Reproductive performance of sexed versus conventional semen in Holstein heifers in various semiarid regions of Iran

Sahereh Joezy-Shekalgolabi, Ali Maghsoudib and Mohammad Reza Mansourianc

Department of Animal Science, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran; Department of Animal Science, Faculty of Agriculture, and Center of Agricultural Biotechnology, University of Zabol, Zabol, Iran; Animal Breeding Centre, Ministry of Jihad Agriculture, Karaj, Iran

ABSTRACT

For comparing reproductive performance of sexed versus conventional semen a total of 3573 heifer insemination records collected from five herds in four provinces of Iran were investigated. The studied provinces were classified into three regions: hot semiarid (Tehran and Alborz provinces), temperate semiarid (Khorasan Razavi province) and cold semiarid (Zanjan province). Various parameters including the conception rate, number of services per conception, calf sex ratio, calf birth weight, gestation length, calving ease score, abortion and stillbirth as well as twining rate were investigated. The logistic regression method was deployed for the analysis of categorical variables while the GLM procedure was applied for the analysis of continuous variables. The average conception rate of sex sorted and conventional semen was 48.3 and 63.8%, respectively. The utilisation of sex sorted semen resulted in 91.1% female calves. The conception rate, number of services per conception, gestation length, calf birth weight and the calf sex ratio were among the reproductive variables that were significantly influenced by the semen sexing. A greater number of services per conception and calving ease score were observed in hot and temperate semiarid areas. These climatic regions also provided a lower incidence of female calf births compared to the cold semiarid regions. Our results highly reaffirm the previous findings on the reproductive performance of sex sorted semen. Yet, climatic and management practices of a herd within a special climate have to be considered when deciding for utilisation of sex sorted semen in a dairy farm.

ARTICLE HISTORY
Received 17 October 2016
Revised 20 February 2017
Accepted 24 March 2017

KEYWORDS
Sexed semen; climate; reproductive performance; heifer; Iran

INTRODUCTION

Nowadays, modern dairy industry management necessitates optimal herd size in addition to culling low or medium performance animals (Seidel 2003). The need for producing replacement heifers in commercial dairy herds has led to development of several methods for separating X and Y bearing chromosomes, in the recent decades. Among the proposed sex pre-selection methods, flowcytometry has been relatively successful in commercial production of sex sorted semen (Hamano 2007; Seidel 2007). This assisted reproductive technology acts based on the inherent difference in DNA content of X and Y bearing sperms. Obtaining an ideal proportion of males and females using sex sorted semen could improve productive efficiency. Moreover, efficient selection of superior replacement heifers would be possible after the use of sex sorted semen.

After introducing sex sorted semen at commercial level, various studies examined its efficiency at herd or population levels. The accuracy of sex pre-selection using flowcytometry is ~90% (Tubman et al. 2004). Generally sexed semen has been proved to have lower conception rate compared to the conventional semen (Andersson et al. 2006; Barba et al. 2006; Cerchiaro et al. 2007; Norman et al. 2010; Norman et al. 2011; Healy et al. 2013; Karakaya et al. 2014). Due to the reduced conception rate reported for sex sorted semen, its use is usually limited to more fertile virgin heifers. It is declared that the use of sex sorted semen in the second or later services could reduce the conception rate compared to the first service heifers (Seidel 2007; DeJarnette et al. 2009; DeJarnette et al. 2011). Improving herd biosecurity and herd turnover rate, reducing the incidence of dystocia, slight improvement of genetic progress and the reduced
price for superior replacement heifers are among the main advantages of using sex sorted semen in virgin heifers. From the economical point of view, the application of the sex sorted semen reduces the costs of milk production (De Vries et al. 2008). In addition, more efficient progeny testing programmes, MOET (multiple ovulation and embryo transfer) and IVP (in vitro embryo production) are expected from the use of sex sorted semen (Weigel 2004; De Vries et al. 2008).

Iran is classified in the hot and dry climate countries. Its weather is influenced by location of Iran between the subtropical aridity of the Arabian Desert areas and the subtropical humidity of the eastern Mediterranean area. In 2008, sex sorted semen was first introduced in Iran’s dairy industry. To the best of our knowledge, no study has evaluated success of using this kind of sperm in the Iran’s dairy farms. The objective of the current study was to compare the reproductive performance of the sexed and conventional semen in some commercial herds of Iran. Moreover, the effect of climate was investigated on the reproductive performance of the sexed or conventional semen.

**Materials and methods**

**Data**

Data were collected during January 2008 to December 2015 on 3573 conventional and sexed semen inseminations on Holstein heifers reared in five commercial farms in four different provinces of Iran, including Tehran (two herds), Khorasan Razavi, Alborz and Zanjan (Table 1). Tehran and Alborz are both classified as provinces with hot semi-arid climate. In addition, Zanjan and Khoesnan Razavi are located in cold-semiarid and temperate-semiarid regions, respectively. Beside the climatic region, data recorded consisted in registration number, insemination date, insemination season, semen type (sexed and conventional), parturition type (natural, abortion, stillbirth), age at calving (months), calving ease score (1–5 scale (Van Tassell et al. 2003)) birth type (single or twin) and the calf sex and birth weight. Only data from 40 sires with both conventional and sex sorted semen inseminations were included in the analysis. The approximate sperm dosage per straw in sexed and conventional semen were $2 \times 10^6$ and $20 \times 10^6$, respectively. In all the studied herds, the regular time of insemination had been performed 12–14 hours after the occurrence of standing oestrus. All inseminations were performed in the uterine body, followed by the evaluation of impending ovulation by palpation per rectum. Conception rate was obtained by the reciprocal of the average number of services per conception. Service numbers greater than six were eliminated from the data. Calving ease scores of 4–5 were regarded as dystocia.

**Statistical analysis**

In the current study, the possible effect of using sex sorted semen on various herd parameters was investigated. For investigation of categorical dependent variables, the statistical analysis was performed using logistic regression. Number of services per conception, parturition type, calving ease score, birth type (single or twin) and calf sex were the main variables analysed by this method. PROC LOGISTIC of SAS 9.4 (Cary, NC) was utilised for performing the logistic regression analysis. The categorical and the continuous independent variables utilised for defining the statistical model of analysis for each of the above traits are summarised in Table 2.

The GLM procedure of SAS 9.4 was also used for analysis of the gestation length (GL) and the calf birth weight (CBW) by employing the following statistical models:

\[
GL_{ijklmn} = \mu + Sex_i + Y_j + S_k + ST_l + Sire_m + BT_n + e_{ijklmn}
\]

\[
CBW_{ijkl} = \mu + Sex_i + Y_j + S_k + ST_l + Sire_m + b_{GL}(GL_i - \overline{GL}) + e_{ijkl}
\]

Where, \(sex_i, Y_j, S_k, ST_l, Sire_m\) and \(BT_n\) refer to the record obtained on the ith sex, jth year, kth season and lth semen type and mth sire and nth birth type, respectively. Moreover, \(b_{GL}\) refers to the regression coefficient of gestation length on calf birth weight.

Analyses were separately performed for each herd. Finally, data of various herds were pooled and the effect of climate type (hot, temperate and cold semi-arid) and herd within climate were included in all the described models in order to find the potential effect of climatic region on the reproductive performance of conventional or sex sorted semen.

### Table 1. Number of available heifer records in various herds and provinces.

| Province (herd) | Conventional semen | Sexed semen |
|----------------|--------------------|-------------|
| Tehran (1)     | 1034               | 476         |
| Tehran (2)     | 156                | 60          |
| Alborz         | 809                | 443         |
| Khorasan Razavi| 168                | 50          |
| Zanjan         | 252                | 125         |
| Total          | 2419               | 1154        |
Results and discussion

Conception rate and the number of services per conception

Not surprisingly, the conception rates obtained in various herds and provinces indicated lower fertility of sexed as a percentage of conventional semen in Holstein heifers (Figure 1). The average empirical conception rate for sex sorted semen was 48.3% (ranging from 41 to 50.4 over various herds). The conception rate of sexed as a percentage of conventional semen ranged from 71.8 to 78.5% in various herds, which were in accordance with the results reported in the literature (Andersson et al. 2006; Cerchiaro et al. 2007; Chebel et al. 2010; Norman et al. 2010; Healy et al. 2013; Karakaya et al. 2014; Noonan et al. 2016).

Besides the damage induced on sperms during the sorting process, the lower number of spermatozoa in sex sorted semen straws was the main reason for lower fertility of this kind of semen. However, it has been declared that high concentration of the bull seminal plasma may decrease the semen sorting efficiency (Steinhauser et al. 2016) which can subsequently result in lower conception rate of this kind of semen. Semen sexing showed a significant effect on the number of services per conception (SpC). The year and the season of insemination had significant influence on this trait. More services per conception were necessary during summer compared to the winter. On the other hand, the lowest number of services per conception was obtained for heifers inseminated during spring. However, age at insemination did not have any effect

Table 2. Categorical and continuous variables applied for analysis of the categorical dependent variables using multivariate logistic regression model.

| Independent variables |
|-----------------------|
| Dependent variable    | Categorical | Continuous |
| Number of service per conception | Y | Sire | S | ST | CS | BT | SpC | HBW | FCA | GL | CBW |
| Abortion, stillbirth   | Y | Sire | S | ST | CS | BT | SpC | HBW | FCA | GL | CBW |
| Calving ease           | Y | Sire | S | ST | CS | BT | SpC | HBW | FCA | GL | CBW |
| Birth type             | Y | Sire | S | ST | CS | BT | SpC | HBW | FCA | GL | CBW |
| Calf sex               | Y | Sire | S | ST | CS | BT | SpC | HBW | FCA | GL | CBW |

Y: insemination year; Sire: inseminating sire; S: insemination season; ST: semen type (conventional or sex); CS: calf sex (male or female); BT: birth type (single or twin); SpC: number of services per conception; HBW: heifer birth weight; FCA: first calving age; GL: gestation length; CBW: calf birth weight.

Figure 1. Conception rate of sexed versus conventional semen in Holstein heifers.
on SpC. The lowest conception rate observed in this study was related to Khorasan Razavi province which was classified in hot semiarid region. The pooled data analysis indicated the significant effect of climatic region on the number of services per conception. A greater SpC was necessary for heifers inseminated in hot or temperate semiarid regions (Odds Ratio (OR) = 3.2 (confidence limit (CL): 1.2–5.3 and \( p = .028 \)) and 2.9 (CL:1.1–3.8 and \( p = .041 \)), respectively). Additionally, the breeding sire had a significant effect on conception rate. Healy et al. (2013) reported significant effect of age at insemination, sperm type, temperature and humidity of breeding station, number of services per conception, breeding sire and inseminating technician on the conception rate of Holstein heifers. The effect of insemination year and the breeding sire beside the sperm type were significant in the study conducted by Cerchiaro et al. (2007). Mellado et al. (2014) reported that the difference in the conception rate among sexed and conventional semen is related to the month of insemination. Lower conception rate of sexed versus conventional semen is highly related to both decreased numbers of sperm per doses of semen in addition to the damage occurred to spermatozoa during the semen sorting process (Bodmer et al. 2005; Garner & Seidel Jr 2008; DeJarnette et al. 2011).

**Sex ratio**

Female calf sex ratios obtained for sexed and conventional semen in various province and herds are illustrated in Figure 2. Average female calf ratio obtained from the use of sex sorted semen was 91.1%. This ratio has been reported between 85 and 91% by previous published studies (Tubman et al. 2004; Borchersen & Peacock 2009; DeJarnette et al. 2009; Norman et al. 2010; Healy et al. 2013; Djedovic et al. 2016). Statistical analysis indicated the considerable influence of climate, semen type, inseminating sire, insemination year and season, and gestation length on the calf sex ratio. The herd effect did not have a significant effect on calf sex ratio. Healy et al. (2013) demonstrated that semen type, gestation length and inseminating sire are the main predictors of calf sex. In comparison with cold semiarid regions, lower probability (\( p < .0001 \)) of getting a female calf was observed in hot and temperate semiarid regions (OR = 0.2 (CL: 0.12–0.33, \( p = .037 \)) and 0.15 (CL: 0.07–0.34, \( p = .023 \)), respectively). Roche et al. (2006) reported greater incidence of male calf birth following periods of elevated temperature during the week prior to conception.

**Calf birth weight**

Generally, average birth weight of calves produced with the sex sorted semen was lower than that of conventional semen (Table 3). Greater frequency of male calves obtained with conventional semen was the result of greater birth weight of calves obtained with this kind of semen. Statistical analysis indicated that calf birth weight was affected by the year and the season at insemination, calf sex and gestation length and inseminating sire. Calves that were born during spring showed greater weights at birth. The effect of semen type was not significant when separate analysis was applied for each farm (except for Zanjan province farm). When the effect of climatic region was included

![Figure 2. Female calf sex ratio for Holstein heifers inseminated with sex sorted or conventional semen.](image-url)
in the analysis, this effect notably influenced the calf birth weight. Calf birth weight is considered as one of the main determinants of dystocia and prenatal death. Comparing with hot semiarid regions, calves born in temperate or cold semiarid regions showed significantly higher birth weights. The significant effect of sex sorted semen on decreasing calf birth weight has been reported in previous studies (Djedovic et al. 2016). However, Tubman et al. (2004) did not find any significant difference among the birth weight of calves obtained with conventional or sex sorted semen.

Gestation length

Least square means of gestation length for male and female calves obtained from insemination of nulliparous heifers with the conventional and sex sorted semen are presented in Table 3. Generally, the average gestation length was longer for heifers inseminated with conventional semen. However, the effect of semen type on gestation length was not significant. Except the fixed effect of climate, the calf sex, insemination year, insemination sire and the incidence of twins were among influential factors on the gestation length. Tubman et al. (2004), Healy et al. (2013) and Djedovic et al. (2016) did not find statistically significant association between the semen type and the gestation length. Although Tubman et al. (2004) indicated that the calf sex and management group were the significant predictors of the gestation length, Healy et al. (2013) reported the importance of the calf sex, insemination sire, inseminating technician and birth type. Generally female sex reduces the gestation length which is reflected in lower birth weight compared to the male calves.

Calving ease score

The incidence of dystocia was not under the control of semen type. The calf birth weight was the main significant factor influencing calving ease score. Moreover, the incidence of dystocia was significantly influenced by the insemination year, insemination season and calf sex in Zanjan province. The gestation length and heifer age at calving were also significant indicators of calving ease score. In accordance with our results, Chebel et al. (2010) observed negligible difference in the incidence of dystocia after insemination of heifers with sex sorted or conventional semen. Norman et al. (2010) reported that the occurrence of dystocia was reduced by 28% as a result of using sex sorted semen in Holstein heifers. Tubman et al. (2004) reported higher average calving ease score in male calves which indicated more prevalence of dystocia. However, in agreement with our results, calving ease score was not affected by the insemination type. Calving ease score was dramatically changed by including the effect of climatic region in the analysis. Lower average calving ease score was observed in hot and temperate semiarid regions (odds ratio = 1.0 (CL = 0.2–1.1, p = .026) and 0.8 (CL = 0.3–1.2, p = .043), respectively) compared to the cold semiarid regions. Increasing feed consumption in the cold weather led to increased blood flow into the uterus; consequently higher calf birth weight and calving difficulties is probable (Colburn et al. 1997).

Abortion and stillbirth

The frequency of abortion and prenatal stillbirth in Holstein heifers inseminated with the conventional and sex sorted semen are illustrated Table 3. Except for the second herd in Tehran province, the effect of the calf sex, insemination year, insemination season and calf birth weight on parturition type (abortion, stillbirth) were not significant. Generally higher stillbirth rate was obtained by the sex sorted semen. However, the effect of semen type on stillbirth rate was inconsiderable except for Zanjan province. A considerably higher stillbirth rate was obtained from sexed semen inseminations in Zanjan province. The damage induced on the sperm during the sorting process might reduce viability of foetus which could escalate the incidence of stillbirth (DeJarnette et al. 2009; Mikkola et al. 2015; Djedovic et al. 2016). Translocation of large DNA content of Y chromosome and its concentration in X chromosome or the incidence of aneuploidy, especially trisomy, may enhance

Table 3. The values of the studied variables according to the semen type and calf sex.

| Variable                  | Calf sex | Conventional semen | Sexed semen |
|---------------------------|---------|--------------------|-------------|
| Calf birth weight, kg, LSmeans ± SD | Male    | 38.36 ± 1.12       | 38.13 ± 0.83 |
|                           | Female  | 38.13 ± 0.94       | 37.12 ± 1.32 |
| Gestation length, days, LSmeans ± SD | Male    | 275.63 ± 8.1       | 276.2 ± 9.1  |
|                           | Female  | 274.1 ± 7.9        | 273.8 ± 8.4  |
| Abortion, %               | Male    | 7.2                | 12.1         |
|                           | Female  | 4.7                | 5.8          |
| Stillbirth, %             | Male    | 5.4                | 6.8          |
|                           | Female  | 4.2                | 5.2          |
the stillbirth rate of male calves obtained from sex sorted semen (Mikkola et al. 2015). Considering the effect of climate in the model of analysis did not change the overall result on the effect of sex sorted semen on stillbirth. For both semen types, the incidence of abortion showed fluctuation over herds. Heifer age at first calving, calf birth weight and gestation length were the significant factors influencing the abortion rate. According to Healy et al. (2013) heifer breeding age was the major factor influencing the abortion in nulliparous heifers. However, they reported the significant influence of twin birth, gestation length and AI technician and the marginal influence of semen type on the calf stillbirth. In accordance with our results, Tubman et al. (2004) and DeJarnette et al. (2009) did not find significant difference among abortion/stillbirth rate resulted from sex sorted or conventional semen.

Twining

Average twining rate obtained from the conventional and sex sorted semen was 0.8 and 0.7%, respectively. The result of statistical analysis indicated that the semen type was not a significant predictor of twinning rate. Similar to our findings, Healy et al. (2013) and Turk et al. (2015) reported that the incidence of twins insignificantly influenced by the semen sexing. Healy et al. (2013) demonstrated that heavier heifers were significantly more prone to twinning. Further result showed that the gestation length was the major predictor of twinning rate. The breeding season had significant influence on the incidence of twining. Similar trend reported in literature revealed higher incidence of twinning for heifers inseminated during summer season (odds ratio = 4.6 (95% CI = 3.4–6.7, p = .04)) (Sartori et al. 2002; Del Río et al. 2007). This phenomenon was a result of photoperiod change or the nutritional flushing at the end of summer (Del Río et al. 2007). Climatic regions did not influence the twining rate. Gender distribution of twins according to the semen type is presented in Figure 3. The occurrence of freemartin twins was considerably lower for sex sorted semen than that of conventional semen. Female homo-sexual twins were the main twining occurrence by sex sorted semen.

Conclusions

Reproductive performance of sex sorted semen was analysed in this research. Some reproductive performances such as conception rate and number of services per conception and calf sex ratio were influenced by the semen type. It was however, neither a major indicator of abortion, stillbirth and dystocia nor the gestation length. The effect of semen type on calf birth weight showed fluctuation in various herds. Considering the effect of herd and climate type, it could be concluded that the selection of sex sorted semen was highly related to herd management practices. On the other hand, climate could play an important role in making decision on the use of sex sorted semen.

Acknowledgements

This article is derived from the research project entitled ‘Comparing the performance of conventional and sex sorted semen in Holstein heifers in Iran’.

Disclosure statement

No potential conflict of interest was reported by the authors.
References

Andersson M, Taponen J, Kommeri M, Dahlbom M. 2006. Pregnancy rates in lactating Holstein-Friesian cows after artificial insemination with sexed sperm. Reprod Domest Anim. 41:95–97.

Barba A, Ducolomb YC, Romo S. 2006. Use of sexed semen for artificial insemination in Holstein heifers in Mexico: preliminary results. Reprod Fertil Dev. 18:113.

Bodmer M, Janett F, Hässig M, Daas N, Reichert P, 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.

Borchersen S, Peacock M. 2009. Danish A.I. field data with sexed semen for cattle. Theriogenology. 69:886

Chebel RC, Guagnini FS, Santos JEP, Fetrow JP, Lima JR. 2010. Sex-sorted semen for dairy heifers: effects on reproductive and lactational performances. J Dairy Sci. 93:2496–2507.

Colburn DJ, Deutscher GH, Nielsen MK, Adams DC. 1997. Effects of sire, dam traits, calf traits, and environment on dystocia and subsequent reproduction of two-year-old heifers. J Anim Sci. 75:1452–1460.

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. J Dairy Sci. 91:847–856.

DeJarnette JM, Leach MA, Nebel RL, Marshall CE, McCleary CR, 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.

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

Del Río NS, Stewart S, Rapnicki P, Chang YM, Fricke PM. 2007. An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. J Dairy Sci. 90:1255–1264.

Djedovic R, Bogdanovic V, Stanojevic D, Nemes Z, Gaspardy A, Cseh S. 2016. Involuntary reduction in vigour of calves born from sexed semen. Acta Veterinaria Hungarica. 64:229–238.

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

Hamano K. 2007. Sex preselection in bovine by separation of X- and Y-chromosome bearing spermatozoa. J Reprod Dev. 53:27–38.

Healy AA, House JK, Thomson PC. 2013. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. J Dairy Sci. 96:1905–1914.

Karakaya E, Yilmazbas-Mectitoglu G, Keskin A, Alkan A, Tasdemir U, Santos JEP, Gumen A. 2014. Fertility in dairy cows after artificial insemination using sex-sorted sperm or conventional semen. Reprod Domest Anim. 49:333–337.

Mellado M, Sepulveda E, Macias-Cruz U, Avendaño L, García J, Veliz F, Rodríguez A. 2014. Effects of month of breeding on reproductive efficiency of Holstein cows and heifers inseminated with sex-sorted or conventional semen in a hot environment. Trop Anim Health Prod. 46:265–269.

Mikkola M, Andersson M, Taponen J. 2015. Transfer of cattle embryos with sexed-sorted semen results in impaired pregnancy rate and increased male calf mortality. Theriogenology. 84:1118–1122.

Noonan EJ, Kelly JC, Beggs DS. 2016. Factors associated with fertility of nulliparous dairy heifers following a 10-day fixed-time artificial insemination program with sex-sorted and conventional semen. Aust Vet J. 94:145–148.

Norman HD, Hutchison JL, 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.

Norman HD, Hutchison JL, VanRaden PM. 2011. Evaluations for service-sire conception rate for heifer and cow inseminations with conventional and sexed semen. J Dairy Sci. 94:6135–6142.

Roche JR, Lee JM, Berry DP. 2006. Climatic factors and secondary sex ratio in dairy cows. J Dairy Sci. 89:3221–3227.

Sartori R, Rosa GJM, Wiltbank MC. 2002. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci. 85:2813–2822.

Seidel GE Jr. 2003. Economics of selecting for sex: the most important genetic trait. Theriogenology. 59:585–598.

Seidel GE Jr. 2007. Overview of sexing sperm. Theriogenology. 68:443–446.

Steinhauser CB, Graham JK, Lenz RW, Seidel GE Jr. 2016. Removing seminal plasma improves bovine sperm sex-sorting. Andrology. 4:1131–1137.

Tubman LM, Brink Z, Suh TK, Seidel GE. 2004. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. J Anim Sci. 82:1029–1036.

Turk G, Yuksel M, Sonmez M, Gur S, Ozer Kaya S, Demirci E. 2015. Effect of semen sexing kits (HeiferplusTM and Bullplus™) supplemented to frozen-thawed bull semen on pregnancy rates, foetal sex ratios and selected reproductive parameters in cows. Veterinarini Medecina. 60:309–313.

Van Tassell CP, Wiggans GR, Misztal I. 2003. Implementation of a sire-maternal grandsire model for evaluation of calving ease in the United States. J Dairy Sci. 86:3366–3373.

Weigel KA. 2004. Exploring the role of sexed semen in dairy production systems. J Dairy Sci. 87:E120–E130.