PHENOTYPIC VARIABILITY OF FERTILITY AND MILK TRAITS IN OFFSPRING OBTAINED BY INSEMINATION BY SEXED AND CONVENTIONAL SEMEN OF HOLSTEIN BREED BULLS

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Abstract
The objective of this paper was to compare heifers reproductive traits and characteristics of their calves after artificial insemination performed by conventional (non-sexed) and sexed sperm on 6 commercial farms in Serbia. Conception rate was 55% and 44% for conventional and sexed semen, an average gestation length being 274.6 and 274.9 days, respectively. Average body mass at birth was 37.47 and 36.75 kg for non-sexed and sexed semen, respectively. The rate of stillbirths and twinning was 6.19 and 3.78% for conventional and 7.54 and 1.13% for sexed semen, respectively. The use of conventional semen exerted no statistically significant (P>0.05) effect on female: male calves relationship (51.96 : 48.04) while artificial insemination by sexed semen highly significantly (P<0.01) changed calf sex-ratio (85.10 : 14.90). First-calf heifers originating from sires whose sperm was obtained in conventional way produced 7880 kg milk with 269 kg milk fat and 242 kg protein in standard lactation, while first-calf heifers originating from sires whose sperm was sex-sorted produced 8184 kg milk with 251 kg milk fat and 242.3 kg protein. Type of insemination (conventional and sexed semen) did not significantly affect the studied milk yield traits (P>0.05).

Keywords: sexed semen, conventional semen, heifers, fertility traits, milk traits.

Introduction

The purpose of the application of sexed semen is to obtain descendants of desired sex (Seidel and Garner 2002; Tubman et al. 2004; Healy et al. 2013). Today sexed semen is widely available to milk producers who use it primarily to increase the number of high genetic potential female calves and heifers. Recommendations for commercial application of sexed semen primarily refer to heifers insemination and rarely that of cows because of lower fertility and higher costs expected thereof (Garner and Seidel 2008). Limitations in sexed semen production may happen in different phases and levels of processing. Seidel (2007) points out that the process of semen sexing is both slow and only a small number of semen doses per hour are obtained (7 - 10 doses / hour). The procedure of semen sexing is very invasive one where spermatozoa are being put under a high pressure and speed what can provoke their damage under the action of physical forces. A high pressure in the system is associated with a reduced fertility (Seidel et al. 2003). For this reason it is recommended to use sexed semen primarily in heifers that show distinct signs of oestrus (Foote, 2010). Heifers are the most fertile segment of the herd and are not burdened by production and this fact is of crucial importance in the application of sexed semen. A smaller number of semen doses, costs of operating equipment and maintenance along with indispensable specialised and capable operators who control the instruments result in higher price of this semen (Prakash, 2014). Besides decreased efficiency regarding the number of doses collected per bull compared to conventional sperm, the semen collected from majority of elite progeny tested bulls is often not sex sorted in order that their semen production should not be decreased, while on the other hand, there is a trend to obtain more sexed semen from young genomic tested bulls. It is estimated that the use of sexed sperm could increase the rate of genetic improvement of milk yield in the herd by 4.4% per year compared to the herds in which sexed semen is not used in insemination. If in larger number of inseminations sexed semen from elite tested bulls was used the rate of genetic improvement could gradually increase up to 7% per year. It is proved that the application of sexed semen can increase the intensity of selection in heifers (due to improving genetic trend), influence the decrease of stillborn calves at birth, cause less difficult calvings and have a particular effect on enlarging the herd by quality breeding offspring (Lavaf et al. 2013). The objective of this paper is to analyse the effects of the application of bull sexed sperm on reproductive and productive traits of descendants compared to the application of conventional, non-sexed sperm.

Material and methods

The research included total of 1922 heifers of Black and White breed raised on 6 farms of the Agricultural Corporation Belgrade AD from January 2012 to January 2014. Heifers included by the research were inseminated 3320 times in total out of which 1205 inseminations were conducted by sexed semen while the remaining 2115 doses applied were that of conventional semen. Sexed semen originated from 6 bulls, while conventional semen originated from 20 bulls. The conditions on all farms on which the animals were raised were equalised.

Following available data were used in the analysis: identification number of heifer, dates of all inseminations, date of calving, number of inseminating bull, type of semen used, calf sex, calf body mass at birth and a calf grading mark (1-calf with inborn anomalies; 2-poorly developed and avital calf; 3- moderately developed and vital calf; 4-5- well developed calf, vital and in type).

$Y_{ijklm} = \mu + T_i + F_j + S_k + P_l + O_m + e_{ijklm}$

In which:

Y_{ijklm} – is the phenotypic manifestation of studied trait,

μ-general average of population

T_i-fixed effect of the type of sperm (i=1,2)

F_j-fixed effect of the j farm (j=1...6)

S_k- fixed effect of the k calving season (k=1...4)

P_l- fixed effect of the calf sex (l=1,2)

O_m- fixed effect of the sire (m=1...26)

e_{ijklm}- random error.

Besides the traits mentioned in the analysis the production traits of standard lactation realised by female descendants of the sires whose semen was sexed (74 first-calf heifers) and female descendants of the same age originating from bulls used in conventional insemination (279 first-calf heifers) were included as well. The analysed traits included milk yield, milk fat content, milk fat yield, protein content and protein yield. The effects were studied by means of GLM procedures within SAS statistical package. Model used for production traits had a following form:

$Y_{ijklm} = \mu + T_i + F_j + O_k + e_{ijk}$

In which:

Y_{ijklm} – is the phenotypic manifestation of studied trait,

μ-general average of population

T_i-fixed effect of the type of sperm (i=1,2)

F_j-fixed effect of the j farm (j=1...6)

O_k- fixed effect of the sire (m=1...26)

e_{ijk}- random error.

λ^2 test, within the same SAS programme package (SAS Institute, 2013) was used to study the calf sex ratio in both types of artificial inseminations.

Results and discussion

Table 1 shows the mean values of the studied reproduction traits observed per type of insemination (conventional and sexed sperm). Rate of conception of the heifers inseminated by conventional sperm was 55% in relation to 44% for sexed sperm for average gestation length of 274.6 and 274.9

days, respectively. The mean values of calf birth weight, calf vigor, neonatal death rate and twinning are statistically highly significantly (P<0.01) dependent on the type of insemination.

Table 1. Mean values of reproduction traits according to the type of semen

Trait	Conventional semen (n)	Sexed semen (n)	P-value
Conception rate (%)	55 (2115)	44 (1205)	<0,001
Gestation length (d), mean ± SD	274,57±9,91 (1163)	274,92±9,04 (530)	=0,492
Birth weight (kg), mean ± SD	37,5±2,5 (1163)	36,8±2,0 (530)	<0,001
Calf vigour (score)	4,5 (1091)	4,1 (490)	<0,001
Stillbirth (%)	6.2 (72)	7.5 (40)	<0.001
Twin births (%)	3,8 (44)	1,1 (6)	<0,001

The effect and statistical significance of the type of sperm, farm, and season of insemination, calf sex and inseminating sire on the gestation length and calf birth weight are shown in Table 2.

Table 2. Analysis of variance for gestation length and birth weight

	Gestation length (d)			
Trait/Source	DF	MS	F-value	P-value
Semen type	1	44,021756	0,51	=0,492
Farm	5	523,579663	6,12	<0,001
Season	3	216,410126	2,53	=0,056
Calf sex	2	1,932,813916	22,58	<0,001
Sire	26	121,929896	1,42	=0,080
Error	1654	85,5898		
	Birth weight (kg)			
Semen type	1	184,906300	49,06	<0,001
Farm	5	122,531210	32,51	<0,001
Season	3	19,754216	5,24	=0,001
Calf sex	2	458,104676	121,55	<0,001
Sire	26	9,409893	2,50	<0,001
Error	1654	3,768798		

DF - degree of freedom; MS - mean square

The relation of the sexes in both types of inseminations determined by λ^2 test is shown in Table 3. The use of conventional sperm has not statistically significantly affected (P>0.05) the relation of male and female calves (52.7:47.3%), while artificial insemination by sexed sperm highly significantly (P<0.01) altered the calf sex relation (85.1 : 14.9%).

Table 3. The sex ratio of calves according to type of semen

Relative frequency (%)	Conventional semen (n=1.091)	Sexed semen (n=490)
females (n)	52.7 (575)	85.1 (417)
males (n)	47.3 (516)	14.9 (73)
$\chi 2$-value	3.19	241.50
P-value	=0.596	<0.001

Table 4 shows mean values of the traits of milk yield obtained by first-calf daughters of examined bull-sires as per type of insemination (conventional and sexed semen).

Table 4. Mean values of milk traits according to the type of semen

Trait	Conventional semen (n=279)	Sexed semen (n=73)	P-value
Milk yield (kg), mean ± SD	7880±1401	8184±1355	=0,093
Milk fat content (%), mean ± SD	3,43±0,17	3,43±0,18	=0,868
Milk fat yield (kg), mean ± SD	269±47	279±43	=0,092
Milk protein content (%), mean ± SD	3,08±0,14	3,07±0,15	=0,755
Milk protein yield (kg), mean ± SD	242±43	251±38	=0,111

The effect of studied factors on milk yield traits as well as their statistical significance is shown in Table 5. As it can be seen from Tables 4 and 5 the type of insemination had no statistically significant (P>0.05) influence on milk yield, milk fat content, milk fat yield, protein content and protein yield.

Table 5. Analysis of variance for milk traits

Trait/Source		Milk yield (kg)		
	DF	MS	F-value	P-value
Semen type	1	5985642,68	3,32	=0,07
Farm	5	1816234,98	1,01	=0,41
Sire	19	4173302,68	2,31	<0,01
Error	326	1805423,1		
		Milk fat content (%)		
Semen type	1	0,01594052	0,93	=0,34
Farm	5	0,76454621	44,43	<0,001
Sire	19	0,08838105	5,14	<0,001
Error	326	0,01720981		
		Milk fat yield (kg)		
Semen type	1	5408,80	2,77	=0,097
Farm	5	9158,06	4,69	<0,001
Sire	19	4159,26	2,13	=0,004
Error	326	1954,3821		
		Milk protein content		
Semen type	1	0,012	1,9	=0,30
Farm	5	0,570	52,50	<0,001
Sire	19	0,038	3,5	<0,001
Error	326	0,0109		
		Milk protein yield		
Semen type	1	6713,94	4,16	=0,04
Farm	5	5465,94	3,39	=0,005
Sire	19	3614,03	2,24	=0,002
Error	326	1613,73		

DF - degree of freedom; MS - mean square

Many studies show that conception rate as a consequence of the application of the sexed sperm is highly variable, and that it is lower in relation to conventional sperm. Obtained rate of conception in this paper was in harmony with the rate of conception obtained by the heifers that were artificially inseminated by sexed sperm in other countries and ranged from 39 to 57% (Cerchiaro et al. 2007;

DeJarnette et al. 2009). The determined rate of conception for sexed sperm was higher than that reported by Veigel (2004), Bodmer et al. (2005) and Healy et al. (2013). A low rate of conception realized by sexed sperm has most probably been provoked by damage of spermatozoa during sorting process and by the decreased concentration of spermatozoa in the applied doses (Seidel et al. 1999; Bodmer, 2005; Garner and Seidel 2008). Conception rate for sexed semen in this study was significantly higher than the same rate established for Holstein heifers by Healy et al. (2013) and is in line with majority of published reports in which it amounts even up to 75% (Cerchiaro et al. 2007; DeJarnette et al. 2009; Norman et al. 2010). At the same time, in a number of studies, we can notice that the highest conception rates were recorded in moderate climate conditions to which studied region also belongs. Improved efficacy of the sorting can in the future increase the rate of conception and thus contribute to wider use of sexed sperm in dairy cattle breeding. Gestation length represent the trait whose duration is characteristic for every species of domestic animals and depends on greater number of factors. Significant differences in the gestation length depending on the parents breed, sires in particular, have been confirmed by many authors (O'Ferrall et al. 1990; Cundiff et al. 1986; Gregory et al. 1997). The results obtained for gestation length were in harmony with several studies (DeJarnette et al. 2009; Norman et al. 2010) which report that the gestation length in heifers is statistically sifgnificantly influenced by the season of insemination, inseminating sire, twinning frequency and calf sex. Male sex prolongs the gestation length what is reflected in higher body mass at birth in relation to the female calves. Type of insemination, in the present study, did not statistically significantly (P= 0.492) shorten or prolong gestation length what is in harmony with the research by Tubman et al., 2004 and Healy et al. (2013). Studying the calf birth weight is significant for a number of reasons, among the others, a great calving mass of calves is one of the major causes of difficult calvings, and later causes the death of calves which can occur immediately after the birth. Calf birth weight is influenced by different genetic and non-genetic effects, such as sex, body mass and the age of dam, sire, calving in order, calving season, breed, gestation length, duration of a dry period (Nelsen et al. 1984; Cundiff et al. 1986). The results obtained by Tubman et al. (2004), correspond to the results obtained in the present study meaning that in addition to the sex, season of insemination and inseminating sire the type of artificial insemination also has a statistically significant (P<0.01) effect on calf birth weight. Calf vigor was evaluated for all live born calves by the marks from 1 to 5. The evaluation is great deal subjective, therefore due to such scoring and partly due to differences originating from dams a statistically significant difference (P<0.01) was confirmed between the calves produced by sexed and those produced by non-sexed semen. The results obtained are not in harmony with the results of Tubman et al. (2004), who proved that there is no significant difference in the results for calf vigor depending on the type of insemination and sex. Many farmers throughout the world today face the constant increase in the calf mortality rate, what highly unfavorably affects the economic value of milk production (Meyer et al. 2000; Steinbock et al. 2003; Zadeh et al. 2008). The rates of single stillborn calves in our research produced by heifers inseminated by sexed semen are lower than the rates obtained by Norman et al. (2010). Healy et al. (2013) report that the rate of stillborns is influenced by following factors: sex, twinning, gestation length, AI technician, semen type, the age of dam and the season of insemination. DeJarnette et al. (2009), in their study suggest that increased rate of stillborns produced by heifers inseminated by sexed semen occurs as a consequence of the process of sorting which damages sperm and leads to reduced vigor of foetus and ultimately to stillbirth. The rates of the twinning for Holstein heifers and White and Black heifers in previous studies ranged from 0.76% to 1.3% (Mee, 1991; Zadeh et al. 2008; Norman et al. 2010). The twinning rate in this study was higher than expected one for the heifers of dairy breeds and statistically depended (P<0.01) on the type of semen. The results obtained are consistent with the values reported by Healy et al. (2013), who, contrary to the results obtained in this study, point out that the type of semen had no statistically significant effect (P>0.05) on twinning rate but that it affected the sex of twins. The inheritance of the sex is the consequence of the random pairing of gametes in the proces of insemination and therefore, it is likely to expect an almost equal sex ratio (1:1) in cattle offspring. By the application of sexed sperm, heifers will deliver female calves in about 90% cases instead of 49% what is an average frequency when we use the sperm which has no been sexed (Seidel, 2003; Cerchiaro et al. 2007; DeJarnette et al. 2009). The relation of sexes accomplished in this study which is obtained by sexed sperm was acceptable and similar to that reported by Bodmer et al. (2005) and Healy et al. (2013). However, it was somewhat lower than majority of reports in literature, which reported that by the application of sexed sperm we can obtain about 90% female calves (Cerchiaro et al. 2007; DeJarnette et al. 2009; Norman et al. 2010). The realised relation of sexes can be considered to be the consequence of reduced accuracy in sorting due to increased rapidity (Seidel, 2003). The relation of sexes in the respective research was statistically significantly ($P<0.01$) altered by the application of sexed sperm. The obtained sex ratio for conventional insemination was not in line with a majority of published results stating that about 50 to 52% male calves are being born (Tubman et al. 2004; Zadeh et al. 2008; DeJarnette et al. 2009). However, similarly to our study, Norman et al. (2010) determined 48.5% birth of male calves. Type of insemination, which involves also the inseminating bull, had a significant effect on sex ratio (Norman et al. 2010; Healy et al. 2013). The results obtained in this paper (Table 4) are consistent with majority of studies comparing the daughter's milk yield traits depending on artificial insemination either by sexed or conventional semen. Hinde et al. 2014 determined that in Holstein breed cows milk production was significantly higher in standard lactation after pregnancy which resulted in birth of female calf. These results are based on 2.39 million lactations and about 1,49 million cows. First-calf heifers which brought forth daughters had 142 ± 5.4 kg more milk in 305 days of lactation compared to the first-calf heifers which delivered of male calves (7.612 vs. 7.470 ± 69 kg). The same authors state that the use of sexed semen has a long-lasting effect on milk yield after the birth of the first female calf. A cumulative rise of milk yield in the first two lactations in cows which at first parity brought forth daughters in relation to the cows which in the first two successive calvings gave birth to sons is about 445 kg. Likewise, Zadeh et al. (2010) concluded that milk yields in heifers inseminated either by sexed or conventional semen were similar with a remark that an overall economic gain was higher in heifers obtained by sexed semen in relation to those obtained by conventional semen. The conclusion stated in the mentioned paper is that the use of sexed semen in the first insemination of heifers can decrease the cost per female calf and that this type of insemination can have a positive effect on milk yield and enable more rapid turnover of invested working capital during the first lactation. In a dairy cattle breeding the sexed semen can be used for production of a larger number of daughters from genetically superior cows. The application of sexed semen offers great possibilities to farmers to increase the efficiency of dairy production by obtaining more female calves. It is particularly important in raising certain cattle breeds (Holstein, Jersey) whose male calves are regarded as much less useful (Korora, 2012). In addition the breeders in dairy cattle breeding can use first-class sexed semen for the production of bulls for progeny and genomic testing from the population of elite cows. The costs of progeny testing of descendants of these bulls are drastically reduced due to reduced necessity for a larger number of inseminations in order that a sufficient number of daughters be produced. It is possible to expect that current method for semen sexing will be improved in the future along with a development of a completely new technology and even obtaining bulls that will produce only one kind (X or Y) of spermatozoa what shall demand a very intensive further research (Prakash, 2014).

Conclusions

For cattle breeders the insemination with sexed semen has certain advantages and privileges. It can be said without doubt that sexed semen neither leads to increased abnormalities nor in a negative way affects studied characteristics in calves. The use of sexed semen increases the rate of genetic improvement of dairy traits in comparison with the use of non-sexed semen. In addition, sexed semen makes possible for producers to use only best replacement females in the herd what results in improving the genetic base of breeding stock and therefore in improving the traits of interest in the herd.

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