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GENETIC AND BIOCHEMICAL CHARACTERISTICS OF SIRE BULLS (*Bos taurus)* IN REPRODUCTIVE BIOTECHNOLOGIES

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ABSTRACT: This study aims to investigate the relationship between polymorphic serum protein systems – transferrin *(Tf)*, amylase (*Am*), and ceruloplasmin, (*Cp*)- and sperm production parameters, as well as fertilization rates, in Red Steppe and Angeln cattle. Electrophoresis was employed to determine protein phenotypes and calculate levels of heterozygosity. The relevance of this study is justified by the necessity of establishing immunogenetic markers to enhance selection efficiency. Analysis revealed breed-specific immunogenetic markers. Regarding the Transferrin (*Tf*) locus in Red Steppe bulls, the highest sperm production indicators were observed in homozygous individuals (Tf^{AA}, Tf^{DD}), whereas in the Angeln breed, superior sperm volume was noted in heterozygous individuals (Tf^{AD}). Cows inseminated with sperm from heterozygous pairs generally exhibited significantly higher fertility rates than those inseminated with sperm from homozygous pairs. The Amylase (*Am*) system confirmed the negative influence of the Am^{CC} phenotype, which is associated with the lowest level of sperm production and fertility. In the Red Steppe breed, homozygous Am^{BB} sire bulls possessed the highest reproductive qualities. The study of the Ceruloplasmin (*Cp*) system revealed that in the Red Steppe breed, heterozygotes (Cp^{BC}) demonstrated the highest fertility, despite the superior sperm productivity of homozygotes (Cp^{AA}). Conversely, in the Angeln breed, Cp^{BB} homozygotes prevailed in both indicators. These results suggest that the optimal level of heterozygosity may depend on breed-specific genetic backgrounds. Red steppe bulls that were homozygous or possessed a 25% heterozygosity level exhibited the highest sperm production and fertilization ability, whereas the optimal level of Angeln bulls was 50%. It is recommended that homozygosity levels of *Tf*, *Am*, and *Cp* be incorporated into selection indices when purchasing sire bulls to improve the reproductive qualities of the herd.

Keywords: Amylase, *Bos taurus*, Ceruloplasmin, Heterozygosity, Semen quality, Sire bull, Transferrin.

INTRODUCTION

In the current globalized and climate changing world, maintaining the genetic diversity of farm animal populations requires a systematic approach. This involves not only obtaining objective information about existing populations, but also creating detailed databases for analyzing their structure and studying genetic processes. The modern strategy for protecting livestock biodiversity in Ukraine necessitates scientific justification that account for internal economic reforms, European integration, and real changes in livestock species composition (Polupan et al., 2017).

The rational preservation and reproduction of domestic breeds that carry unique, economically valuable traits are of increasing importance (Davidescu et al., 2023; Engdawork et al., 2024). Red steppe cattle, for example, were developed under the conditions of a dairy sector with one-sided specialization (Suyunova et al., 2024) and are distinguished by exceptional endurance and adaptability to extreme heat (Bodnaruk et al., 2020).

In improvement and refinement of cattle breeds and types, significant attention is given to genetic and population analysis. Employing various genetic methods, such as studying blood groups and polymorphic systems of proteins and enzymes, allows for a more objective analysis of the current state and outlines prospects for genetic and selection processes at different stages of breed development and functioning (Chumel, 2003; Shcherbatyj et al., 2016; Zhmur et al., 2025).

One of the most effective tools for addressing these issues is the implementation of modern genetics achievements, particular the use of polymorphic protein systems as markers of breeding value (Bodnaruk et al., 2022). The study of systems such as transferrin (*Tf*), amylase (*Am*) and ceruloplasmin (*Cp*) allows not only for the assessment of population genetic structure (Guha et al., 2012, Hrytsiienko et al., 2019, Sanni et al., 2023), but also for the identification of relationships between these markers and the reproductive function and sperm productivity of sire bulls.

The investigation of sperm production indicators and factors affecting the quality of sire bulls of farm animals remains a pertinent issue (Piddubna and Zakharchuk, 2020). Recently, increased attention has been directed towards the immunology and immunogenetics of male reproductive qualities, particularly the distribution of phenotypes and the frequency of polymorphic protein systems (e.g., transferrin, amylase, and ceruloplasmin). These systems determine the

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level of heterozygosity and establish relationships with sperm production and the fertility of sire bulls of the Red Steppe and Angler breeds.

Studies by Fedorovych et al. (2008) have demonstrated a relationship between genotype specificity and the fertilization of cows, as well as the volume of ejaculates in breeding bulls. Hrytsiienko et al. (2019) concluded that allelic variants of the studied polymorphic genetic and biochemical systems can be used to evaluate the genetic composition of breeds and intra-breed groups. This is crucial for organizing breeding and selection activities in animal husbandry and enables the practical application of genetic-mathematical methods to predict the milk productivity of cows.

Recent studies by Stadnytska and Kaplinsky (2021) indicated that certain genotypes at serum protein loci (e.g., *AA* homozygotes for transferrin) exhibit better fertility rates and shorter service periods compared to other variants. Therefore, combining strategic biodiversity management with the application of genetic markers is necessary to increase the competitiveness of domestic livestock and preserve its gene pool (Kryvoruchko et al., 2024).

An analysis of the genetic structure of the Black-and-White dairy breed by Bodnaruk et al. (2022) revealed specific features: the transferrin locus demonstrates the highest heterozygosity (0.729) and was in a balanced state according to the Hardy–Weinberg law (*P* – 0.104). The dominant allele *Tf A* had a frequency of 0.438 owing to heterozygotes AD_1 and AD_2. The allele *Tf* D_1 had a low frequency (0.208), while *Tf* D_2 was quite high (0.333), which is typical for meat breeds.

The *Tf E* allele is rare, with a frequency of 0.021 in heterozygotes. The amylase locus (*Am-l*) is in an unbalanced state due to a deficiency of heterozygotes and an excess of homozygotes (P – 0.013), with equal frequencies of the *Am-l B* and *Am-l C* alleles (0.500 each). The heterozygosity for this locus is 31.8%. The ceruloplasmin (CP) locus is also unbalanced (*P* – 0.041) and shows an excess of *AB* heterozygotes. The frequency of the *Cp A* allele is 0.489, while that of the *Cp B* allele is 0.511.

The relationship between transferrin types and reproductive abilities has also been studied in other farm animals (Weitkamp et al., 1982; Demir and Mert, 2015; Jawad et al., 2025). Research on the genetic polymorphism of local goat breeds in Turkey revealed significant variability of biochemical markers, indicating three hemoglobin phenotypes and six transferrin phenotypes (*AA*, *AB*, *AC*, *BB*, *BC*, *CC*). Notably, interbreed differentiation in ceruloplasmin levels was observed: in the Honamli breed, the level was 1.793 ± 0.109 mg/dL, which is twice that of the Kilis breed (0.822 ± 0.055 mg/dL). These findings indicate significant differences in metabolic process intensity and antioxidant protection (Demir et al., 2015).

To study potential relationships between transferrin type and litter size (multiparity) across five breed groups (Durok, selected Durok, Yorkshire, selected Yorkshire, and selected Durok × Yorkshire crosses), transferrin (*Tf*) typing was performed on 343 boars and 1094 gilts, which were sires of 1208 litters (Huang and Rasmusen, 1982). Boars with the BB genotype produced a higher number of live-born piglets (8.67) than those with the *AB* genotype (7.76). Crossbreeding *BB* males with *AB* females resulted in the highest number of live-born (8.71) and weaned (8.39) piglets in the selected Duroc group. Crossbreeding *BB* males with *AB* females in crossbreeds resulted in higher multiparity rates (10.85 born; 8.87 live-born; and 8.19 weaned) compared to breeding *AB* males with *BB* females (9.84; 7.65; and 7.13, respectively).

However, no statistically significant effects of paternal or maternal transferrin genotype, or their interaction, was found on the number of piglets born, born alive, or weaned per litter in any of the five breed classes. This suggests that transferrin typing may have limited value in increasing multiparity in pigs. In Ukraine, the use of molecular genetic markers is integral to preserving the animal gene pool by identifying valuable genotypes and creating the conditions necessary for their targeted use in biodiversity conservation programs for cattle populations (Kopylova et al., 2013).

This study aims to establish a connection between polymorphic serum systems and the reproductive function of bulls to develop molecular genetic criteria for selecting highly productive animals.

MATERIALS AND METHODS

The research was conducted at JSC "Oblplempidpryemstvo" and the breeding enterprises of Mykolaiv Oblast, focusing on semen and blood serum of *Bos Taurus* sire bulls. A total of 233 breeding bulls, comprising Red Steppe (*n*=165) and Angeln (*n*=68) breeds, were examined. Data were also sourced from laboratory records pertaining to the breeding bulls' utilization, sperm production evaluation, and breeding cards (1 MOL). Experimental groups were formed considering breed, age, and heterozygosity level for marker genes, adhering to all zootechnical requirements. Feeding, housing, and the management of breeding bulls complied with generally accepted zootechnical standards (Ibatullin et al., 2014).

Semen was collected from the bulls using an artificial vagina, following the procedures outlined in the artificial insemination instructions (Koreyba, 2016). The ejaculate volume, sperm concentration, and activity were evaluated using methods described by Koreyba (2016), after which samples were collected for further analysis.. Testing for polymorphic protein systems (*Tf*, *Am*, and *Cp*) in sire bulls was performed at the immunogenetics laboratory of the M.F. Ivanov Institute of Animal Husbandry of Steppe Regions, "Askania-Nova" NAAS. This was conducted according to the current procedures described by Harris and Hopkinson (1976) and Gahne et al. (1977). The level of heterozygosity was calculated as the percentage of heterozygous loci among the examined *Tf*, *Am*, and *Cp* loci relative to the total number of loci analyzed. For

instance, if one heterozygous locus was identified out of four analyzed, the heterozygosity level was considered 25%; if two were found, it was 50%, and so on.

RESULTS AND DISCUSSION

An analysis of the distribution of phenotypes and transferrin gene frequencies in breeding bulls (Table 1) revealed the presence of Red Steppe breeds with possible phenotypes *AA, AD, AE, DD* and *DE*. The *EE* phenotype was absent; while bulls of the Angeln breed exhibited *AE* and *EE* phenotypes. Male offspring of both breeds showed the highest concentrations of the *D* gene allele (0.519 and 0.516), whereas the concentrations of *A* and *E* were lower (0.446 and 0.476 and 0.035 and 0.008, respectively). Red Steppe bulls displayed a higher level of heterozygosity at the *Tf* locus (50.9%) compared to Angeln bulls (39.7%). Similar genotypes were observed in studies by Chumel (2003), Shcherbatyj et al. (2016), Hrytsiienko et al. (2019), Stadnytska and Kaplinsky (2021), although the frequencies differed.

The analysis of sperm production in relation to transferrin types in Red Steppe bulls (Table 2) showed that males with the *DD* type produced the fewest ejaculates per year compared to other groups. This difference was significant - 10.6 ejaculates fewer (t_d = 2.236; $P < 0.05$), than in bulls with the *AA* type.

The largest total volume of semen per year was obtained from bulls with the Tf^{AA} type (718.4 ± 26.4 ml), and the smallest from bulls of the *AE* or *DE* type (506.0 ± 60.5 and 570.8 ± 53.8 ml). These differences were significant (t_d = 3.218 and 2.502). Bulls with Tf^{AD} phenotype also significantly outperformed their Tf^{AE} and Tf^{DE} counterparts (t_d = 2.784 and 1.966). The largest average ejaculate volume was recorded in bulls with the *Tf AA* and *DD* type (3.88 ± 0.13 and 3.78 ± 0.12 ml), while the lowest was in bulls with the *AE* type (2.73±0.28 ml). The differences were statistically significant (t_d = 3.720 and 3.322; $P<0.001$). Sperm concentration was highest in bulls with the *Tf AE* type (1.100 ± 0.032 billion/ml) significantly exceeding those of bulls with the *AA* and *AD* types (t_d = 2.209 and 2.654; $P<0.05$ and $P<0.01$). Regarding total sperm count, bulls with the Tf^{DD} and Tf^{AA} types (4.049 ± 0.158 and 3.912 ± 0.182 billion, respectively) had higher counts than bulls with the Tf^{AE} type (3.010 ± 0.383 billion; t_d = 2.509 and 2.127; $P<0.02$ and $P<0.05$). Bulls with the *AA* and *AD* types also exhibited the highest sperm motility (8.39 ± 0.08 and 8.33 ± 0.04, respectively), significantly exceeding that of bulls with the *DE* type (8.13 ± 0.07; t_d = 2.452 and 2.439; $P<0.02$).

The fertilization rate of cows from the first insemination was highest when sperm from bulls with the *AA* and *DD* types was used (64.3 ± 1.04% and 63.7 ± 1.01%, respectively), and lowest when semen from bulls with the Tf^{AE} type was used (61.0 ± 3.8%). These patterns were consistent with the observed correlations between transferrin types and sperm quality (Table 3). Notably, Red Steppe bulls with the *D1D1* transferrin type produced the greatest number of ejaculates (195.7 ± 0.9 per year) and the largest total volume of semen (828.3 ± 49.2 ml). They also had the highest ejaculate volume (4.23 ± 0.25 ml), total sperm count (4.24 ± 0.57 billion), and sperm activity (8.43 ± 0.09). In contrast, bulls with the *D2E* transferrin type produced the fewest ejaculates (175.8 ± 5.9) and the least semen volume (496.0 ± 38.8 ml), with the lowest ejaculate volume (2.81 ± 0.15 ml), sperm concentration (0.98 ± 0.04 billion/ml), total sperm count (2.74 ± 0.18 billion), and sperm activity (8.08 ± 0.07). Interestingly, cows inseminated with semen from the *D1D1* bulls had lower fertilization rates, whereas those inseminated with semen from *D2E* bulls exhibited the highest rates.

This observation may be influenced by additional factors, such as female's reproductive status and insemination management practices, which were not directly controlled in this study. No significant differences were foun in the number of ejaculates obtained from Angeln bulls with different transferrin types (Table 4). However, bulls with the *AD* type demonstrated a significant advantage over those with the *AA* type in terms of total annual sperm volume (799.2 ± 89.6 ml and 690.2 ± 32.7 ml, respectively; td = 2.472, $P < 0.02$). Consequently, the average ejaculate volume was significantly higher in bulls with the *AD* type (4.35 ± 0.09 ml) compared to the *AA* type (3.76 ± 0.16 ml; td = 3.207). Sperm concentration was highest in bulls *with* the *AA* type (1.073 ± 0.038 billion/ml), although this difference was not statistically significant. The total number of sperm per ejaculate was highest in bulls with the *AD* type (4.442 ± 0.152 billion), with slightly lower counts in bulls of the *DD* and *AA* types. Sperm activity was highest in bulls with the *AA* type (8.44 ± 0.06), but no significant difference was observed compared to other transferrin types. The fertilization rate of females following the first insemination was highest when using sperm from bulls with the *TfDD* type (66.7 ± 1.4%), a result that differed significantly (td = 2.827) from that of bulls with the *TfAD* phenotype (61.3 ± 1.3%) and also significantly (td = 1.902) from bulls with the *TfAA* type (62.8 ± 1.5%). Therefore, based on the results in Table 4, establishing a consistent and close relationship between quantitative and qualitative sperm production indicators and transferrin types in Angeln bulls is not always possible across all parameters.

A comparison of gene frequencies at the amylase locus (Table 1) indicates that both breeds are characterized by a higher frequency of the *B* allele. For Red Steppe bulls, this frequency is 0.630, and for Angeln bulls, it is 0.792, respectively. The level of heterozygosity at the amylase locus for Red Steppe bulls is 33.3%, and for Angeln bulls, it is 31.7%, respectively. These findings may differ from those reported in studies by Bodnaruk et al. (2020), Bodnaruk et al. (2022), Zhmur et al. (2025).

Table 1 - Phenotype distribution and frequency of in sire bulls

Tf alleles													
Breed	***n***	**Distribution of phenotypes**										**Allele frequency,** $\overline{X} \pm S_{\overline{X}}$	
		AA		***AD***		***AE***		***DD***		***DE***			
		n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***A***	***D***	***E***
Red Steppe	157	34	21.7	69	43.9	3	1.9	43	27.4	8	5.1	0.446±0.0280	0.519±0.0301	0.035±0.0104
Angeln's	63	18	28.6	24	38.1	-	-	20	31.7	1	1.6	0.476±0.0443	0.516±0.0445	0.008±0.0079
Am alleles													
Breed	***n***	**Distribution of phenotypes**										**Allele frequency,** $\overline{X} \pm S_{\overline{X}}$	
		BB		***BC***		***CC***							
		n	**%**	***n***	**%**	***n***	**%**					***B***	***C***
Red Steppe	144	68	47.2	48	33.3	28	19.4					0.630±0.0283	0.361±0.0283
Angeln's	60	38	63.3	19	31.7	3	5.0					0.792±0.0371	0.208±0.0371
Cp alleles													
Breed	***n***	**Distribution of phenotypes**										**Allele frequency,** $\overline{X} \pm S_{\overline{X}}$	
		AA		***AB***		***BB***							
		n	**%**	***n***	**%**	***n***	**%**					***A***	***B***
Red Steppe	147	59	40.1	64	43.5	24	16.3					0.619±0.0283	0.381±0.0283
Angeln's	66	38	57.6	23	34.8	5	7.6					0.750±0.0119	0.250±0.0119

Table 2 - Quantitative and qualitative indicators of sperm production of sire bulls of the Red Steppe breed different genetic predisposition according to Tf types

Transferrin type	Number of bulls, criterion of reliability	Number of ejaculates	Semen received from bull per year, ml	Average volume ejaculate, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
AA	34	184.9±3.4	718.4±26.4	3.88±0.13	1.005±0.028	3.912±0.182	8.39±0.08	64.3±1.04
AD	69	183.5±5.8	681.0±17.1	3.69±0.07	1.005±0.016	3.713±0.091	8.33±0.04	62.9±1.0
	td	0.208	1.189	1.284	-	0.978	0.674	0.972
AE	3	185.3±7.2	506.0±60.5	2.73±0.28	1.100±0.032	3.010±0.383	8.30±0.15	61.0±3.8
	t d	0.050	3.218**	3.720***	2.209*	2.127*	0.529	0.837
	$t d_1$	0.196	2.784**	3.322***	2.654*	1.784	0.194	0.483
	td_2	1.388	2.563*	3.443***	0.956	2.509*	-	0.687
DD	43	174.3±3.3	672.6±23.7	3.78±0.12	1.057±0.021	4.049±0.158[a]	8.30±0.054	63.7±1.01
	t d	2.236*	1.290	0.560	1.485	0.568	0.957	0.414
	td_1	1.379	0.288	0.647	1.970	1.846	0.469	0.563
DE	8	179.0±4.4	570.8±53.8	3.37±0.30	1.021±0.033	3.485±0.386	8.13±0.07	63.3±1.8
	t d	1.061	2.502	1.560	0.372	1.001	2.452*	0.714
	$t d_1$	0.618	1.966	1.038	1.416	0.574	2.409*	0.015
	$t d_2$	0.855	1.731	1.269	0.795	1.352	1.977	0.679

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$), (*** - $P < 0.001$) based on *t*-Student criteria (t_d).

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Table 3 - Quantitative and qualitative indicators of sperm production of sire bulls of the Red Steppe breed different genetic predisposition according to *Tf* types

Transferrin type	Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
A A	34	184.9±3.4	718.4±26.4	3.88±0.13	1.005±0.028	3.912±0.182	8.39±0.08	64.3±1.04
A D₁	21	173.2±5.8	619.9±24.4	3.77±0.22	1.035±0.027	3.884±0.242	8.32±0.07	61.3±2.0
A D₂	48	186.2±2.3	707.8±21.2	3.78±0.09	0.992±0.020	3.760±0.120	8.33±0.06	63.6±1.2
A E	3	185.3±7.2	506.0±60.5	2.73±0.28	1.100±0.032	3.010±0.383	8.30±0.15	61.0±3.8
D₁D₁	3	195.7±0.9	828.3±49.2	4.23±0.25	0.990±0.082	4.240±0.570	8.43±0.09	59.3±0.9
D₂D₂	34	169.5±3.9	628.3±28.1	3.70±0.14	1.074±0.024	3.978±0.188	8.28±0.06	63.2±1.2
D₁D₂	6	184.0±7.1	732.2±52.9	3.98±0.24	1.002±0.047	4.027±0.386	8.33±0.07	68.5±2.5
D₁E	3	184.3±6.8	695.3±97.7	4.31±0.24	1.097±0.015	4.72±0.240	8.20±0.15	59.3±2.6
D₂E	5	175.8±5.9	496.0±38.8	2.81±0.15	0.980±0.040	2.74±0.180	8.08±0.07	64.0±2.0

Table 4 - Quantitative and qualitative indicators of sperm production of sire bulls of the Angeln breed of different genetic predisposition by *Tf* types

Transferrin type	Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
A A	18	184.6±5.4	690.2±32.7	3.76±0.16	1.073±0.038	4.017±0.207	8.44±0.06	62.8±1.5
A D	24	183.1±4.3	799.2±29.6	4.35±0.09	1.022±0.025	4.442±0.152	8.42±0.08	61.3±1.3
	t d	0.217	2.472*	3.207**	1.133	1.655	0.200	0.758
D D	20	181.2±5.7	761.3±35.6	4.23±0.21	1.011±0.024	4.278±0.250	8.42±0.09	66.7±1.4[a]
	t d	0.433	1.471	1.780	1.381	0.803	0.185	1.902
	t d₁	0.268	0.819	0.526	0.257	0.560	-	2.827**
A A	18	184.6±5.4	690.2±32.7	3.76±0.16	1.073±0.038	4.017±0.207	8.44±0.06	62.8±1.5
A D₁	8	174.9±12.5	788.3±67.4	4.58±0.35	0.969±0.035	4.438±0.398	8.44±0.08	64.5±1.9
A D₂	16	183.5±5.4	804.6±30.7	4.38±0.09	1.048±0.032	4.596±0.190	8.40±0.10	59.7±1.5
A E	-	-	-	-	-	-	-	-
D₁D₁	1	194	848	4.37	1.07	4.68	8.3	59.3±0.9
D₂D₂	12	184.3±7.8	715.3±40.8	3.88±0.12	1.018±0.028	3.943±0.169	8.41±0.12	66.8±1.7
D₁D₂	7	184.0±7.1	732.2±52.9	3.98±0.24	1.002±0.047	4.027±0.386	8.33±0.07	68.5±2.5
D₁E	1	198	870	4.39	1.12	4.92	7.9	

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

A study of the sperm production of Red Steppe bulls in relation to amylase types (Table 5) determined that bulls with the Am^{CC} phenotype produced the least ejaculate volume (180.6 ± 2.8 ml) and sperm count (666.4 ± 23.7 ml) annually. In contrast, homozygous *BB* sires produced the largest ejaculate volume (3.79 ± 0.08 ml), which significantly exceeded that of males with the Am^{BC} (3.55 ± 0.09 ml; t_d = 2.000*; P < 0.05)* and Am^{CC} (3.54 ± 0.11 ml, t_d = 1.838) types. It was also observed that homozygous *BB* bulls for the amylase locus secreted the largest number of sperm per ejaculate (3.968 ± 0.116 billion) with a higher concentration (1.025 ± 0.018 billion/ml) and sperm activity (8.35 ± 0.04). Fertilization rates of females after the first insemination with their sperm were also the highest (62.8 ± 0.94). Sperm production indicators of homozygous Am^{CC} bulls were, as noted, worse across all parameters. They exhibited the lowest sperm concentration (0.989 ± 0.25 billion/ml, t_d =1.61) and sperm count per ejaculate (3.482 ± 0.124 billion, t_d = 2.862), as well as the lowest sperm activity (8.28 ± 0.06, t_d = 0.970). Consequently, the lowest fertilization rate was obtained when inseminating females with their sperm (60.4 ± 1.6, t_d = 1.293). Bulls with the Am^{BC} phenotype occupied an intermediate position for most of the studied indicators.

In the Angeln breed, it was not possible to establish a consistent and close relationship between different amylase types and sperm production quality (Table 5). Although bulls with the Am^{CC} phenotype produced the fewest ejaculates (179.3 ± 17.7), and the least amount of sperm (669.7 ± 125.9 ml) per year, they also had the smallest ejaculate volume (3.68 ± 0.41 ml), the lowest total sperm count in the ejaculate (3.807 ± 0.571 billion), sperm activity (8.37±0.03) and the lowest fertilization rate of females from the first insemination (59.0 ± 5.3%). Homozygous *BB* and heterozygous *BC* males at the amylase locus outperformed their *CC* counterparts in both quantitative and qualitative sperm production parameters. However, this advantage was not consistent: either *BB* bulls prevailed in one case or *BC* bulls in another. These minor differences among these fertile individuals explain the variability in the outcomes.

The genetic polymorphism of ceruloplasmin, characterized by two codominant alleles, *A* and *B,* was determined (Table 1). The data show that the frequency of allele *A* was higher in both breeds (0.619 and 0.750) compared to allele *B* (0.381 and 0.250, respectively). The level of heterozygosity at the ceruloplasmin locus was 43.5% for Red Steppe bulls and 34.8% for Angeln bulls. These frequencies and heterozygosity levels are consistent with previous studies by Hrytsienko et al. (2019), Bodnaruk et al. (2022), and Zhmur et al. (2025).

Analysis of the relationship between bulls' sperm production parameters and ceruloplasmin types (Table 6) revealed that the highest number of ejaculates (185.1 ± 2.1) and sperm (696.9 ± 17.9 ml) per year were obtained from bulls with the *AA* genotype. These sire bulls also produced the largest ejaculate volumes (3.76 ± 0.08 ml) the highest sperm concentration (1.040 ± 0.19 billion/ml) the greatest total sperm count in the ejaculate (3.930 ± 0.128 billion), and the highest sperm activity (8.37 ± 0.05). In contrast, all these indicators were lowest in homozygous Cp^{BB} bulls, with only 661.1 ± 18.9 ml of sperm obtained (t_d = 1.144), an ejaculate volume of 3.61 ± 0.10 (t_d = 1.172), sperm concentration of 0.985 ± 0.034 billion/ml (t_d = 1.413), total sperm count of 3.582 ± 0.168 billion, and sperm activity of 8.27 ± 0.07. The fertility rate of females following the first insemination was also lowest in this group, at 60.5% ± 1.4% compared to 62.6 ± 0.9% in the *AA* group (t_d = 1.265).

Similar patterns were observed in the Angeln breed (Table 6): the quantitative and qualitative sperm production indicators were closely associated with the ceruloplasmin types. The highest number of ejaculates (191.0 ± 4.1) and sperm volume (830.2 ± 58.8 ml) per year was obtained from homozygous Cp^{BB} bulls. These bulls also exhibited the highest sperm concentration (1.106 ± 0.056 billion/ml), total sperm count (4.762 ± 0.239 billion), sperm activity (8.40 ± 0.07), and the highest fertilization success rate after the first insemination (65.8 ± 2.1%). Lower quantitative and qualitative sperm production indicators were observed in heterozygous Cp^{AB} bulls, from which 183.5 ± 5.4 ejaculates with a total volume of 742.1 ± 33.6 ml (td = 1.302) were obtained per year. Each ejaculate had an average volume of 4.05 ± 0.14 ml (td = 1.064), with a sperm concentration of 1.013 ± 0.022 billion/ml (td = 1.407), and a total sperm count of 4.075 ± 0.141 billion (td = 2.475). These parameters were minimal, and the fertilization rate after the first insemination was lower than that of the Cp^{BB} group, at 64.1 ± 1.3%. The lowest fertilization rate was observed in the homozygous Cp^{AA} bulls, at 62.5 ± 1.2%.

The potential importance of different locus combinations in the studied systems for determining sperm production indicators in breeding bulls necessitates further analysis. Genetic markers were therefore used to assess the complexity of heterozygosity at these loci and to analyze the relationship with sperm production in breeding bulls (Tables 7 and 8). The entire population of Red Steppe breeding bulls was divided into five groups based on their degree of heterozygosity, while the Angeln breed was divided into four groups. The study's findings indicated that, in the Red Steppe breed, there was a slight decrease in sperm production indicators as the level of heterozygosity for marker genes increased. The largest number of ejaculates was obtained from bulls with a 25% level of heterozygosity (181.5 ± 2.8), while the smallest number was obtained from bulls with 100% heterozygosity (166.0 ± 11.4, td = 1.320). In terms of total sperm volume obtained (674.3 ± 39.7 ml), homozygous bulls (with 0% heterozygosity) surpassed all other groups. Subsequently, a linear decrease in volume was observed, with a significant difference compared to bulls with 100% heterozygosity (486.0 ± 83.5 ml, t_d = 2.036). Similar results were found for ejaculate volume, which was largest in homozygous bulls (3.83 ± 0.156 ml) and significantly exceeded that of bulls with 25% heterozygosity (3.74 ± 0.08 ml). It also exceeded the volumes from bulls with 75% heterozygosity (3.41 ± 0.13 ml) and 100% heterozygosity (2.85 ± 0.35 ml). The linear relationship for sperm concentration was not consistently maintained. It decreased from homozygosity to a 50% heterozygosity level (from 1.052 ± 0.37 to 1.013 ± 0.016 billion/ml), increased at 75% heterozygosity (to 1.068 ± 0.24 billion/ml), and then decreased again at 100% heterozygosity.

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Table 5 - Quantitative and qualitative indicators of sperm production of sire bulls of different genetic predisposition by types Am

Amylase type		Number of bulls, heads	Ejaculates received	Semen obtained from one bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	General number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows after 1st insemination, %
Red Steppe breed	*BB*	68	184.1±2.1	692.3±17.9	3.79±0.08	1.025±0.018	3.968±0.116	8.35±0.04	62.8±0.94
	BC	48	185.9±1.8	668.4±18.5	3.55±0.09	1.036±0.021	3.708±0.124	8.32±0.05	62.1±1.07
		td	0.650	0.930	2.0*	0.397	1.529	0.469	0.492
	CC	28	180.6±2.8	666.4±23.7	3.54±0.11	0.989±0.025	3.482±0.124	8.28±0.06	60.4±1.6
		td	1.000	0.872	1.838	1.161	2.862**	0.970	1.293
		td_1	1.592	0.066	0.070	1.424	1.291	0.500	0.895
Angeln breed	*BB*	38	188.5±2.9	775.2±24.2	4.11±0.10	1.037±0.023	4.249±0.135	8.49±0.09	62.98±0.96
	BC	19	185.6±4.8	801.2±36.5	4.30±0.15	1.022±0.019	4.384±0.162	8.42±0.08	62.9±1.45
		td	0.516	0.605	0.333	0.503	0.640	0.583	-
	CC	3	179.3±17.7	669.7±125.9	3.68±0.41	1.030±0.065	3.807±0.571	8.37±0.03	59.0±5.3
		td	0.512	0.823	1.018	0.102	0.753	1.263	0.724
		td_1	0.344	0.317	1.419	0.118	0.971	0.588	0.710

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Table 6 - Quantitative and qualitative indicators of sperm production of sire bulls of different genetic predisposition by Cp types

Ceruloplasmin type		Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows after 1st insemination, %
Red Steppe breed	*AA*	59	185.1±2.1	690.9±17.9	3.76±0.08	1.040±0.019	3.930±0.128	8.37±0.05	62.6±0.9
	AB	64	181.7±1.9	666.1±18.1	3.66±0.09	1.010±0.023	3.748±0.115	8.28±0.04	63.34±1.0
		td	1.201	0.939	0.833	1.007	1.057	1.406	0.548
	BB	24	182.9±3.7	661.1±18.9	3.61±0.10	0.985±0.034	3.582±0.168	8.27±0.07	60.5±1.4
		td	0.517	1.144	1.172	1.413	1.648	1.163	1.265
		td_1	0.288	0.184	0.370	0.610	0.814	0.123	1.651
Angeln breed	*AA*	38	186.5±2.8	780.7±27.4	4.17±0.11	1.035±0.019	4.317±0.145	8.39±0.06	62.5±1.2
	AB	23	183.5±5.4	742.1±33.6	4.05±0.14	1.013±0.022	4.075±0.141	8.40±0.06	64.1±1.3
		td	0.492	0.890	0.674	0.759	1.198	0.117	0.791
	BB	5	191.0±4.1	830.2±58.8	4.33±0.24	1.106±0.056	4.762±0.239	8.40±0.07	65.8±2.1
		td	0.907	0.763	0.606	1.203	1.592	0.109	1.369
		td_1	1.106	1.302	1.064	1.407	2.475*	-	0.688

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$) based on *t*-Student criteria (t_d).

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Table 7 - Characteristics of sperm production of sire bulls of the Red Steppe breed at different levels of heterozygosity for marker genes

Heterozygosity, %	Number of bulls, heads	Ejaculates received	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows from the 1st insemination, %
0	22	173.7±5.1	674.3±39.7	3.83±0.156[a]	1.052±0.037	3.995±0.237	8.38±0.06	64.4±1.56
25	57	181.5±2.8	671.3±20.7	3.74±0.08[b]	1.026±0.018	3.890±0.108	8.39±0.05	63.8±0.95
	td	1.345	0.067	0.514	0.634	0.404	0.128	0.327
50	51	180.3±2.2	667.8±17.6	3.62±0.08[c]	1.013±0.016	3.674±0.105	8.31±0.05	62.1±0.91
	td	1.179	0.149	1.200	0.975	1.239	0.897	1.271
	td_1	0.337	0.129	1.062	0.542	1.912	1.127	1.288
75	22	178.9±4.8	646.2±26.2	3.41±0.13	1.068±0.024	3.634±0.159	8.25±0.06	60.6±1.25
	td	0.743	0.630	2.068*	0.364	1.267	1.529	1.900
	td_1	0.467	0.751	2.156*	1.400	0.813	1.795	2.038
	td_2	0.265	0.684	1.373	1.910	0.211	0.769	0.968
100	8	166.0±11.4	486.0±83.5	2.85±0.35[a]	1.035±0.064	2.904±0.331	8.31±0.12	56.3±2.99
	td	0.616	2.036	2.559*	0.230	2.973**	0.522	2.403*
	td_1	1.320	2.154*	2.479*	0.136	2.833**	0.615	2.389*
	td_2	1.233	2.131*	2.145*	0.333	2.219*	-	1.924
	td_3	1.040	1.831	1.514	0.485	1.989	0.448	1.327

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Table 8 - Characteristics of sperm production of sire bulls of the Angeln breed at different levels of heterozygosity for marker genes

Heterozygosity, %	Number of bulls, heads	Ejaculates received	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows from the 1st insemination, %
0	13	187.5±2.8	779.8±51.9	4.14±0.25	1.062±0.028	4.388±0.273	8.36±0.08	60.6±1.78
25	31	184.9±3.5	758.4±22.6	4.09±0.11	1.050±0.020	4.200±0.142	8.37±0.07	63.9±1.04
	td	0.580	0.378	0.185	0.353	0.610	0.094	1.602
50	14	175.9±7.9	782.0±44.3	4.43±0.12	0.994±0.020	4.394±0.127	8.43±0.05	64.6±1.19
	td	1.384	0.023	1.036	2.000	0.020	0.638	1.869
	td_1	1.042	0.474	2.086*	2.800**	1.016	0.698	0.443
75	4	189.5±6.2	786.8±88.1	4.13±0.37	0.980±0.075	3.985±0.430	8.35±0.03	62.8±6.2
	td	0.294	0.680	0.022	1.025	0.729	0.118	0.341
	td_1	0.646	0.301	0.103	0.897	0.475	0.263	0.175
	td_2	1.360	0.049	0.771	0.180	0.912	1.379	0.285

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Thus, the highest sperm concentration was observed in bulls with a 75% heterozygosity level, and a relatively high concentration was found in homozygous bulls. However, no significant differences were established between the groups for this indicator. A similar pattern was observed for other indicators. In terms of the total number of sperm per ejaculate (3.995 ± 0.237 and 3.890 ± 0.108 billion), bulls with 0% and 25% heterozygosity bulls exceeded other groups, with a significant difference compared to bulls with 100% heterozygosity level (2.904 ± 0.331 billion, t_d = 2.973 and 2.833). The highest sperm activity (8.38±0.06 and 8.39±0.05) was also found in bulls with 0% and 25% heterozygosity, while the lowest (8.25 ± 0.06, t_d = 1.529 and 1.795) was observed in bulls with 75% heterozygosity. With an increase in the level of heterozygosity, there was also a decrease in the fertilizing ability of bull sperm. The highest fertilization rate of females at the first insemination (64.4 ± 1.56 and 63.8 ± 0.95%) was observed in the group using sperm from homozygous bulls and bulls with a 25% heterozygosity. The lowest rates were recorded in the 4th and 5th groups (75% and 100% heterozygosity, respectively), with rates of 60.6 ± 1.25% (td = 1.900 and 2.038) and 56.3 ± 2.99% (td = 2.403 and 2.389). Studies by Gopinathan et al. (2018) and Piddubna and Zakharchuk (2020) have established the dependence of sperm productivity in breeding bulls on genotypic factors.

When examining the sperm production indicators of Angeln bulls (Table 8), a similar pattern related to heterozygosity levels was not found. The largest ejaculate volume (4.43 ± 0.12 ml) was observed in bulls with a 50% level of heterozygosity, and the smallest (4.09 ± 0.11 ml, t_d = 2.086) was in bulls with a 25% level. Regarding sperm concentration, a linear decrease was observed as the level of heterozygosity increased. The highest concentration (1.062 ± 0.028 billion/ml) was characteristic of homozygotes, while the lowest concentrations (0.994±0.02 billion/ml and 0.980 ± 0.075 billion/ml) were found in bulls with 50% and 75% heterozygosity. In terms of the total number of sperm in the ejaculate (4.394 ± 0.127 and 4.388 ± 0.273 billion/ml), bulls with 50% heterozygosity and homozygotes showed an advantage. Sperm activity was higher in bulls with 50% heterozygosity (8.43 ± 0.05), and they also exhibited higher fertilization ability (64.6 ± 1.19%). The lowest fertilization rate of females from the first insemination was obtained in homozygous bulls (60.6 ± 1.78%, t_d = 1.869).

CONCLUSIONS

1. A significant difference was observed in the volume of semen obtained per year between bulls with the transferrin types *AA*, *AB*, *AE* and *DE*. The ejaculate volume and the total number of sperm count were highest in homozygous bulls (*AA*, *DD*) of the Red Steppe breed, while in the Angeln breed, the highest values were found in heterozygous bulls (*AD*). The highest sperm activity was characteristic of bulls with the *AA* transferrin type. The fertilization rate of females from the first insemination was the highest in Red Steppe bulls with the *AA* and *AD* transferrin types, and in Angeln bulls with the *DD* type.
2. Homozygous *BB* at the *Am* locus in the Red Steppe breed, and heterozygous *BC* bulls in the Angeln breed exhibited the highest sperm production and fertilization ability.
3. Homozygous bulls of the Red Steppe breed with the *Cp AA* type showed the highest ejaculate volume, sperm concentration, total sperm count in the ejaculate, and sperm activity. The fertilization ability from the first insemination of cows and heifers was highest in heterozygous (*BC*) bulls. Homozygous (*BB*) bulls in the Angeln breed demonstrated the highest sperm production and fertilization ability.
4. Homozygous bulls and those with a 25% level of heterozygosity for marker genes in the Red Steppe breed exhibited the highest indicators of sperm production and fertilization ability. An increase in the level of heterozygosity in these bulls was associated with a decrease in the noted indicators of reproductive function. In the Angeln breed, bulls with a 50% level of heterozygosity demonstrated the highest indicators of sperm production and fertilization ability.

Suggestions

1. These findings suggest that consideration of homozygosity levels at the *Tf*, Am, and Cp loci may be useful when selecting sire bulls, although validation in independent populations is warranted.
2. To perform typing of genetic and biochemical features of sperm production of bulls of Bos taurus breeds currently widespread in Ukraine.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

V. Melnyk focused on the conceptual and methodological part of the study, as well as on the primary data processing, development of the main scientific concept of the work and definition of key tasks. Direct participation in the collection of material. M. Gill was critical for the analysis, interpretation of results and their scientific justification, conducting

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comprehensive statistical analysis, including calculating the reliability of differences and correlations. Interpreting the obtained numerical data and comparing them with literary sources. Formulation of final conclusions based on the data obtained and development of practical recommendations (proposals for breeding enterprises), structuring and critical review of the scientific text. M. Tymofiiv provided the collection, systematization, and critical analysis of existing scientific publications used in the literature review. Carrying out general scientific and stylistic editing of the text, ensuring its compliance with publication requirements, and being responsible for the final version of the article.

Consent to publish

All authors agreed to the publication of this manuscript.

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Competing interests

The authors declare that they have no competing interests.

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