Original paper

THE EFFECT OF THE USE OF SEXED SEMEN ON REPRODUCTIVE TRAITS AND SEX RATIO OF BLACK AND WHITE CALVES

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Abstract

Sperm sexing is a relatively new biotechnological procedure which offers us the possibility to choose the offspring sex. This method is widely used today in the insemination of the dairy cattle breeds and it is based on the difference in total DNA quantity between X and Y chromosome spermatozoa. In this research paper we have analysed the fertility, sex ratio and characteristics of female Black and White breed calves inseminated by the sexed sperm produced by 6 bulls on 6 commercial farms of the PK Belgrade. Conception rate was 44% with average gestation length of 274.9 days. Average calf birth weight was 36.75 kg. The stillbirths and twinning rates were 7.16 and 1.13%, respectively. Artificial insemination by sexed sperm assessed by χ^2 test highly significantly ($P<0.01$) alters the sex ratio between female and male calves (85:15%). By means of GL model we have assessed the effects of farm, insemination season, sex of the calves and inseminating bull on gestation length and calf birth weight. All mentioned factors have manifested statistical significance ($P<0.01$) on studied traits, except for the inseminating bull trait which did not statistically significantly affect ($P>0.05$) gestation length. Regardless the low conception rate, the application of sexed sperm in dairy cattle breeding is very significant since it enables the breeders to use the best replacement female animals what may result in the improvement of genetic basis of breeding stock.

Key words: *Black and White breed, fertility traits, sex relationship, sexed sperm*

Introduction

Artificial insemination of dairy cattle by sexed semen facilitates faster genetic improvement. By the application of sexed semen we can, in the first place, produce a greater number of descendants of desired sex in shorter time period in relation to a conventional artificial insemination (Seidel, 2002; De Vries et al., 2008). So far a several methods for separating X and Y spermatozoa have been developed but most often used method recently has been the method of flow cytometry which provides a satisfying degree of separating and is based on difference in total DNA quantity between spermatozoa with X and Y chromosomes.

The research shows that the fertility with sexed sperm is lower in relation to the fertility obtained by use of conventional non-sexed sperm (Seidel, 1999; Tubman et al., 2004; Norman et al., 2010; DeJarnette et al., 2011; Healy et al., 2013). Thus, Seidel et al. (2002) reported that in Holstein heifers the pregnancy rate ranged from 21 to 35% for sexed sperm compared with 58% for conventional. Lower fertility is caused by the damages occurring on spermatozoa during sorting of sperm. The process with spermatozoa during sorting is very invasive; they are placed under high pressure and great speeds and abruptly halted thereupon, causing the damage of the spermatozoa by such physical forces. High pressure within the system has been associated with decreased fertility (Seidel et al., 2003). For this reason it is recommended that sexed sperm is to be used primarily in heifers that show distinct signs of estrus (Foote, 2010). The heifers are the most fertile part of the herd and they are not burdened by the production, and that fact is of crucial importance in the use of sexed sperm.

There was no increase in calf abnormalities that have been reported to date with sexed sperm relative to controls. There is a possibility that early embryonic mortality may be increased slightly with sexed sperm (Seidel et al., 1999; Tubman et al., 2004; Healy et al., 2013).

The aim of this paper was to determine the conception rate, gestation length, calf birth weight, sex ratio, calf vigor, neonatal death rate and calf twinning rate after insemination of Black and White breed heifers by sexed semen.

Materials and methods

Study Design, Animals, and Housing

Data for the analysis was collected, from January 2012 until January 2014, from the total of 1205 inseminations in 530 heifers of Black and White breed raised on 6 commercial farms of the Agricultural Corporation of Belgrade. The conditions on all farms were uniformed and there were minimal differences between the heifers regarding the feeding and care conditions.

The heifers were grouped according to height and weight, and fed mixed rations to meet all or part of their nutritional requirements according to pasture availability. All of the heifers enrolled in the breeding program were grouped together and, as such, minimal differences exist between these heifers.

Reproductive Management

Artificial insemination was performed once daily, on the same morning as estrus detection. Insemination was carried out by multiple AI technicians using sexed semen straws collected from 6 dairy sires, provided by CRI (Wisconsin, USA) and sorted by flow cytometry. Sperm viability and semen quality were not evaluated before insemination. In most cases, sex-sorted straws were used only for the first or second breeding and heifers returning to estrus were inseminated with conventional semen for subsequent breedings.

Data and Statistical Analysis

Data available for analysis included: heifers identity, date of insemination, date of successful conception, date of calving (birth), identity of the calf sire, type of sperm (sexed or non-sexed), calf sex, calf birth weight, calf vigor (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), incidence of twinning and stillbirths. Gestation length was calculated as the difference of the date of successful conception and the date of calving. All stillborn calves were recorded, but their sex was not determined. During the trial no anatomic anomaly was perceived in calves. Both fertility traits and characteristics for total of 530 calves obtained by the application of sexed semen were analyzed. After the records obtained on different farms have been completed and edited the establishing of average values, standard deviation and frequency of studied traits took place.

By means of GLM procedures of the Statistical Analysis System (SAS Institute, 2013), the assessment of the effect of farm, insemination season, calf sex and inseminating sires on gestation length and calf birth weight was carried out.

The following GL model was used:

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

In which:

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

μ-general average of population

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 l sire (m=1...6)

e_{jklm}- random error.

χ^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 disscusion

Table 1 shows the mean values for the conception rate, gestation length, calf birth weight, calf vigor, neonatal death rate and calf twinning rate obtained after the insemination of heifers by sexed semen.

Table 1. *Average values of studied traits*

Traits	n=530
Conception,%	44
Gestation length, days Mean ±SD	274,92±9,04
Birth weight, kg Mean ±SD	36,75±2,0
Vitality of calves[a]	4,12
Stillbirth, %	7,16
Twinning, %	1,13

[a]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

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

Table 2. *Analysis of variance for gestation length*

Source	DF	Mean square	*F*-value	Significance
Farm	5	611.241966	8.54	<.0001***
Season	3	253.087765	3.53	0.0147*
Calf sex	2	615.512068	8.60	<.0001***
Sire	5	72.581875	1.01	0.4304 ^{NS}
Error	510	71.59860		

DF- Degrees of Freedom; MS- mean square; F-value; P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

From the results displayed in the table we can observe that the farm and calf sex have a very high significant effect (P<0.001) on gestation length whereas inseminating sire does not statistically significantly affect mentioned trait. Similarly to a previous table, Table 3 displays the significance of studied factors on calf birth weight.

Table 3. Analysis of variance for birth weight

Source	DF	Mean square	*F*-value	Significance
Farm	5	35.6066081	14.34	<.0001***
Season	3	8.8772715	3.57	0.0140*
Calf sex	2	151.4725914	60.99	<.0001***
Sire	5	12.2940260	4.95	<.0001***
Error	510	2.483482		

DF- Degrees of Freedom; MS- mean square; F-value; P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

The farm, calf sex and inseminating sire have a very highly significant (P<0.001) effect on birth weight. The season significantly (P<0.01) affected a given trait.

Table 4. Mean values of gestation length and calf birth weight observed per sex

Sex	Traits	
	Gestation length, days	Birth weight, kg
Female	274,73	36,61
n	425	425
Male	276,23	37,74
n	75	75
P	<.0001***	<.0001***

P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

Table 4 shows mean values of gestation length and calf birth weight observed per sex. The calf sex statistically very highly significantly ($P<0.001$) affects the observed traits.

The sex ratio and statistical significance determined by χ^2 test are shown in Table 5. Artificial insemination by sexed semen very highly significantly ($P<0.001$) alters the calf sex ratio (85:15).

Table 5. *The sex ratio of calves and χ^2 value*

	Sex ratio		χ^2	P
Sexed semen, n	Female, %	Male, %		
	85	15	245,00	<0.001***
	500	500		

χ^2-value; P- probability; NS non-significant, * $P<0.05$, **$P<0.01$; ***$P<0.001$

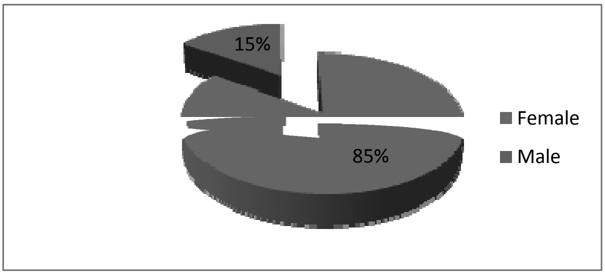

Figure 1. The sex ratio of calves

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 Weigel (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 ($P= 0.4734$) 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 use of sexed semen (Table 5) increases significantly the frequency of the pairs of female twins at the expense of the male ones (Tubman et al., 2004; Norman et al, 2010; Healy et al., 2013).

The inheritance of the sex is the consequence of the random pairing of gamets 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).

Conclusion

The results obtained in this study are consistent with a majority of studies which compared the fertility traits, sex ratio and calf characteristics realised by artificial insemination with sexed semen. It can be said that the application of sexed sperm does not lead to the increase in the abnormalities and that it does not affect in a negative way the studied calf characteristics. Sexed sperm enables the breeders to use only the best replacement heifers in the herd what will result in improvement of the genetic base of breeding stock. Sexed sperm should be used only for the insemination of sound heifers on the farms with good organisation of reproduction because otherwise the fertility can additionaly decrease. Regardless all limitations it has, the use of sexed sperm is very significant because in this way we can obtain the animals of desired sex and superior hereditary traits.

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References

1. Bodmer M, Janett F, Hässig M, Daas Nd, Reichert P and Thun R 2005. Fertility in heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. Theriogenology 64,1647–1655.

2. Cerchiaro I, Cassandro M, Dal Zotto R, Carnier P and Gallo L 2007. A field study on fertility and purity of sex-sorted cattle sperm. J. Dairy Sci. 90, 2538–2542.

3. Cundiff LV and MacNeil MD 1986. Between and within-breed genetic analysis of calving traits and survival to weaning in beef cattle. J. of Animal Sci. 63, 1, 27-33.

4. De Vries A, Overton M, Fetrow J, Leslie K, Eicker S and Rogers G 2008. Exploring the impact of sexed semen on the structure of the dairy industry. J. Dairy Sci. 91, 847–856.

5. DeJarnette JM, Nebel RL and Marshall CE 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology 71, 49–58.

6. DeJarnette JM, Leach MA, Nebel RL, Marshall CE, McCleary CR and Moreno JF 2011. Effects of sex-sorting and sperm dosage on conception rates of Holstein heifers: Is comparable fertility of sex-sorted and conventional semen plausible J. Dairy Sci. 94 ,3477–3483.

7. Foote RH 2010. The history of artificial insemination: Selected notes and notables. J. Anim. Sci, 80, 1-10.

8. Garner DL and Seidel GE 2008. History of commercializing sexed semen for cattle. Theriogenology 69, 886–895.

9. Gregory KE, Bennet GL, Van Vleck LD, Echternkamp S and Cundiff LV 1997. Genetic and anviromental parameters for ovulatin rate, twining rate, and weigth in a cattle population for twining. J. Anim Sci. 75, 1213-1222.

10. Healy AA, House JK and Thomson PC 2013. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. J. Dairy Sci. 96, 1905-14.

11. Mee JF 1991. Factors affecting the spontaneous twining rate and the effect of twinning on calving problems in nine Irish dairy herds. Ir.Vet J. 44, 14-20.

12. Meyer CL, Berger PJ and Koehler KJ 2000. Interactions among factors affecting stillbirths in Holstein Cattle in the United States. J. Dairy Sci. 83, 2657–2663.

13. Nelsen TC, Short RE, Urick JJ and Reynolds WL 1984. Genetic variance components of birth weight in a herd of unselected cattle. J. Anim. Sci. 59, 1459-1466.

14. Norman HD, Hutchison JL and Miller RH 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J. Dairy Sci. 93, 3880–3890.

15. O′Ferrall GJM and Ryan M. 1990. Beef crossing: effects of genotype of the foetus on the performance of dairy cows. Irish Journal of Agricultural Reserch. 29, 101-108.

16. SAS 2013. SAS Version 9.1.3, SAS Institute Inc. Cary, NC, USA.

17. Seidel GE, Schenk JL, Herickhoff LA, Doyle SP, Brink Z and Green RD 1999. Insemination of heifers with sexed sperm. Theriogenology 52, 1407–1420.

18. Seidel GE 2007. Overview of sexing sperm. Theriogenology 68, 443–446.

19. Seidel GE 2003. Sexing mammalian sperm—Intertwining of commerce, technology, and biology. Anim. Reprod. Sci. 79, 145–156.

20. Seidel GE Jr 2002. Sexing sperm for beef and dairy cattle breeding. Pages 281–286 in Factors Affecting Calf Crop: Biotechnology of Reproduction. M. J. Fields, R. S. Sand, and J. V. Yelich, ed. CRC Press, Boca Raton, FL.
21. Seidel GE Jr. and Garner DL 2002. Current status of sexing mammalian spermatozoa. Reproduction 124, 733–743.
22. Steinbock LA, Näsholm AB, Berglund K, Johansson K. and Philipsson J. 2003. Genetic Effects on Stillbirth and Calving Difficulty in Swedish Holsteins at First and Second Calving. J. Dairy Sci. 86, 2228-2235.
23. Tubman LM, Brink Z, Suh TK and Seidel GE 2004. Characteristics of calves produced with sperm sexed by flow cytometry cell sorting. J. Anim Sci. 82, 1029-36.
24. Weigel KA 2004. Exploring the role of sexed semen in dairy production systems. J. Dairy Sci. 87(Suppl. 13), 120–130.
25. Zadeh GH, Javaremi AN, Miraei-Ashtiani SR and Kohram H 2008. An Observational Analysis of Twin Births, Calf Stillbirth, Calf Sex Ratio, and Abortion in Iranian Holsteins. J. Dairy Sci. 91,4198-4205.