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Interrelation of Kinetic Parameters of Sperm of Servicing Bulls of the Holstein Breed with Its Fertilising Ability

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Abstract. Sperm motility is an essential indicator that characterises the viability and structural integrity of sperm. This parameter is closely related to the fertilisation capacity of male germ cells, so its assessment is an integral part of sperm analysis. The purpose of this study is to investigate the possibility of predicting the fertilisation capacity of sperm of servicing bulls based on kinetic indicators. The study involved laboratory, zootechnical, and statistical methods. A significant variability in the kinetic parameters of sperm of servicing bulls was revealed – from 6.2 to 16.1%. The variation in the percentage of motile sperm in the ejaculate was 78.9-89.8; the percentage of progressive sperm – 50.0-74.5; velocity average path of sperm (VAP) – 132.6-163.7 $\mu\text{m/s}$; velocity straight line (VSL) – 99.2-138.2 $\mu\text{m/s}$; curvilinear velocity (VCL) – 223.7-272.3 $\mu\text{m/s}$; straightness of sperm (STR) – 73.9-85.0%, linearity (LIN) – 45.1-56.1%, wobble (WOB) – 57.2-63.8%. The difference between the minimum and maximum values for all the parameters under study is highly likely ($P < 0.001$). A correlation between various kinetic parameters of sperm is established. Sperm movement rates and their relative values are largely conditioned upon the percentage of progressive sperm in the ejaculate ($r = +0.231-0.761$). VAP, VSL, and VCL indicators are interrelated ($r = +0.550-0.887$). The study investigated the average fertilising ability of the sperm of servicing bulls based on the results of insemination of 8,594 cows and 992 heifers in four farms of Zhytomyr and Kyiv oblasts, the variation was within 40.7-61.4%. It was proved that bulls with higher and average fertilising capacity are described by a higher percentage of progressive sperm in ejaculates, and VAP, VSL, and VCL indicators of sperm movement compared to bulls with low fertilising capacity. This pattern is confirmed by correlation analysis ($r = +0.538-0.675$). Kinetic parameters identified using the CASA system allow predicting the fertilising capacity of sperm and identify servicing bulls with reduced reproductive function

Keywords: bull, CASA system, sperm motility, VAP, VSL, VCL, fertilising capacity of sperm



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INTRODUCTION

The dairy industry is an important link in the food market of Ukraine, which accounts for about 11% of the total sales of the food industry. Due to their beneficial properties, milk and dairy products are in high demand among consumers. Approximately 18% of the total food costs of the Ukrainian population belong to dairy products (Korman *et al.*, 2022). Currently, there is a rapid decline in milk production, one of the reasons for which is a substantial decrease in the number of cattle. The solution to this problem is partly possible due to the proper organisation of herd reproduction.

Pedigree bulls with high genetic potential play a vital role in the successful breeding of highly productive cows, since thousands of offspring can be obtained from a single male by artificial insemination in a short period of time (Kyzebnyy & Boiko, 2018).

Assessment of the reproductive capacity of producers is based on the analysis of sperm quality. One of the indicators that is considered most associated with the fertilisation capacity of sperm is motility (Gillan *et al.*, 2008; Hering *et al.*, 2014; David *et al.*, 2015). It is the ability of male cells to actively move that allows them to overcome the anatomical and physiological barriers of the female genital organs and fertilise the egg (Suarez & Pacey, 2006). Assessment of sperm motility by the conventional microscopic method is quite subjective (Singh *et al.*, 2021), and therefore it does not always correlate with fertilising capacity (Farrell *et al.*, 1998). In the 1980s, Computer Assisted Semen Analysis (CASA) was created, which, unlike conventional analysis methods, provides objective, accurate, and fast results of the quality of ejaculates (Yaniz *et al.*, 2018). The basic elements of this system include a phase contrast microscope, a video camera, and a computer with software. Apart from the concentration and quantification of motile and progressive sperm, CASA technology determines a wide range of kinetic parameters for each cell: velocity average path (VAP), velocity straight line (VSL) and curvilinear velocity (VCL), $\mu\text{m/s}$; straightness (STR), linearity (LIN) and wobble (WOB), %; amplitude of lateral head displacement (ALH), μm ; beat cross frequency (BCF), Hz (Tanga *et al.*, 2021). Furthermore, this technology allows identifying subpopulations of sperm in each field – stationary, slow, medium, and fast (Ibanescu *et al.*, 2020).

Numerous scientific studies indicate a relationship between various sperm counts determined by CASA and its fertilising capacity in males (Ahmed *et al.*, 2016; Suliman *et al.*, 2020; Barquero *et al.*, 2021; Gliozzi *et al.*, 2017) found that Holstein bulls with high reproductive potential significantly outnumbered animals with low sperm motility, STR and LIN indicators, and the percentage of active cells. Zăhan *et al.* (2018) found a significant correlation between sperm fertilising capacity and sperm motility ($r=0.878$), VSL ($r=0.648$), and VAP ($r=0.684$) motility in Simmental bulls. Bernecic

et al. (2021), having investigated a wide range of functional, morphological, and intracellular parameters of cryopreserved sperm, concluded that only the viability and integrity of the acrosome can serve as indicators of bull fecundity.

The purpose of this study is to figure out the kinetic parameters of native sperm of servicing bulls and investigate the relationship of these parameters with the fertilising capacity of sperm to predict it. To fulfil the purpose of the study, the following tasks were set: to find and investigate the kinetic parameters of sperm using the IVOS semen analyser; to figure out the fertilising capacity of sperm of experimental bulls; to investigate the relationship between the kinetic parameters of sperm and its fertilising capacity.

MATERIALS AND METHODS

The experiment was conducted in the conditions of "Ukrainian genetic company" LLC. The study used 11 servicing bulls of the black and red-spotted Holstein breed, aged from 4 to 8 years. Bulls of foreign origin, imported to pedigree enterprise from Germany and the Netherlands.

Ejaculates from servicing bulls were obtained according to the schedule twice a week by a double mount with an interval of 5-10 minutes, using a dummy bull. Sperm was taken into a shortened artificial vagina using a disposable sperm receiver, which was sealed and passed through a sterile airlock to a production laboratory.

At the initial stage, the obtained ejaculates were evaluated visually, after which the following indicators were identified using the IVOS computer analysis system (Hamilton Thorne Research, USA): percentage of motile and progressive sperm in the ejaculate, velocity average path (VAP, $\mu\text{m/s}$), straight-line velocity (VSL, $\mu\text{m/s}$), curvilinear velocity (VCL, $\mu\text{m/s}$), straightness (STR, %), linearity (LIN, %), wobble (WOB, %). The quality of native sperm was evaluated according to DSTU 3535-97.

Dilution of sperm suitable for cryopreservation took place at a temperature of $+35^{\circ}\text{C}$ with AndroMed medium (Germany). One spermodose contained at least 20 million active sperm cells.

Rarefied sperm was packed into 0.25 ml straws using IS4 automatic equipment (IMV Technologies, France). After packaging and labelling, the spermodoses were kept in a refrigerated display case (IMV Technologies, France) for 3-4 hours to undergo the balancing process. The straws cooled to 4°C were cryopreserved in a MiniDigitcool programmable freezer (IMV Technologies, France) in three stages: I – the rate of lowering the temperature was $5^{\circ}\text{C}/\text{min.}$, cooling from $+4$ to -10°C ; II – speed of $40^{\circ}\text{C}/\text{min.}$, freezing from -10 to -100°C ; III – speed of $20^{\circ}\text{C}/\text{min.}$, freezing from -100 to -140°C . Cryopreserved sperm products were stored in HB-200 bio-storage facilities in liquid nitrogen at -196°C .

Cryopreserved straws were thawed in a water bath at $+35^{\circ}\text{C}$ for 30 s. The motility of thawed sperm

was determined on an IVOS analyser. Sperm survival was studied by incubating samples at +38±0.5°C for 5 hours. Sperm products that had an initial motility of less than 4 points and a survival rate of less than 5 hours were subject to culling.

To determine the fertilising capacity of sperm, 8594 were used cows and 992 heifers of Ukrainian black-and red-piebald dairy breeds, which were inseminated with sperm products of experimental bulls in the farms of Zhytomyr (PAF “Yerchiki”, SE EF “Nova Peremoha”, ALLC “Ptahoplezavod “Korobovskiy”) and Kyiv (AF “Kyivska”) oblasts. Females were artificial inseminated according to the recto-cervical method, twice at one sexual excitement with an interval of 10-12 hours. Fecundation was determined on the 90th day after insemination using an ultrasound machine.

The indicator of the average fertilising capacity of sperm was determined according to the formula (Maiboroda et al., 2019):

$$AFC_j = \frac{\sum N_{ij} \frac{kK_{ij} + T_{ij}}{N_{ij}}}{\sum N_{ij}} \times 100, \quad (1)$$

where AFC_j is the average fertilising capacity of j -th bull's sperm, %; k is the correction factor for cows for the level of fertilisation of heifers; K_{ij} is the number of cows fertilised from the first sperm insemination by the j -th bull in the i -th herd; T_{ij} is the number of heifers fertilised from the first sperm insemination by the j -th bull in the i -th herd; N_{ij} is the total number of cows and heifers fertilised from the first sperm insemination by the j -th bull in the i -th herd.

The obtained data were processed according to the methods of mathematical statistics on a personal computer using the programs “STATISTICA 10.0” and MS Excel.

RESULTS AND DISCUSSION

Experimental servicing bulls of the Holstein breed differ substantially in all the indicators considered (Table 1).

Table 1. Kinetic parameters of sperm of servicing bulls

Nickname and bull identification No.	Quantity ejaculates	Motile sperm, %	Progressive sperm, %	VAP, µm/s	VSL, µm/s	VCL, µm/s	STR, %	LIN, %	WOB, %
Argonaut DE 538441348	61	89.7±0.55	67.3±1.00	157.2±1.20	127.1±1.81	271.0±1.85	79.7±0.47	47.7±0.45	58.0±0.30
Asall DE 579542573/42573	24	82.2±1.56	55.3±2.39	133.0±3.16	104.9±2.04	223.7±5.75	77.8±1.10	48.6±1.15	59.6±0.67
Bugatti DE 538441328/41328	57	83.5±0.59	66.3±0.90	163.7±0.94	138.2±1.06	265.8±1.87	82.5±0.40	51.9±0.45	61.7±0.24
Glamour NL 713313332	35	78.9±0.77	59.9±1.04	152.5±1.20	126.4±1.08	267.5±3.33	81.7±0.45	48.4±0.58	57.2±0.46
Chancellor DE 768305280/5280	21	83.3±1.10	50.0±2.25	132.6±1.92	99.2±2.04	228.6±4.00	73.9±0.98	45.1±1.04	58.1±0.70
Carmello DE 349214122/14122	18	81.3±1.91	50.8±2.74	132.6±2.47	104.0±2.61	227.0±3.40	76.3±1.10	46.6±1.06	58.4±0.80
Lasky NL 762041879/41879	43	84.8±0.75	61.5±1.14	155.6±1.46	125.6±1.84	272.3±2.15	79.0±0.62	47.1±0.65	57.2±0.50
Lafard DE 121030279	22	84.4±0.90	66.7±1.57	147.0±2.69	127.2±2.42	231.5±6.05	85.0±0.59	56.0±0.72	63.8±0.68
Levitz DE 356447182	30	89.8±0.60	74.5±1.45	158.6±2.00	137.9±2.16	253.6±3.84	84.5±1.28	56.1±1.27	62.7±0.57
Masiro DE 354071654/71654	47	83.2±0.63	68.6±0.92	149.7±1.25	129.2±1.35	255.5±2.85	85.0±0.50	51.7±0.65	58.8±0.46
Fawn DE 356552537	71	89.4±0.55	73.6±0.91	155.8±1.72	134.0±1.58	247.3±3.03	84.9±0.34	54.7±0.52	63.1±0.37
Max-min		10,9 ^c	24,5 ^c	31,1 ^c	39,0 ^c	48,6 ^c	11,1 ^c	11,0 ^c	6,6 ^c
Cv, %		7,0	16,1	9,2	12,3	10,0	6,2	10,9	6,2

Note: The results are statistically significant at $a - P<0.05$, $b - P<0.01$, $c - P<0.001$

Source: compiled by the authors

The variability of kinetic parameters was within 6.2-16.1%. Traditionally, when evaluating sperm at pedigree enterprises, the main and only kinetic indicator that determines the suitability of the ejaculate for use is sperm motility. In the samples under study, it varies from 78.9 (Glamour) to 89.8 (Levitz), i.e., the max-min difference is 10.9%. More variable is the percentage of

progressive sperm in the ejaculates of bulls, its variation is 50.0% (Chancellor) – 74.5% (Levitz), max-min 24.5%. Therewith, there were cases when the difference between the number of motile and progressive sperm was 20-30%, and a bull with a motility of 81-83% had only 50% of progressive sperm (Chancellor, Carmello), or vice versa with a motility of 78.9% – almost 60%

of progressive sperm (Glamour). The largest percentage of progressive sperm in ejaculates (66.3-74.5%) was recorded in the bulls Agronaut, Bugatti, Lafard, Levitz, Masiro, Fawn.

Significant differences in servicing bulls were also found in the velocity of sperm. The minimum VAP value is 132.6 $\mu\text{m/s}$ (Chancellor), the maximum is 163.7 $\mu\text{m/s}$ (Bugatti); the minimum VSL value is 99.2 $\mu\text{m/s}$ (Chancellor), the maximum is 138.2 $\mu\text{m/s}$ (Bugatti); the minimum VCL value is 223.7 $\mu\text{m/s}$ (Asall), the maximum is 272.3 $\mu\text{m/s}$ (Lasky). The highest sperm movement rates (VAP over 155 $\mu\text{m/s}$) were found in bulls Argonaut,

Bugatti, Lasky, Levitz, Fawn. Three calculated indicators that characterise the velocity ratio were also found. The minimum STR value is 73.9% (Chancellor), the maximum is 85.0% (Masiro); the minimum LIN value is 45.1% (Chancellor), the maximum is 56.1% (Levitz), the minimum WOB value is 57.2% (Glamour), the maximum is 63.8% (Lafard).

The difference between the extreme values according to VAP is 31.1 $\mu\text{m/s}$, VSL – 39.0 $\mu\text{m/s}$, VCL – 48.6 $\mu\text{m/s}$, STR – 11.1%, LIN – 11.0%, WOB – 6.6%. In all cases without exception, the difference is highly probable ($P < 0.001$).

Correlation analysis revealed the interdependence of sperm parameters of servicing bulls (Table 2).

Table 2. Relationship between kinetic parameters of servicing bull sperm ($n=429$)

Correlation coefficient	Progressive sperm, %	VAP, $\mu\text{m/s}$	VSL, $\mu\text{m/s}$	VCL, $\mu\text{m/s}$	STR, %	LIN, %	WOB, %
Motile sperm, %	+0.396 ^c	+0.275 ^c	+0.225 ^c	+0.105 ^a	+0.020	+0.133 ^b	+0.247 ^c
Progressive sperm, %		+0.555 ^c	+0.743 ^c	+0.231 ^c	+0.761 ^c	+0.641 ^c	+0.458 ^c
VAP, $\mu\text{m/s}$			+0.887 ^c	+0.807 ^c	+0.320 ^c	+0.242 ^c	+0.180 ^c
VSL, $\mu\text{m/s}$				+0.550 ^c	+0.649 ^c	+0.556 ^c	+0.435 ^c
VCL, $\mu\text{m/s}$					-0.054	-0.281 ^c	-0.431 ^c
STR, %						+0.706 ^c	+0.575 ^c
LIN, %							+0.844 ^c

Note: The results are statistically significant at a – $P < 0.05$, b – $P < 0.01$, c – $P < 0.001$

Source: compiled by the authors

A positive relationship of average strength is observed between the percentage of motile and progressive sperm (+0.396, $P < 0.001$). Sperm motility rates positively and reliably correlate with the percentage of motile and progressive sperm in the ejaculate, but the dependence on the percentage of progressive sperm is much higher – VAP+0.555 vs +0.275, VSL +0.743 vs +0.225, VCL +0.231 vs + 0.105. It is clear that all velocities are interrelated, which is confirmed by high and probable correlation coefficients between them – from +0.550 to +0.887.

As for the relative velocity indicators that describe the STR, LIN, and WOB degrees, they are most conditioned upon the percentage of progressive sperm in the ejaculate (+0.458-0.761) and VLS (+0.435-0.649), and negatively correlate with VCL (-0,431-0,054).

Fertilising capacity is the main criterion for sperm quality. To evaluate it, the results of insemination of 9,586 cows and heifers of mating age with the sperm of experimental bulls in 4 farms of Zhytomyr and Kyiv oblasts were analysed (Table 3).

Table 3. Fecundity rate of livestock with sperm of experimental servicing bulls

Nickname and bull identification No.	Farm	Number of inseminations			Fertilising capacity capacity, %*	Average fertilising capacity of sperm, %*
		Cows	Heifers	Total		
Argonaut DE 538441348	“AF “Kyivska” LLC	921	–	921	61.4	61.4 \pm 1.60
	PAF “Yerchyky”		40	40	70.0	
Asall DE 579542573/42573	“Ptahoplemzavod “Korobivskiy” ALLC	61	32	93	40.2	49.2 \pm 4.33
Bugatti DE 538441328/41328	“AF “Kyivska” LLC	487	–	487	44.0	51.2 \pm 1.05
	PAF “Yerchyky”	1604	102	1706	52.7	
	“Ptahoplemzavod “Korobivskiy” ALLC	65	28	93	60.2	
Glamour NL 713313332	“Ptahoplemzavod “Korobivskiy” ALLC	123	19	142	54.2	54.2 \pm 4.18
Chancellor DE 768305280/5280	PAF “Yerchyky”	172	20	192	41.9	41.9 \pm 3.56

Table 3, Continued

Carmello DE 349214122/14122	PAF "Yerchyky"	263	29	292	61.8	49.7±1.14
	"AF "Kyivska" LLC	1190	10	1200	49.7	
	SE "EF "Nova Peremoha"	330	88	418	41.3	
Lasky NL 762041879/41879	PAF "Yerchyky"	230	7	237	52.2	52.2±3.24
Lafard DE 121030279	PAF "Yerchyky"	95	10	105	40.7	40.7±4.79
Levitz DE 356447182	PAF "Yerchyky"	301	64	365	44.4	60.0±1.41
	"AF "Kyivska" LLC	383	361	744	67.1	
	SE "EF "Nova Peremoha"	60	42	102	64.4	
Masiro DE 354071654/71654	PAF "Yerchyky"	175	–	175	54.2	54.4±1.19
	"AF "Kyivska" LLC	1546	42	1588	54.4	
Fawn DE 356552537	PAF "Yerchyky"	299	46	345	51.7	53.9±1.90
	"AF "Kyivska" LLC	94	–	94	52.6	
	"Ptahoplemzavod "Korobivskiy" ALLC	195	52	247	57.5	

Note: Corrective factor for cows for the fecundity level of heifers is 1.3

Source: compiled by the authors

For some servicing bulls, the fertilisation rate of livestock did not significantly differ between farms (Masiro, Fawn), for others it varied quite widely (Asall, Bugatti, Carmello, Levitz). This is because apart from the quality of sperm products of the servicing bull, the results of livestock fertilisation are affected by factors such as the state of reproductive health of the herd, the method of insemination, the level of training of artificial insemination technician, the organisation of feeding and conditions of keeping animals on the farm, etc. (Kebede, 2018).

Sperm fertilisation rate of 60% or more is found in bulls Argonaut and Levitz; 50% or more – Bugatti,

Glamour, Lasky, Masiro, Fawn; 40-50% – Asall, Chancellor, Carmello, Lafard. In general, there is a tendency that bulls with higher and average fertilising capacity are described by a higher percentage of progressive sperm (66.3-74.5%), VAP (149.7-163.7 $\mu\text{m/s}$), VLS (125.6-138.2 $\mu\text{m/s}$) and VCL (247.3-272.3 $\mu\text{m/s}$) indicators of sperm motility compared to bulls with low fertilising capacity, in which these parameters were 50.0-66.7%; 132.6-147.0; 99.2-127.2, and 223.7-231.5 $\mu\text{m/s}$, respectively.

The results of correlation analysis confirm the relationship between the parameters of native sperm and its fertilising capacity (Table 4)

Table 4. Relationship between kinetic parameters of servicing bull sperm and fertilising capacity

Indicator. units of measurement	Correlation coefficient($r\pm m$)	td
Motile sperm, %	+0.491±0.229	2.15
Progressive sperm, %	+0.550±0.210	2.61 ^a
VAP, $\mu\text{m/s}$	+0.604±0.192	3.15 ^a
VSL, $\mu\text{m/s}$	+0.538±0.214	2.51 ^a
VCL, $\mu\text{m/s}$	+0.675±0.164	4.11 ^b
STR, %	+0.317±0.271	1.17
LIN, %	+0.116±0.297	0.39
WOB, %	-0.136±0.296	0.46

Note: The results are statistically significant at a – $P<0.05$, b – $P<0.01$, c – $P<0.001$

Source: compiled by the authors

Sperm kinetic parameters, in addition to the degree of deviation of sperm movement, are positively correlated with fertilising capacity. The relationship between the conventional sperm motility index in bull ejaculates and fertilising capacity is positive of medium strength, but improbable (+0.491). Probable correlation coefficients were obtained with the percentage of progressive sperm (+0.550) and their movement rates (+0.538-0.675).

Modern comprehensive assessment of sperm motility of producers includes determination of total motility (percentage of sperm showing any movement), progressive

motility (percentage of sperm with rectilinear translational movement) and kinetic parameters (Berg *et al.*, 2018). Analysis of sperm movement patterns, their velocity, and head movement trajectory helps to better understand the functional capacity of sperm and select the highest quality bull ejaculates for further cryopreservation (Perumal *et al.*, 2014). The possibility of selecting the highest quality ejaculates is clearly confirmed by the authors' research, as they prove their high variability in the investigated kinetic parameters in different bulls. Variability can be caused by both the bull genotype and a wide range of other factors,

such as age, season, mode of use of the servicing bull, the interval between ejaculations, etc. (Gopinathan *et al.*, 2018; Islam *et al.*, 2018; Murphy *et al.*, 2018).

Studies have found a correlation between different kinetic parameters of sperm. The strongest positive relationship is observed between VSP and VSL ($r=+0.887$), VAP and VCL ($+0.807$), and LIN and WOB ($+0.844$). Similar results were obtained in the study of native (Khan *et al.*, 2017) and cryopreserved sperm of gayals (Perumal *et al.*, 2014) and Simmental bulls (Inanç *et al.*, 2018). All parameters of sperm movement correlate with the percentage of progressive sperm, which indicates the possibility of sperm with straight-line movement to pass the distance in a short period of time (Inanç *et al.*, 2018).

Numerous scientific studies indicate a relationship between sperm motility indicators and sperm fertilising capacity. Singh *et al.* (2016) found that buffaloes with high fecundity have a considerably higher percentage of motile sperm, curvilinear (VCL), and average (VAP) rates compared to buffaloes with medium and low fecundity. Guilherme *et al.* (2020), having investigated the fertilising capacity of bull sperm of the local Brazilian breed Girolando, concluded that VCL, VSL, and VAP are the main indicators by which it is possible to predict ovum fertilisation in vitro.

According to the results of studies, the fertilising capacity of Holstein bull sperm depends on the percentage of sperm with progressive movement ($r=+0.550$), VCL, VAP, and VSL values ($+0.538-0.675$). A strong positive association of fertilisation results with the percentage of progressive sperm in bulls was also recorded by foreign researchers (Kathiravan *et al.*, 2008; Li *et al.*, 2016). The results obtained in this study are in good agreement with Nagy *et al.* (2015), who established a high-probability correlation between sperm motility velocities (VAP, VSL, and VCL) of Holstein bulls based on the results of insemination of 9,000 cows.

Therewith, Nagy *et al.* (2015), Page & Rosenkrans (2019) consider VAP to be the most informative indicator of native sperm quality and the main factor affecting fertilising capacity. According to the results of studies, among all indicators, VCL correlates most with the fertilising capacity of sperm. Fertilisation of the ovum is impossible without hyperactivation of sperm. It is hyperactive sperm that is described by energetic curvilinear movement due to an increase in the amplitude of tail beating (Harayama, 2018). This movement allows them

to overcome the viscous environment of the reproductive organs of the female and penetrate the Zona Pellucida of the ovum (Perez-Cerezales *et al.*, 2015). According to Mortimer *et al.* (1998), spermatozoa are classified as hyperactive if they show high curvilinear velocity ($\geq 150 \mu\text{m/s}$), amplitude of lateral head displacement ($\geq 7 \mu\text{m}$), and low linearity ($\leq 50\%$), while the criteria of De Lamirande & Gagnon (1993) are, respectively, $\geq 80 \mu\text{m/s}$; $6.5 \mu\text{m}$; $\leq 65\%$.

Studies have not found a reliable correlation between the relative velocity parameters (STR, LIN, WOB) and the fertilising capacity of sperm, which is consistent with data from Inanç *et al.* (2018).

CONCLUSIONS

The results of the evaluation of Holstein bulls of the "Ukrainian genetic company" LLC according to the kinetic parameters of native sperm indicate their difference in the quality of ejaculates. Variation limits of the percentage of motile sperm 78.9-89.8%, progressive – 50.0-74.5%, VAP – 132.6-163.7 $\mu\text{m/s}$, VSL – 99.2-138.2 $\mu\text{m/s}$, VCL – 223.7-272.3 $\mu\text{m/s}$, degree STR – 73.9-85.0%, LIN – 45.1-56.1%, WOB – 57.2-63.8%. In all cases without exception, the difference between the extreme values is highly probable ($P<0.001$).

Sperm motility parameters and their relative indicators depend to a greater extent on the percentage of progressive sperm in the ejaculate ($r=+0.231-0.761$) than motile ones ($r=+0.020-0.275$). VAP, VLS, and VCL indicators are strongly correlated with each other ($r=+0.550-0.887$).

According to the results of insemination of brood stock in farms of the Zhytomyr and Kyiv oblasts, the average fertilising capacity of the sperm of experimental servicing bulls ranges from 40.7% to 61.4%. The fertilising capacity of frozen-thawed sperm largely depends on the kinetic parameters of native sperm – a probable correlation is observed with the percentage of progressive sperm in the ejaculate ($r=+0.550$) and the velocities of its movement ($r=+0.538-0.675$).

The results of this study prove the possibility and feasibility of a comprehensive assessment of ejaculates obtained from bulls by motility, including kinetic parameters. Indicators such as the percentage of progressive sperm can be used to predict the fertilising capacity of sperm, the velocity average path (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL).

REFERENCES

- [1] Ahmed, H., Andrabi, S.M.H., & Jahan, S. (2016). Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during low-breeding season. *Theriogenology*, 86(6), 1516-1522. doi:10.1016/j.theriogenology.2016.05.010.
- [2] Barquero, V., Roldan, ERS., Soler, C., Vargas-Leitón, B., Sevilla, F., Camacho, M., & Valverde, A. (2021). Relationship between fertility traits and kinematics in clusters of boar ejaculates. *Biology (Basel)*, 10(7), article number 595. doi: 10.3390/biology10070595.
- [3] Berg, H.F., Kommisrud, E., Bai, G., Gaustad, E.R., Klinkenberg, G., Standerholen, F.B., & Alm Kristiansen, A.H. (2018). Comparison of sperm adenosine triphosphate content, motility and fertility of immobilized and conventionally cryopreserved Norwegian Red bull semen. *Theriogenology*, 121(11), 181-187. doi:10.1016/j.theriogenology.2018.08.016.

- [4] Bernecic, N.C., Donnellan, E., O'Callaghan, E., Kupisiewicz, K., O'Meara, C., Weldon, K., Lonergan, P., Kenny, D.A., & Fair S. (2021). Comprehensive functional analysis reveals that acrosome integrity and viability are key variables distinguishing artificial insemination bulls of varying fertility. *Journal of Dairy Science*, 104(10), 11226-11241. doi: 10.3168/jds.2021-20319.
- [5] David, I., Kohnke, P., Lagriffoul, G., Praud, O., Plouarboué, F., Degond, P., & Druart, X. (2015). Mass sperm motility is associated with fertility in sheep. *Animal Reproduction Science*, 161, 75-81. doi: 10.1016/j.anireprosci.2015.08.006
- [6] De Lamirande, E., & Gagnon, C. (1993). Human sperm hyperactivation in whole semen and its association with low superoxide scavenging capacity in seminal plasma. *Fertility and Sterility*, 59, 1291-1295.
- [7] Farrell, P.B., Presicce, G.A., Brockett, C.C., & Foote, R.H. (1998). Quantification of bull sperm characteristics measured by computer assisted sperm analysis (CASA) and the relationship to fertility. *Theriogenology*, 49, 871-879. doi: 10.1016/S0093-691X(98)000.
- [8] Gillan, L., Kroetsch, T., Maxwell, W.M.C., & Evans, G. (2008). Assessment of in vitro sperm characteristics in relation to fertility in dairy bulls. *Animal Reproduction Science*, 103, 201-214. doi: 10.1016/J.ANIRE PROSCI.2006.12.010.
- [9] Gliozzi, T.M., Turri, F., Manes, S., Cassinelli, C., & Pizzi, F. (2017). The combination of kinetic and flow cytometric semen parameters as a tool to predict fertility in cryopreserved bull semen. *Animal*, 11, 1975-1982. doi: 10.1017/S1751731117000684.
- [10] Gopinathan, A., Sivaselvam, S.N., Karthickeyan, S.K., Kulasekar, K., Kirubaharan, J.J., & Venkataramanan, R. (2018). Effect of non-genetic factors on semen quality traits of crossbred holstein friesian bulls (bos taurus x bos indicus) in organized farming conditions at Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences*, 7(11), 3219-3229. doi: 10.20546/ijcmas.2018.711.370.
- [11] Guilherme, C., dos Santos Filho, A., Vieira, J., Fantin, B., Nascimento, P., Guerra, M., Batista, A., & Wischral, A. (2020). Correlation between sperm kinetics and in vitro fertilisation potential of girolando bulls. *Revista Agraria Academica*, 3, 44-53. doi: 10.32406/v3n52020/44-53/agrariacad.
- [12] Harayama, H. (2018). Flagellar hyperactivation of bull and boar spermatozoa. *Reproductive Medicine and Biology*, 17(4), 442-448. doi: 10.1002/rmb2.12227.
- [13] Hering, D.M., Olenski, K., & Kaminski, S. (2014). Genome-wide association study for poor sperm motility in Holstein-Friesian bulls. *Animal Reproduction Science*, 146, 89-97. doi: 10.1016/j.anireprosci.2014.01.012.
- [14] Ibanescu, I., Siuda, M., & Bollwein, H. (2020). Motile sperm subpopulations in bull semen using different clustering approaches – Associations with flow cytometric sperm characteristics and fertility. *Animal Reproduction Science*, 215, article number 106329. doi: 10.1016/j.anireprosci.2020.106329.
- [15] Inan, Ç.M., Çil, B., Tekin, K., Alemdar, H., & Daşkin, A. (2018). The combination of CASA kinetic parameters and fluorescein staining as a fertility tool in cryopreserved bull semen. *Turkish Journal of Veterinary and Animal Sciences*, 42, 452-458. doi: 10.3906/vet-1801-83.
- [16] Islam, M., Apu, A., Hoque, S., Ali, M., & Karmaker, S. (2018). Comparative study on the libido, semen quality and fertility of Brahman cross, Holstein Friesian cross and Red Chittagong breeding bulls. *Bangladesh Journal of Animal Science*, 47(2), 61-67. doi: 10.3329/bjas.v47i2.40236.
- [17] Kathiravan, P., Kalatharan, J., Edwin, M.J., & Veerapandian, C. (2008). Computer automated motion analysis of crossbred bull spermatozoa and its relationship with in vitro fertility in zona-free hamster oocytes. *Animal Reproduction Science*, 104, 9-17. doi: 10.1016/j.anireprosci.2007.01.002.
- [18] Kebede, A. (2018). Review on factors affecting success of artificial insemination. *International Journal of Current Research and Academic Review*, 6(5), 42-49. doi: 10.20546/ijcrar.2018.605.008.
- [19] Khan, M., Sinha, P., & Hazarika, S. (2017). Study on sperm motility and velocity parameters of freshly collected mithun semen through computer-assisted sperm analyzer (CASA). *Indian Journal of Animal Sciences*, 87(3), 293-296.
- [20] Korman, I., Lementovska, V., & Semenda, O. (2022). Marketing research of the milk and dairy products market of Ukraine. *Economy and State*, 4, 62-68. doi: 10.32702/2306-6806.2022.4.62.
- [21] Kyzebnyy, S.V., & Boiko, O.V. (2018). Obtaining, evaluating, storing and using sperm of sires of farm animals. In M.V. Hladii, & Yu.P. Polupan (Eds.), *Breeding, genetic and biotechnological methods of improvement and preservation of the gene pool of farm animals* (pp. 709-720). Poltava: LLC "Techservice Company".
- [22] Li, Y., Kalo, D., Zeron, Y., & Roth, Z. (2016). Progressive motility – a potential predictive parameter for semen fertilisation capacity in bovines. *Zygote*, 24(1), 70-82. doi: 10.1017/S0967199414000720.
- [23] Maiboroda, M.M., Hermanchuk, S.H., Polupan, Yu.P., & Basovs'kyi, D.M. (2019). *Methods of calculation the breeding value of bulls, cows and young animals of the cattle and selecting them by selectoin indices*. Chubynske: Institute of Animal Breeding and Genetics.
- [24] Mortimer, S.T., Swan, M.A., & Mortimer, D. (1998). Effect of seminal plasma on capacitation and hyperactivation in human spermatozoa. *Human Reproduction*, 13, 2139-2146.
- [25] Murphy, E.M., Kelly, A.K., O'Meara, C., Eivers, B., Lonergan, P., & Fair S. (2018). Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. *Journal of Animal Science*, 96(6), 2408-2418. doi: 10.1093/jas/sky130.

- [26] Nagy, A., Polichronopoulos, T., Gaspard, A., Solti, L., & Cseh, S. (2015). Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. *Acta Veterinaria Hungarica*, 63, 370-381. doi: 10.1556/004.2015.035.
- [27] Page, R., & Rosenkrans, Jr.Ch. (2019). Bovine sperm motility as affected by alpha tocopherol and ascorbic acid during storage. *Advances in Reproductive Sciences*, 7, 39-49. doi: 10.4236/arsci.2019.72006.
- [28] Perez-Cerezales, S., Boryshpolets, S., & Eisenbach, M. (2015). Behavioral mechanisms of mammalian sperm guidance. *Asian Journal of Andrology*, 17, 628-632. doi: 10.4103/1008-682X.154308.
- [29] Perumal, P., Srivastava, S.K., Ghosh, S.K., & Baruah, K.K. (2014). Computer-assisted sperm analysis of freezable and nonfreezable Mithun (*Bos frontalis*) semen. *Journal of Animals*, 3, 1-6. doi: 10.1155/2014/675031.
- [30] Singh, A., Kumar, A., & Bisla, A. (2021). Computer-assisted sperm analysis (CASA) in veterinary science: A review. *The Indian Journal of Animal Sciences*, 91, 419-429.
- [31] Singh, R., Kumaresan, A., Mir, M., Kumar, P., Chillar, S., Tripathi U., Rajak S., Nayak, S., & Mohanty, T. (2017). Computer assisted sperm analysis: Relationship between the movement characteristics of buffalo spermatozoa and sire fertility. *Indian Journal of Animal Research*, 51, 660-664. doi: 10.18805/ijar.10768.
- [32] Suarez, S.S., & Pacey, A.A. (2006). Sperm transport in the female reproductive tract. *Human Reproduction Update*, 12, 23-37. doi: 10.1093/HUMUPD/DMI047.
- [33] Suliman, Y., Becker, F., Tuchscherer, A., & Wimmers, K. (2020). Seasonal variations in quantitative and qualitative sperm characteristics in fertile and subfertile stallions. *Archives Animal Breeding*, 63(1), 145-154. doi: 10.5194/aab-63-145-2020.
- [34] Tanga, B.M., Qamar, A.Y., Raza, S., Bang, S., Fang, X., Yoon, K., & Cho, J. (2021). Semen evaluation: Methodological advancements in sperm quality-specific fertility. *Animal Bioscience*, 34(8), 1253-1270. doi: 10.5713/ab.21.0072.
- [35] Yáñez, J.L., Silvestre, M.A., Santolaria, P., & Soler, C. (2018). CASA-Mot in mammals: An update. *Reproduction, Fertility and Development*, 30(6), 799-809. doi: 10.1071/RD17432.
- [36] Zăhan, M., Pall, E., Cenariu, M., Miclea, I., & Dascăl, A. (2018). Relationship between in vitro semen parameters and bull fertility. *ABAH Bioflux*, 10(2), 156-163.

Взаємозв'язок кінетичних параметрів сперми бугаїв-плідників голштинської породи з її запліднювальною здатністю

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Анотація. Рухливість сперми є важливим показником, який характеризує життєздатність та структурну цілісність сперміїв. Цей параметр тісно пов'язаний із запліднювальною здатністю чоловічих статевих клітин, тому його оцінка є невід'ємною частиною аналізу сперми. Метою досліджень є вивчення можливості прогнозування запліднювальної здатності сперми бугаїв-плідників за кінетичними показниками. В ході досліджень використано лабораторні, зоотехнічні та статистичні методи. Виявлено значну мінливість кінетичних параметрів сперми бугаїв-плідників – від 6,2 до 16,1%. Варіація відсотка рухливих сперміїв у еякуляті склала 78,9–89,8; відсотка прогресивних сперміїв – 50,0–74,5; середньої швидкості руху сперміїв (VAP) – 132,6–163,7 мкм/с, прогресивної (VSL) – 99,2–138,2 мкм/с, трекової (VCL) – 223,7–272,3 мкм/с; ступеня прямолінійності руху сперміїв (STR) – 73,9–85,0%, ступеня лінійності (LIN) – 45,1–56,1%, ступеня відхилення (WOB) – 57,2–63,8%. Різниця між мінімальними і максимальними значеннями за усіма дослідженими параметрами є високовірогідною ($P < 0,001$). Встановлено кореляційний взаємозв'язок між різними кінетичними параметрами сперміїв. Швидкості руху сперміїв та їх відносні показники значною мірою обумовлені відсотком прогресивних сперміїв у еякуляті ($r = +0,231-0,761$). Середня, прогресивна та трекова швидкості взаємопов'язані ($r = +0,550-0,887$). Досліджено середню запліднювальну здатність сперми бугаїв-плідників за результатами осіменіння 8594 корів та 992 телиць у чотирьох господарствах Житомирської та Київської областей, варіація склала від 40,7 до 61,4 %. Доведено, що бугаї із вищою та середньою запліднювальною здатністю характеризуються вищим відсотком прогресивних сперміїв у еякулятах, середньою, прогресивною та трековою швидкостями їх руху порівняно з бугаями із низькою запліднювальною здатністю. Цю закономірність підтверджено кореляційним аналізом ($r = +0,538-0,675$). Кінетичні параметри, визначені за допомогою системи CASA, дають можливість прогнозувати запліднювальну здатність сперми та виявляти бугаїв-плідників зі зниженою репродуктивною функцією

Ключові слова: бугай, система CASA, рухливість сперміїв, VAP, VSL, VCL, запліднювальна здатність сперми