradiol binding globulin, which inhibits the function of free testosterone and reduces the number of mature spermatozoa.

1. The proportion (%) of Holstein sires conventionally assigned to groups of different estradiol levels by the seasons ($X \pm x$, n = 18, the animals aged 30±6 months, Moscow Province, 2012)

Date of testing (number of animals examined)	Estradiol, nmol/l		
	up to 0.100 (group I)	0.101-0.200 (group II)	> 0.200 (group III)
April 23 (n = 18)	77.80±9.80	5.56±5.40	16.80±8.78
July 24 (n = 18)	61.11±11.49	33.33±11.10	5.56±5.40
September 5 and October 18 (n = 35)	5.71±3.92	48.57±8.45	45.71±8.42

N o t e. Differences between groups are significant at P < 0.001.

2. Quantitative characteristics of sperm production in Holstein sires conventionally assigned to groups of different estradiol levels ($X \pm x$, n = 18, the animals aged 30±6 months, Moscow Province, 2012)

Parameter	Estradiol, nmol/l		
	up to 0.100 (group I)	0.101-0.200 (group II)	> 0.200 (group III)
Number of sires, n	7	4	7
Estradiol, nmol/l	0.06±0.02	0.16±0.03*	0.26±0.04*
Ejaculate volume, ml	4.6±0.9	3.4±1.1	3.5±0.9
Sperm counts in ejaculate, bln/ml	1.3±0.3	1.4±0.3	1.3±0.2
Doses frozen per one ejaculate	170.6±69.4	146.7±37.4	112.1±32.3

* Differences vs. group I are significant at P < 0.001.

In assessing semen fertility, sires were divided into groups depending on the estradiol level on the day of semen collection (autumn 2013), i.e. 0.240-0.320 (min), 0.321-0.360 (mean) and > 0.361 nmol/l (max). We evaluated the pregnancy occurred from the first insemination (cows that were inseminated for the first time after calving) and from singel insemination (regardless of the multiplicity of coming in heat). As seen from the data (Table. 3), the lower the endogenous blood estradiol levels on the day of the semen collection, the more effective the insemination. At the lowest (within this investigation) concentration of estradiol in sires (0.240-0.320 nmol/l), the performance of one insemination by their sperm was the highest (62.03±3.86 %), while at maximum estrogenization level it was the lowest (45.45 %). Similar data were obtained for the first insemination. In both cases, differences vs. minimum values were significant at P < 0.05.

3. Insemination by sperm from Holstein sires having different estradiol levels on the day of semen collection ($X \pm x$, the sires aged 30±6 months, Klenovo-Chegodaevo Experimental Farm, New Moscow, 2013-2014)

Estradiol, nmol/l	Number of cows							
	inseminated		pregnant					
			from one insemination			from first insemination		
	total	first	total	%	vs. control, %	total	%	vs. control, %
0.240-0.320 (control)	79	52	49	62.03±3.86		31	59.62±4.81	
0.321-0.360	91	59	46	50.55±3.71	–11.98*	25	42.37±4.55	–17.25*
> 0.361	44	29	20	45.45±5.31	–16.58*	9	31.03±6.07	–28.59*
Total	214	140	115	53.74±2.41	–8.29	65	46.43±2.98	–13.19*

* Differences vs. control are significant at P < 0.05.

A probable cause of the observed variations in the amount of endogenous estradiol depending on the season might be an unequal hypothalamic response to incoming signals. In some cases, this may result in the deficiency of sex hor-

mone binding globulin, as well as in increased levels of LH and FSH, leading to manifestation of the primary and secondary testicular failure that may cause a drastic change in blood estradiol concentrations [19]. Our findings are consistent with the results showing that hyperestrogenization increases the amount of testosterone-estradiol binding globulin, and this in turn suppresses the free testosterone function and leads to deterioration of spermatologic parameters [15], including reduced sexual activity and number of spermatozoa in the ejaculate. Increased serum estradiol concentrations may also serve as an indicator of the onset of fatty liver disease (especially if sires are fed with concentrates).

Thus, the level of endogenous blood serum estradiol in sires significantly ($P < 0.001$) varies depending on the season, that affects the sperm productivity ($P < 0.001$) and the insemination ($P < 0.05$). Low levels of estradiol exert a positive effect on quantitative characteristics of sperm production with an increase in ejaculate volume by 31 %, and in the output of high-quality doses by 52 %. The number of successful inseminations, resulted in pregnancy from single or from the first insemination, is inversely proportional to the blood estradiol level in sires on the day of semen collection. These data can be used as an additional test in predicting the results of artificial insemination.

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