

Morpho-biological traits of sexed and non-sexed sperm of Holstein bulls

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Received: 01.06.2021. Accepted: 03.07.2021.

Presented results of the comparative analysis of non-sexed and sexed sperm of the same Holstein bulls after defrosting considering: the concentration of spermatozoa in 1 ml, their motility, survival, the dynamic characteristics of the movement, the acrosome integrity (intactness), the level of microbial contamination. It has been established that the motility and survival of sexed sperm were lower by 15–20% compared with similar traits of non-sexed sperm. The sperm of the bull Vioris Sleeman HOCANM7817774 had the highest activity rate in non-sexed and sexed forms. This demonstrates the possibility of breeding bulls based on this quality trait. The experimental results indicate the necessity to improve the technology of freezing and thawing sexed bull sperm and the relevance of training specialists of breeding centers and farms in Ukraine.

Keywords: Holstein bulls, sexed and traditional semen, spermatozoa motility and survival.

Introduction

The Holstein cattle breed is one of the best-specialized dairy breeds. Holsteins are world leaders in terms of the number of life-long milk yield (100 thousand kg of milk and more), high daily milk yield (100 kg of milk and more), the amount of milk fat and protein for a single lactation and for the period of economic use. Long-term (more than 100 years) unilateral selection in milk yield ensured the formation of the classic exterior type of dairy cows. (Goncharenko, 2002).

Surprisingly, this specialized dairy breed is quite suitable for the production of medium-quality beef due to the size of the animals (live weight of full-aged cows 650–700 kg, bulls – 1000–1100 kg) (Goncharenko, 2002).

However, long-term unilateral selection in levels of milk yield negatively affected several essential indicators of milk quality and especially on the reproductive capacity of the broodstock (67–72% in farms with intensive industrial technology) and a short period of economic use of animals (2–2.5 lactation). The average content of fat and protein in milk (3.4 and 2.8%, respectively) is not enough to ensure the mass production of butter and cheeses. The fertility of females of this breed does not provide for expanded herd reproduction due to their offspring (on average, 70 calves per 100 cows, which is not enough with an annual herd turnover of 30–35%) (Albarrán-Portillo & Pollott, 2012).

It would seem that in such a situation, the use of sexed bull sperm is an ideal solution to many adverse accumulated problems in the breeding of Holstein dairy cattle, especially since the published works have confirmed the expected results of new biotechnology (the fertilizing capacity of sperm at the level of 50–70% of the first insemination and obtaining heifers at 92% of the offspring population). It is strange that biologists and breeders do not recommend the use of sexed sperm to the maximum extent at the first stages but emphasize the expediency and necessity of a comprehensive assessment of the offspring by the reproductive capacity of the broodstock and bulls, the sex ratio (bulls: heifers) in the offspring of cows and heifers in the breeding 'in themselves' of broodstock in F1, F2, F3, obtained using sexed sperm. (Hutchinson et al., 2013; Butler et al., 2014; Murphy et al., 2016; Maicas et al., 2019).

In this aspect, it is necessary to evaluate not only the results of the use of sexed sperm in breeding and commercial herds (taking into account the interaction 'genotype x environment'), but also the theoretical premises formulated by geneticists from different countries of the world (Butler et al., 2014; Maicas et al., 2019).

The stated prerequisites make it possible to decide on the advisability of studying the zoometric parameters of sexed bull sperm following the requirements of scientific research. Therefore, we researched semen from the same Holstein bulls - natural and sexed-supplied in sequins and offered for use in Ukrainian farms.

Materials and Methods

The technological equipment assessed the motility and morphological characteristics of spermatozoa in the cryopreservation laboratory of the LNPC LLC 'Zakhidplemresursi' in the Lviv region of the German company 'Minitub' according to the software package CASA (Computer Assisted Semen Analysis)–Sperm Vision. Defrosted bull semen was examined in 7 fields, with an average of 100 cells per field of view. The computer program provides graphic support of the analysis results with a color image of the trajectories of sperm movement.

The analysis was performed on individual samples, in the field of view, and on individual sperm. The total duration of the analysis was 15–20 seconds. The research was carried out on unsexed and sexed sperm of bulls-producers of the Holstein breed of Canadian selection from LLC 'Simex Alliance Ukraine'.

A study of the semen of 4 breeding bulls of different lines was carried out and investigated 24 sperm doses, of which 12 sperm doses of sex and 12 sperm doses of non-sexed sperm (Table 1).

The sexed bovine semen was subjected to a separation process using high-speed flow cytometry to obtain fractions containing 87–92% of the Y-chromosome sperm. After separation, the semen was cryopreserved in 0.25 ml straws (Goncharenko&Fychak, 2014)

Name Individual. No bull	Line	Number of sperm doses	
		Sexed sperm	Traditional sperm
Benjamin Red CANM 7866444	Bell	3	3
Ardent HOUSAM 137922325	Chif	3	3
Mathys CANM 103439288	Chif	3	3
Vioris Sleeman HOCANM7817774	Valiant	3	3

Table 1. Researches of bulls producers sperm.

Sperm quality was assessed according to the following indicators: the concentration of sperm in 1 ml, their motility after defrosting, the number of sperm with progressively motile, non-progressively motile and non-motile, as well as after incubation at 37 °C after 60, 120, 180 minutes; intact acrosome, the level of microbial contamination (Dunin, 2011). Additionally, the dynamic characteristics of sperm movement were studied. The level of microbial contamination was determined immediately after thawing using standard microbiological methods. The results were statistically processed using Microsoft Excel software.

Results and Discussion

The results of studies of spermatozoa's morphological and biological properties isolated from sexed and traditional (non-sexed) sperm, taking into account mobility, survival, activity, and other generally accepted indicators in zootechnical practice, are shown in Table 2, 3. The digital material gives reason to state the following identified observations. Microbiological analysis of defrosted sexed sperm samples confirmed their sterility in all samples.

Comparison of the integrity of the acrosomes in cryodeconserved spermatozoa with sexed and traditional pies showed that the process of sperm separation did not significantly negatively affect this indicator. Incubation of sperm for three hours led to a slight decrease in germ cells with intact acrosome (Table 2, 3).

Index	Bulls			
	Benjamin CANM7866444	Ardent HOUSAM 137922325	Mathys CANM 103439288	Vioris Sleeman HOCANM 7817774
Concentration (billion. in 1 ml)	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Activity, (%):	49.33 ± 4.71	60.00 ± 5.52	58.00 ± 12.33	74.00 ± 1.22

after defrosting,				
3 hours after defrosting	37.67 ± 2.48	34.00 ± 3.08	30.33 ± 5.89	42.33 ± 2.48
Sperm with intact acrosome, (%):				
after defrosting	88.33 ± 2.04	85.00 ± 3.54	88.33 ± 2.04	88.33 ± 2.04
3 hours after defrosting	81.33 ± 1.63	76.67 ± 5.40	80.67 ± 0.82	76.67 ± 2.04
Microbial contamination, (biol. obj. in 1 ml)	Sterile	Sterile	Sterile	Sterile

Table 2. Biological indicators of thawed sexed sperm of sire bulls.

In the studied samples of thawed sexed sperm, the concentration of spermatozoa was 33–49 million in 1 ml, and their activity (motility) immediately after thawing was in the range of 49–74%, while this indicator in traditional (non-sexed) sperm was higher by 15–33%.

Index	Bulls			
	Benjamin CANM 7866444	Ardent HOUSAM 137922325	Mathys CANM 103439288	Vioris Sleeman HOCANM 7817774
	Concentration (billion. in 1 ml)	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
Activity, (%):				
after defrosting,	49.33 ± 4.71	60.00 ± 5.52	58.00 ± 12.33	74.00 ± 1.22
3 hours after defrosting	37.67 ± 2.48	34.00 ± 3.08	30.33 ± 5.89	42.33 ± 2.48
Sperm with intact acrosome, (%):				
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Microbial contamination, (biol. obj. in 1 ml)	Sterile	Sterile	Sterile	Sterile

Table 3. Analysis of motility and survival of sexed sperm.

The mobility and survival rate of sexed spermatozoa compared to similar indicators of traditional sperm of the same breeding bulls is 15–20% lower, which should be considered in further improving the used technology. Similar results were obtained in the study of sperm motility, which was significantly lower in samples of sexed sperm.

Comparative characteristics of spermatozoa's rectilinear-translational movement in individual bulls indicate that in traditional sperm, they are much more mobile than in sexual sperm. This is especially noticeable 180 minutes after thawing, where spermatozoa with the rectilinear-translational movement of bulls Benjamin CANM7866444, Ardent HOUSAM137922325, Mathys CANM103439288 of traditional sperm were 2–2.5 times higher than those of the same bulls with sexed semen.

The highest activity was exhibited by the sperm of the bull Vioris Sleeman HOCANM7817774. It should be emphasized that this manufacturer's indicators of sperm activity in sexed and unsexed semen were relatively high (74% and 78%, respectively—Fig. 1). This indicates the possibility of selecting bulls for this indicator of semen quality. Therefore, a comprehensive assessment of breeding bulls should be supplemented with their sperm production and fertility (Lebedev, 2000; Pyzhova, 2011).

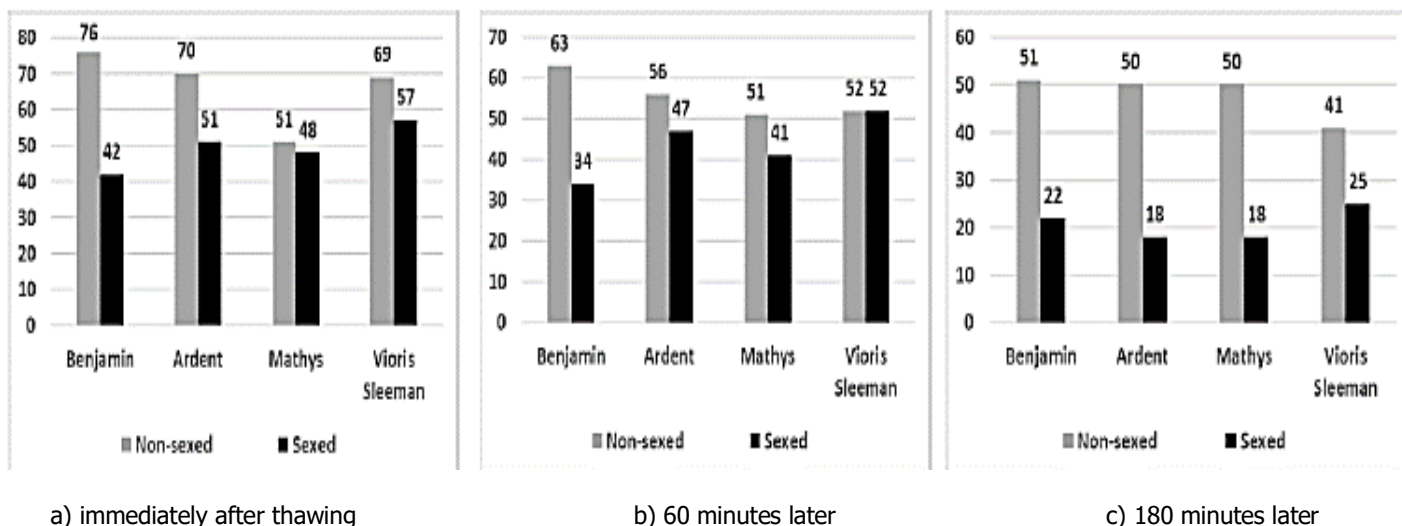


Fig. 1. Comparative characteristics of rectilinear motion of sperm

Sperm quality scores in sexed sperm samples have, on average, minor variation with high negative scores (Fig. 2, 3).

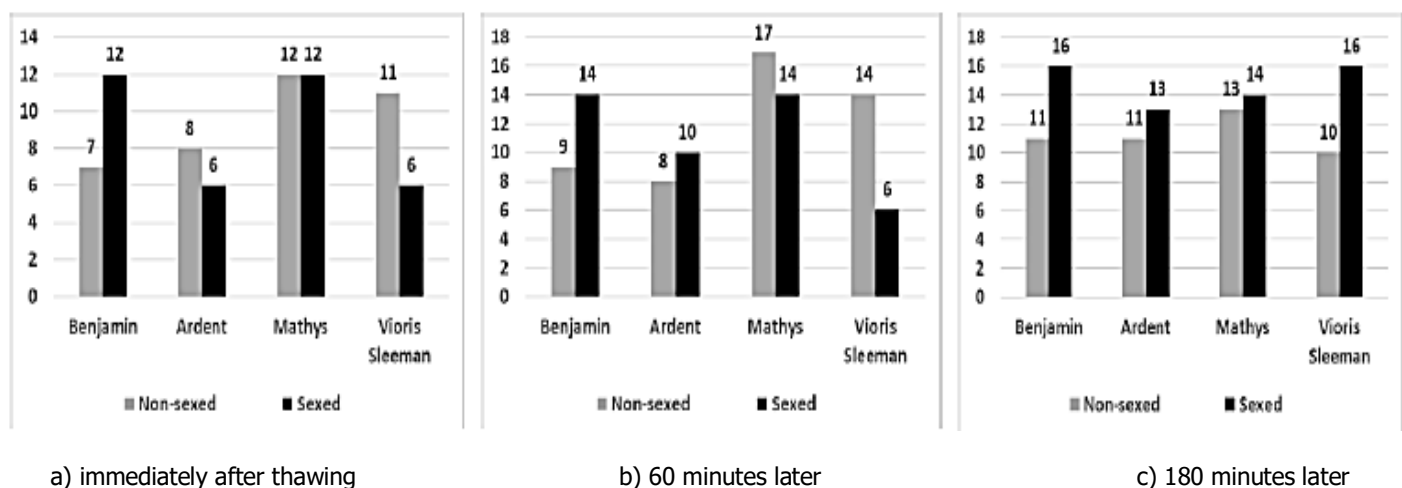


Fig. 2. Comparative characteristics of sperm with manege movement.

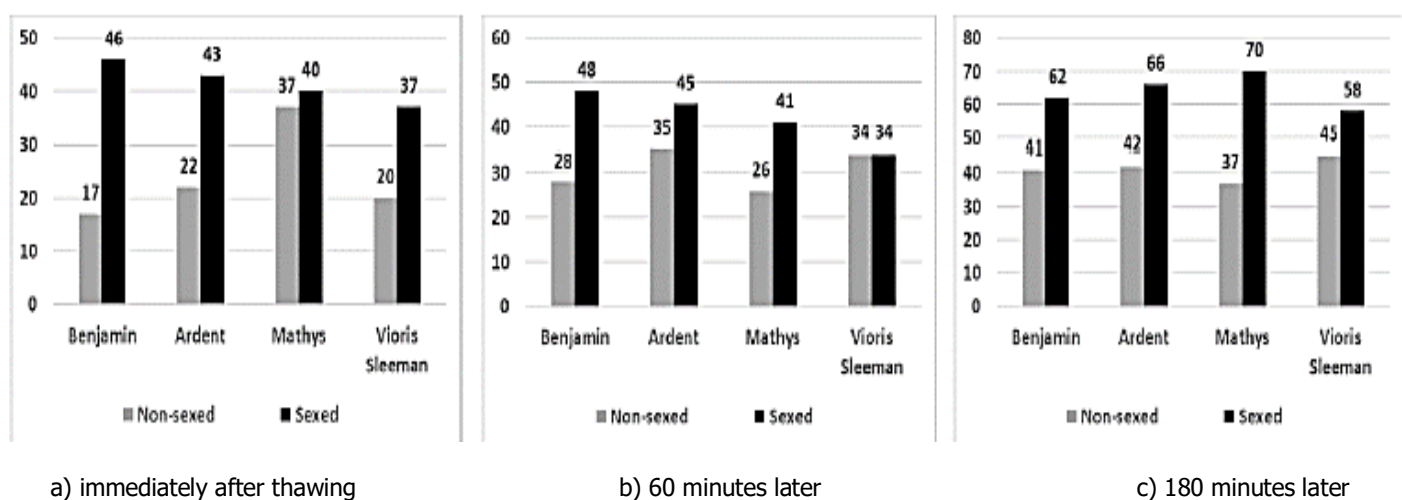


Fig. 3. Comparative characteristics of sperm of individual bulls with immobile sperm.

A similar situation is observed in Fig. 4, 5 regarding the survival rate of cryopreserved sperm. In all studied bulls, the survival rate of cryopreserved spermatozoa is worse in sexed sperm samples.

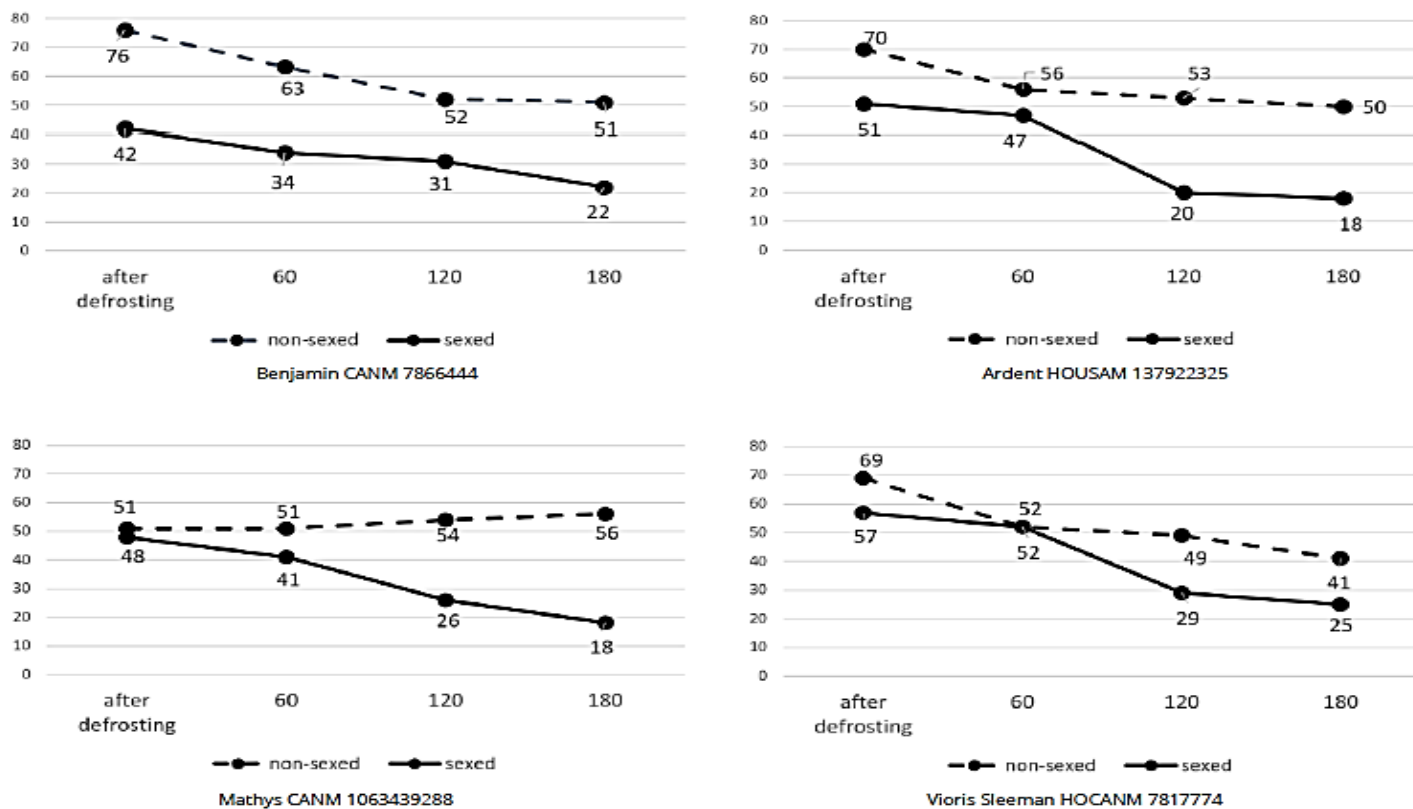


Fig. 4. The survival rate of cryopreserved spermatozoa in non-sexed and sexed sperm of bulls.

One should not be too pessimistic about the experimental results obtained. Similar problems arose at the initial stages of developing and mastering the technology of freezing and thawing of native bull sperm. It is known that these problems have been successfully solved.

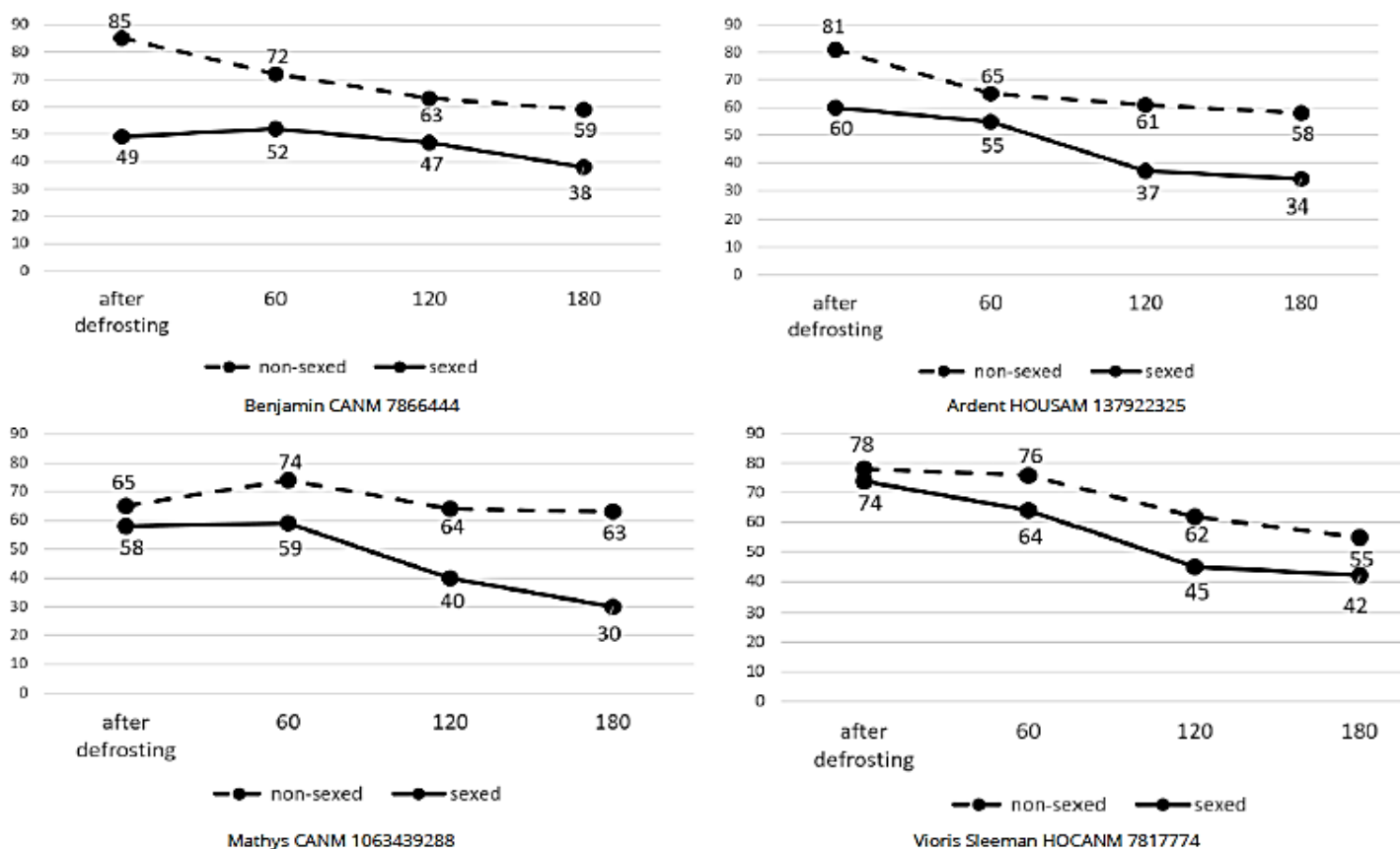


Fig. 5. The activity of cryopreserved spermatozoa in non-sexed and sexed sperm.

Therefore, it is necessary to systematically study the genotype of some generations of offspring obtained with sexed sperm, including analyzing the balance of chromosomes in tissues and the body as a whole. The development and practical application of sexual sperm from bulls-producers is the most considerable achievement of biological science in the breeding and reproducing of farm animals in the XXI century. It will also affect the methods of preserving the gene pools of living organisms, the pace of their evolution, and the intensity of the breeding process, especially with the creation and functioning of paternal and maternal lines, the use of the matroclinous effect, and other aspects.

The technology for separating spermatozoa carrying the X or Y chromosome should be improved considering the experience and results of the development of traditional artificial insemination of farm animals. In connection with the introduction of sexed semen into world practice, assessing the quality of sperm from producers becomes especially relevant. Separation of semen by sex is a very aggressive procedure, and therefore it is necessary to use semen with high quantitative and qualitative indicators.

In subsequent experiments, it is necessary to study the efficiency of insemination of heifers with sexed sperm taking into account the following indicators: fertility (%), the sex ratio in the offspring (%), characteristics by a set of indicators of first-calf cows obtained using sexed sperm in comparison with their mothers.

Conclusion


1. In the studied samples of thawed sexed sperm, the sperm concentration was 33–49 million in 1 ml, and their activity (mobility) immediately after thawing was within 49–74%, while this figure is traditional (not divided by sex) sperm was higher at 15–33%.
2. The mobility and survival of divided by sex sperm compared with similar indicators of traditional sperm of the same breeding bulls are 15–20% lower, which should be considered in further improving the technology used.
3. The highest sperm activity was possessed by bull Vioris Sleeman HOCANM7817774 (78 and 74% respectively), which indicates the possibility of selecting bulls for this indicator of sperm quality. Therefore, a comprehensive assessment of breeding bulls should be supplemented by their sperm production and fertility indicators.

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Citation:

Goncharenko, I., Getya, A., Svyrydenko, N., Pelich, Y. (2021). Morpho-biological traits of sexed and non-sexed sperm of Holstein bulls. *Ukrainian Journal of Ecology*, 11 (3), 322-327.

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