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A field study on the reproductive efficiency of sex-sorted semen in Holstein heifers

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Abstract Sex-sorted semen can increase the profitability of dairy cattle by producing offspring of the desired sex. The objective of the present study was to compare conception rate, stillbirth, calving difficulty and sex ratios from sexed and conventional semen in a commercial dairy herd in Isfahan province, Iran. Data were collected from April 2009 until December 2015 on 13,343 inseminations in 8,011 nulliparous Holstein heifers. Based on the proportion of female calves, purity of the sexed sperm was 86.45%, indicating that sexed semen technology was effective in producing heifers. However, sex ratio at birth from conventional semen was 49.05% female. Empirical conception rate in heifers was 42.65% for sexed and 54.85% for conventional semen ($P < 0.05$). Based on the obtained results, approximately 77.75% of conception rates in heifers were compromised by the sperm sorting procedure. No significant difference was observed for stillbirth due to sexed *vs.* conventional semen ($P > 0.05$). The results showed that the higher percentage of female calves in the sexed semen heifers was accompanied by lower risk of dystocia. Despite the higher cost and reduced conception rate, sexed semen can be recommended for insemination of heifers as its use is associated with lower rate of dystocia and birth of more heifer calves.

Keywords: pregnancy rate, stillbirth, calving difficulty, sex ratio, sexed semen

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Introduction

Reproductive technologies such as artificial insemination (AI), multiple ovulation and embryo transfer (MOET) and semen sexing are effective tools in achieving greater genetic improvement in dairy cattle relative to the conventional natural mating systems. The main goal in the use of sexed semen is to produce calves of a specific sex. In mammalian species, males produce semen in which 50% of the sperm carry the X-chromosome and 50% carry the Y-chromosome. For sexed semen production, the proportion of X-bearing (female) and Y-bearing (male) sperm have been modified from the natural mix through sorting with 85 to 95% accuracy (De Vries et al., 2008; Garner and Seidel, 2008). Sperm sorting is based on flow cytometry of DNA content because bovine X-chromosome bearing sperm have approximately 4% more DNA than bovine Y-chromosome bearing sperm (Seidel, 2014). Commercial dairy farmers who produce and market milk may use sexed semen to produce replacement heifers from genetically superior cows. In beef cattle production, meat and weight gain are the main traits and therefore male-sexed semen are often preferred (Dominguez et al., 2011). Norman et al. (2010) reported that the availability of sexed semen is about 37% for active Holstein bulls. Active AI bulls with marketed sexed semen are superior for their yield traits, productive life, somatic cell score, daughter pregnancy rate, service-sire calving ease, service-sire stillbirth, final score, sire conception rate, and lifetime net merit (Norman et al., 2010). Some bovine field studies (Norman et al., 2010; DeJarnette et al., 2009) have reported an approximate 90% production of female offspring achieved by separating X-chromosome and Y-chromosome bearing sperm. In addition to achieving a desirable sex ratio, reduced dystocia risk is another advantage of using sexed semen because on the average, female calves are 2.5 kg lighter at birth than the male calves (Tubman et al., 2004). Norman et al. (2010) also reported that dystocia was decreased by 28% for heifers and 64% for cows through the use of female-designated sexed semen in US Holstein breed.

The technology of semen cell sorting is continuously being improved but the viability and quality of sperm

during sorting procedure will be reduced (De Vries et al., 2008; Seidel Jr., 2014). Side effects with sex-sorted semen results in compromised conception rates and concerns related to the incidence of stillbirths (Schenk et al., 2009; Seidel Jr., 2014). Several studies have reported that conception rate due to sexed semen was approximately 70% to 90% of that achieved with conventional breeding (Schenk et al., 2009; Dominguez et al., 2011). Based on the mean conception rates, Norman et al. (2010) reported that 2.6 sexed semen services were needed for a heifer pregnancy but only 1.8 services were required with conventional semen. DeJarnette et al. (2009) found no significant difference in heifer stillbirth rate between conventional and sexed semen methods after adjustment was made for calving age. Profitability of sexed semen was reported based on the population structure of Iranian Holstein under a simulation-based study (Hossein-Zadeh et al., 2010). Currently, limited on-farm data are available about sexed semen application, and little effort has been made to evaluate the performance of sex-sorted semen in dairy cows in Iran. Therefore, the objective of this study was to compare the performance of conventional and sexed semen in terms of sex ratio, conception rate, stillbirth and calving difficulty in a commercial Holstein herd in Iran.

Materials and methods

Description of data and farm

On-farm records were collected on the reproductive traits from the FKA commercial dairy herd. The farm is located in Isfahan province, Iran. The location has a hot and dry climate with a mean annual temperature of 16.5°C (±1.4) and total annual precipitation of 123 mm. To date, the herd has been under official performance recording and pedigree registration by the National Animal Breeding Center and Promotion of Animal products. Based on the latest data, total herd size of FKA farm was about 4000 lactating cows with an annual milk production of more than 60000 tons. Lactating cows were milked 3 times per day in Herringbone milking parlors, and the average daily milk production was 42±2 kg per cow. About 99% of cows were artificially inseminated, and about 1% were subjected to embryo transfer from superior cows. More than 90% of semen was from the US and Canadian proven sires. All cows were raised under intensive production systems within free stall barns. Cows were fed 3 times per day with a balanced total mixed ration that consisted of corn silage, alfalfa hay, dehydrated beet pulp, barley grain, ground corn grain, soybean meal, canola meal, cotton seed, cotton seed meal, corn gluten meal, extruded soybean, fish meal, rumen-protected fat powder, sodium bicarbonate, salt, macro- and micro-minerals, vitamin supplements, and feed additives.

Description of traits

Reproductive data were extracted from the farm database from April 2009 to December 2015 on 13,343 inseminations of 8,011 nulliparous Holstein heifers. The studied traits were included the sex ratio, conception rate, stillbirth and calving difficulty. Sex ratio is used as a criterion for accuracy of sperm sorting and purity of semen. Sex ratio was defined as the number of heifer calves to total calves born in the population. Conception rate, as a measure fertility at service, was calculated by dividing the number of pregnant heifers by the total number of heifers inseminated. Pregnancy diagnosis was performed between day 26 and 35 after AI using ultrasonography. Stillbirth parturition was defined as calves that were either born dead and or died within 48 h after calving. Stillbirth is a binary trait which was recorded as 0 for alive and 1 for dead calves. Dystocia (calving difficulty) was defined as a difficult birth resulting in prolonged calving or severe assisted extraction of the calf at birth. The degree of assistance (force) that is provided during assisted parturition determines the degree of dystocia. Calving difficulty events were converted and categorized to 1 to 5 scale, including 1 = no assistance, 2 = assistance by one person without the use of mechanical traction, 3 = assistance by two or more persons, 4 = assistance with mechanical traction and 5 = surgical procedure.

Statistical analysis

Quality control and data validation were conducted by Microsoft Excel 2016. The GLM procedure (SAS Institute, 2015) was used with the following linear model to determine the impact of semen type (sexed vs conventional) on traits:

$$y_{ij} = \mu + Semen_i + YS_j + e_{ij}$$

where, y_{ij} is the record on stillbirth (defined as a binary variable as 1 for alive and 0 for dead) and dystocia (defined as a categorical trait ranged from1 to 5 score) affected by i^{th} type of semen ($Semen_i$), fixed effects of year-season of calving (YS_j) and the random effect of residual error (e_{ij}).

The $\chi 2$ test for a contingency table was performed to investigate the associations between semen type (conventional and sexed) and incidence of stillbirths and dystocia.

Results

Primary analysis showed that 911 cases of the total 8011 calving events in the herd (i.e., 11.37% of all births), were based on sexed semen. In addition, sexed semen was primarily used at first and second services.

The proportions of heifer calves and bull calves at birth resulting from insemination of sexed semen were 86.45% and 13.55%, respectively (Table 2). Sex was not recorded for 599 calves which died at birth. The purity of sexed semen as a criterion of accuracy of sperm sorting was 86.45%. The Arithmetic means of conception rate by semen type are shown in Table 2. Significant difference was observed for conception rates between sexed and conventional semen ($P < 0.05$). The overall mean of conception rate was 42.65% for conventional semen and 54.85% for sexed semen. On the other words, relative to the conventional semen, the conception rate of the sexed semen was only 77.75%. Based on the mean overall conception rates, 2.34 sexed semen services were needed for a heifer pregnancy but only 1.82 services for conventional semen ($P<0.05$). The stillbirth rates for heifers inseminated with sexed and conventional semen (Table 3) were 7.68% and 7.18%, respectively ($P>0.05$). Dystocia was more prevalent (Table 3) for conventional semen (17.07%) than for sexed semen (15.92%).

Discussion

Practical application of sexed semen by commercial dairy herds depends on several factors, but fertility and accuracy of sexed semen (i.e., purity) are probably the most important criteria for evaluating the reliability of the procedure (Cerchiaro et al., 2007; Borchersen and Peacoc, 2009).

Sex ratio

The purpose of sexing sperm is to produce more offspring of the desired sex. Majority of trials have used X-chromosome sorted sperm to produce more heifers. Results of the sexed sperm purity obtained in the present study were in agreement with the literature (Borchersen and Peacock, 2009; Seidel Jr., 2014) and provide a field evidence that semen sorting is a reliable method for sex pre-selection in cattle. Tubman et al. (2004) reported that the sexing accuracy of X-chromosome sorted sperm was 87.8% for female calves. An increase of 37.4% in female calves was observed using sexed semen in in comparison to the conventional semen. This will enable farmers to increase the number of available replacement heifers in the herd. The sex ratio for unsorted semen was 49.05% for female calves, implying that 1:1 male to female sex ratio was naturally observed in the progenies. The availability of genetically desirable replacement heifers significantly influences the profitability of a dairy enterprise (Healy et al., 2013). Chebel et al. (2010) proposed that the application of sex-sorted semen for first insemination of the virgin heifers reduced the cost per female calf produced and increased the economic return during the first lactation. Increasing the proportion of heifer calves through the sexed semen method might be used to accelerate the genetic gain in dairy herds by selecting only the highest-ranking cows to breed as replacements.

Conception Rate

The process of sorting sperm cells reduces the concep-

Table 1. Characteristics of sexed and conventional semen

	Conventional Semen	Sexed Semen
No. of sperm per straw	10 Million	2 Million
Proportion of female sperm	50%	~ 90%
Semen wastage during processing	Minimal	> 50%

Table 2. Conception rate and sex ratio for sexed and conventional semen in Holstein heifers

Semen type	Calf sex	No. of calves born	Conception rate (%)	Sex ratio* (%)
Sexed	Female	727	42.65	86.45
	Male	114		
Conventional	Female	3223	54.85	49.05
	Male	3348		

*Sex ratio was defined as the number of female calves to total calves born.

Table 3. Empirical incidence (%) of stillbirths and dystocia in heifers for sexed and conventional semen

Semen type	Calving difficulty score (% heifers)					Dystocia (%)	Stillbirth (%)
	1	2	3	4	5		
Sex-sorted	84.08	14.49	1.21	0.22	0.00	15.92	7.68
Conventional	82.93	15.48	1.46	0.08	0.04	17.07	7.18

tion rate. Numerous field studies have been conducted for addressing the concerns associated with the potential damage of sexed bovine semen during flow cytometry/cell sorting (De Vries et al., 2008; Schenk et al., 2009; Seidel Jr., 2014). The difference between conception rates of the heifers inseminated with sexed and conventional semen (12.2%) was less than the pregnancy rate difference (>16 percentage units) in Holstein cows (De Jarnette et al., 2011). The mean conception rate reported by Norman et al. (2010) for heifers was 56% under conventional semen and 39% for sexed semen; the corresponding conception rates for cows were 30 and 25%, respectively. DeJarnette et al. (2009) reported that the conception rates from sexed sperm (2.1×10^6 sperm/dose) were 85±3% of those obtained at the first service using conventional semen (20×10^6 sperm/dose) in the US Holsteins. In general, the conception rate in field trials involving virgin heifers have typically ranged from 35 to 40% with sexed semen compared to 55 to 60% for conventional semen, and this limits the willingness of dairy farmers to pay a premium price for the product (Weigel, 2004). Schenk et al. (2009) reported that sexed semen reduced the pregnancy rates for cows by >12 percentage. Based on Danish A.I. field data, Borchersen and Peacock (2009) found that the conception rate using sorted semen was 12% lower than that of conventional doses for Holsteins. In nulliparous Australian Holsteins, Healy et al. (2013) reported empirical conception rates of 31.6% and 39.6% for sexed and conventional semen, respectively, both being lower than the results achieved in the present study. Cerchiaro et al. (2007) found significant difference among bulls in fertility of their sexed semen. Therefore, to avoid declines in fertility, careful selection of the candidate sires used for sorted semen production will be necessary. Because of financial considerations, Norman et al. (2010) proposed sexed semen could be used primarily for heifer breeding because only 2.6 sexed semen services are needed per pregnancy for heifers compared with 4.0 services for cows. The number of required services for a heifer pregnancy by applying sexed semen were significantly higher that of obtained for heifers under conventional semen (2.34 vs. 1.82), implying that the process of sorting sperm cells may reduce the conception rate. The decrease in conception rates was in agreement with the literature (DeJarnette et al., 2011; Norman et al., 2010) and implies that sexed semen should be reserved for the first and second services because increased service number results in low cost-to-benefit ratios.

Stillbirth

Calving process and the postpartum period are the most stressful parts of a cow's life cycle. Thus, post-natal mortality of calves is not only costly for cattle producers but also a problem for animal welfare and health (Meyer et al., 2001). Field trials with sexed semen have shown the unpredictable increases in stillbirths because of the stress of semen sorting. Stillbirth rate in our study was slightly lower than that of Norman et al. (2010) who reported 10.4%, and 11.3% stillbirth under the conventional and sexed semen usage, respectively. DeJarnette et al. (2009) reported that mean incidence of stillbirths in Holstein heifers carrying female calves was 9.2% for sexed semen and 10.5% for conventional semen. Meyer et al. (2001) reported that the incidence of stillbirth increased from 9.5 to 13.2% in primiparous, and 5.0 to 6.6% in multiparous cows from 1985 to 1996. Calving difficulty has been implicated as the major cause of stillbirth, and yet, approximately 50% of stillborn calves are from unassisted births (Philipsson, 1996). The calves requiring assistance due to dystocia have higher rates of death. As suggested by DeJarnette et al. (2009), the slight increase in stillbirth from sexed semen compared with conventional semen might have been related to the current state of sex-sorting technology and efficiency. However, it would be logical to expect a significant concurrent increase in stillbirth which was not observed.

Dystocia

Few studies have examined the differences in dystocia and stillbirth incidence in dairy cows when female and male calves are produced from conventional and sexed semen. Application of X-chromosome sorted semen would be expected to decrease dystocia in heifers as there is an increased incidence of dystocia in dams giving birth to male calves (Norman et al., 2010). Using X-chromosome bearing sperm to inseminate virgin dairy heifers substantially decreases the incidence of dystocia because female calves are smaller in size than males. Because sexed semen method greatly increased the percentage of female calves, it reduced the percentage of dystocia for heifers. Higher frequency of female calves obtained from sexed semen and consequently smaller calf sizes are expected to reduce the incidence of dystocia, in particular for nulliparous heifers (Weigel 2004; Seidel, 2014). In the present study, statistically significant differences in calving difficulty were observed between the sexed and non-sexed semen. These findings are in agreement with the work of Norman et al. (2010). However, the present study contradicts Tubman et al. (2004) who reported no identifiable effect of semen type on the calving difficulty. Meyer et al. (2001) reported that the average incidence of dystocia in the first-parity

of US Holsteins was more than 28%, when dystocia was defined as any birth other than those with "no assistance". Therefore, it appears that the application of sexed semen method might reduce dystocia-associated costs in first-calving heifers.

Conclusions

The present study indicated that sexing sperm by flow cytometry can be approximately >85% accurate and sex-sorted semen allows farmers to significantly skew the sex ratio in offspring. No difference between stillbirth from sexed and conventional sperm was detected. These results imply that, despite concerns about the potential damage incurred on sperm DNA during sorting, stillbirth is not affected by the sperm sorting process. Conception rates achieved with sexed semen was 77.75% of the conventional semen that can be explained by the mechanical damage to the sperm during the process of sorting. Calving ease was more frequent for the sexed semen, probably as a consequence of smaller calf size and birth weight of female calves. Sexed semen is recommended for use in virgin heifers because they are more fertile and more prone to dystocia.

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References

Borchersen, S., Peacoc, M., 2009. Danish AI field data with sexed semen. *Theriogenolgy* 71, 59-63.

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

Chebel, R.C., Guagnini, F.S., Santos, J.E.P., Fetrow, J.P., Lima, J.R., 2010. Sex-sorted semen for dairy heifers: Effects on reproductive and lactational performances. *Journal of Dairy Science* 93, 2496-2507.

DeJarnette, J.M., Nebel, R.L., Marshall, C.E., 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. *Theriogenology* 71, 49-58.

DeJarnette, J.M., Leach, M.A., Nebel, R.L., Marshall, C.E., McCleary, C.R., Moreno J.F., 2011. Effects of sex-sorting and sperm dosage on conception rates of Holstein heifers: Is comparable fertility of sex-sorted and conventional semen plausible? *Journal of Dairy Science* 94, 3477-3483.

De Vries, A., Overton, M., Fetrow, J., Leslie, K., Eicker, S., Rogers., G., 2008. Exploring the impact of sexed semen on the structure of the dairy industry. *Journal of Dairy Science* 91, 847-856.

Dominguez, J.H., Costa, D.S., Centurion, V.J., Faria, F.J., 2011. Pregnancy rate of Nelore females inseminated with male-sexed semen. *Animal Reproduction Science* 129, 127-131.

Garner, D.L., Seidel Jr, G.E., 2008. History of commercializing sexed semen for cattle. *Theriogenology* 69, 886-895.

Healy, A.A., House, K., Thomson, P.C., 2013. Artificial insemination field data on the use of sexed and conventional semen in Holstein heifers. *Journal of Dairy Science* 96, 1905-1914.

Hossein-Zadeh, N.G., Nejati-Javaremi, A., Miraei-Ashtiani, S.R., Kohram, H., 2010. Bio-economic evaluation of the use of sexed semen at different conception rates and herd sizes in Holstein populations. *Animal Reproduction Science* 121, 17-23.

Meyer, C.L., Berger, P.J., Koehler, K.J., Thompson, J.R., Sattler, C.G., 2001. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. *Journal of Dairy Science* 84, 515-523.

Norman, H.D., Hutchison, J.L., Miller, R.H., 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. *Journal of Dairy Science* 93, 3880-3890.

Philipsson, J., 1996. Strategies to reduce problems in calving performance and stillbirths by selection and differential use of bulls. *Proceedings of the International Workshop on Genetic Improvement of Functional Traits in Cattle. Gembloux, Belgium. Interbull Bulletin* 12, 65–71.

SAS Institute Inc. 2015. SAS User's Guide, Version 9.4m3. SAS Institute, Cary, NC, USA.

Seidel Jr., G.E., 2014. Update on sexed semen technology in cattle. *Animal* 8, 160-164.

Schenk, J.L., Cran, D.G., Everett, R.W., Seidel Jr., G.E., 2009. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. *Theriogenology* 71, 717-728.

Tubman, L.M., Brink, Z., Suh, T.K., Seidel Jr., G.E., 2004. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. *Journal of Animal Science* 82, 1029-1036.

Weigel, K.A., 2004. Exploring the role of sexed semen in dairy production systems. *Journal of Dairy Science* 87, 120-130.

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بررسی کارایی تولید مثلی کاربرد اسپرم تعیین جنسیت شده در تلیسه‌های هولشتن

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چکیده اسپرم تعیین جنسیت شده با افزایش شمار گوساله‌های ماده می‌تواند سبب بهبود بازده اقتصادی گاوهای شیری شود. هدف پژوهش کنونی، بررسی پیامدهای کاربرد اسپرم تعیین جنسیت شده بر درصد ماده‌زایی، نرخ آبستنی آغازین (نرخ گیرایی)، سخت‌زایی و نرخ مرده‌زایی در تلیسه‌های هولشتن بود. به این منظور از رکوردها و اطلاعات مربوط به ۱۳۳۴۳تلقیح در ۸۰۱۱ تلیسه از مجتمع شرکت کشت و دام فکا اصفهان بین سال‌های ۱۳۸۸ تا ۱۳۹۵ استفاد شد. نرخ ماده زایی برای اسپرم های تعیین جنسیت شده ۸۶/۴۵ درصد و ۱/۷۶ برابر بیشتر از نرخ ماده زایی اسپرم‌های معمولی (۴۹/۰۵ درصد) بود. نرخ آبستنی در تلیسه‌های تلقیح شده بااسپرم تعیین جنسیت شده ۴۲/۶۵ درصد و برای اسپرم‌های معمولی ۵۴/۸۵ درصد بود که نشان می‌دهد نرخ‌گیرایی اسپرم تعیین جنسیت شده ۷۷/۷۵ درصد اسپرم معمولی بود. نرخ مرده‌زایی در تلیسه‌های تلقیح شده با اسپرم‌های معمولی و تعیین جنس شده تفاوت معنی داری نداشت ($P > 0.05$). نرخ سخت‌زایی در تلیسه‌های تلقیح شده با اسپرم تعیین جنسیت شده نسبت به تلیسه‌های تلقیح شده با اسپرم معمولی کمتر بود. به طور کلی، با وجود قیمت بیشتر و کاهش نرخ‌گیرایی، کاربرد اسپرم تعیین جنسیت شده در تلیسه‌ها به علت کاهش سخت‌زایی وافزایش نرخ ماده‌زایی، می تواند سودمند باشد.