



# Parallel distribution of sexes within left and right uterine horns in Holstein dairy cows: Evidence that the effect of side of pregnancy on sex ratio could be breed-specific in cattle

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## ABSTRACT

Dissimilar distribution of male and female calves within left and right uterine horns has been observed in beef cows. A retrospective study was conducted to investigate the effect of side of pregnancy on secondary sex ratio in Holstein dairy cows. Data associated with sex of calves, side of pregnancy, sire, dam, parity number of dam, AI technician, season and year were retrieved from the database of a Holstein dairy farm. In total, data consisted of 6515 birth records from 3155 dams and 244 sires across years 2001–2010. Data were analyzed using logistic regression. There was no difference in proportion of male and female calves between left (52.9% and 47.1%, respectively) and right (53.2% and 46.8%, respectively) uterine horns ( $P > 0.05$ ). AI technician, year, season and parity of dam did not affect secondary sex ratio ( $P > 0.05$ ). Secondary sex ratio of left and right uterine horns, and consequently, overall secondary sex ratio (53.1%) were skewed toward males as compared with hypothetical secondary sex ratio of 50% ( $P < 0.05$ ). Incidence of right pregnancy (60.5%) was higher than hypothetical 50% incidence of right pregnancy. In conclusion, the present study revealed similar secondary sex ratio of calves between left and right uterine horns in Holstein dairy cows.

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## 1. Introduction

Although it is generally assumed that mammals produce equal proportion of male and female offspring (Rosenfeld and Roberts, 2004), various factors have been reported to alter sex ratio of offspring toward one gender, including maternal body condition (Roche et al., 2006b; Trivers and Willard, 1973), nutrition (Rosenfeld and Roberts, 2004; Green et al., 2008), parity of dam (Hosseini-Zadeh, 2012; Huck et al., 1988; Roche et al., 2006a,b), maternal hormonal profile (Grant and Chamley, 2010; Helle et al., 2008b),

stress (Akbarinejad et al., 2012; Krackow and Hoeck, 1989), climatic parameters (Helle et al., 2008a; Hosseini-Zadeh, 2012; Roche et al., 2006a; Youssefi et al., 2013), time of insemination relative to onset of estrus (Martinez et al., 2004; Wehner et al., 1997) and site of semen deposition (Zobel et al., 2011).

Asymmetric distribution of male and female fetuses within left and right uterine horns has been demonstrated in various species of rodents (Clark et al., 1991; Endo et al., 1987; YoungLai et al., 1981). In cattle, it has been indicated that conception in the right uterine horn was more likely to result in male calves while conception in the left uterine horn was more likely to result in female calves (Giraldo et al., 2010; Hylan et al., 2009). However, most of the studies investigating the effect of side of ovary and/or uterine horn on sex ratio of calves have been carried out in beef

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cows (Giraldo et al., 2010; Hylan et al., 2009) or in excised uteri obtained from a mixture of slaughtered beef and dairy cows without considering the effect of breed (Giraldo et al., 2010).

Therefore, a retrospective study was conducted to investigate the effect of side of pregnancy on secondary sex ratio (proportion of male calves at birth; SSR) in Holstein dairy cows, in which sex of calf is of economic importance as production of more heifers could improve milk production efficiency and genetic progress (Seidel, 2003).

## 2. Materials and methods

### 2.1. Location

The present study was conducted at a commercial Holstein dairy farm located in Varamin county, Tehran province, Iran (latitude: 35°46'N; longitude: 51°65'E; altitude: 1200 m).

### 2.2. Data

To investigate the effect of side of pregnancy on SSR, data associated with sex of calves, side of pregnancy, sire and dam ID, parity number of dam at conception, AI technician, season and year were collected from the database of the herd. Data associated with multiple births, unidentified sire, natural breeding and sexed semen were excluded from the dataset. In total, data consisted of 6515 birth records from 3155 dams and 244 sires across years 2001–2010. Across years 2001–2007, inseminations were carried out by the same AI technician, coded as AI technician A. Thereafter (from 2008 to 2010), inseminations were carried out by another AI technician, coded as AI technician B. Season and year of conception were determined based on the date of insemination. Uterine side of pregnancy had been determined based on transrectal palpation of amniotic vesicle between Days 40 and 45 after insemination. The sex of calves, which had been determined at birth, was coded as dichotomous variable (0: female; 1: male). Parity of cows was coded into four classes including 1, 2, 3 and  $\geq 4$ .

### 2.3. Statistical analysis

The effect of side of pregnancy, AI technician, year, season and parity (included in the model as fixed effects) on the probability of a male calf being born was analyzed using univariable logistic regression; sire and dam were considered as random effects in all univariable logistic regression analyses. The observed SSR in the left and right uterine horns in the present study compared with hypothetical SSR of 50% and the SSR reported by Hylan et al. (2009) in the left and right uterine horns were analyzed using univariable logistic regression. Additionally, the observed overall SSR in the present study versus hypothetical SSR of 50% as well as the observed incidence of right pregnancy in the present study compared with hypothetical 50% incidence of right pregnancy and the incidence of right pregnancy reported by Hylan et al. (2009) were analyzed using univariable logistic regression. All analyses were conducted in SAS version 9.2 (SAS, 2008) using GLIMMIX procedure

including function link logit in the model. The univariable logistic regression analyses with random effects (sire and dam) produced adjusted odds ratios (AORs) as the estimates of strength of association between each potential risk factor (side of pregnancy, AI technician, year, season and parity) and the birth of male calves. Univariable logistic regression analyses of observed and expected SSR and incidence of right pregnancy produced odds ratios (ORs) as the estimates of strength of difference between the observed and expected SSR and incidence of right pregnancy, respectively. The effect of factors on SSR and differences between observed and expected SSR and incidence of right pregnancy were considered statistically significant at  $P < 0.05$  level. The statistical power of tests comparing observed and expected SSR and incidence of right pregnancy was computed (considering  $\alpha = 0.05$ ) using SigmaPlot version 12.0. Statistical power of 80.0% was considered as standard statistical power to reject the null hypothesis.

## 3. Results

Proportion of male and female calves was similar between left (52.9% and 47.1%, respectively) and right (53.2% and 46.8%, respectively) uterine horns (Table 1;  $P > 0.05$ ). AI technician, year, season and parity did not significantly affect SSR (Table 1;  $P > 0.05$ ). SSR in the left and right uterine horns and overall SSR (53.1%) were higher than the hypothetical SSR of 50% (Table 2;  $P < 0.05$ ). Moreover, the observed SSR in the left uterine horn was higher than that reported by Hylan et al. (2009) (34.4%;  $P < 0.0001$ ), whereas the observed SSR in the right uterine horn was lower than that reported by Hylan et al. (2009) (67.4%; Table 2;  $P < 0.0001$ ). Except for the analysis comparing the observed SSR in the left uterine horn with hypothetical SSR of 50%, all analyses of the observed and expected SSR had statistical power of more than 80.0% (Table 2). Incidence of right pregnancy in the present study (60.5%) was higher than hypothetical 50% incidence of right pregnancy and that reported by Hylan et al. (2009) in beef cows (53.2%; Table 3;  $P < 0.0001$ ). Statistical power was more than 80.0% in both analyses of observed and expected incidence of right pregnancy (Table 3).

## 4. Discussion

Skewness of SSR toward male calves in the present study agrees with previous reports (Berry et al., 2011; Hossein-Zadeh et al., 2008; Roche et al., 2006b). Examining excised uteri obtained from mixed-breed beef and dairy cows at abattoir, Giraldo et al. (2010) has also observed 53.1% male fetuses as similar to the present study. It has been postulated that greater proportion of male calves at birth ensures equal numbers of two sexes after the occurrence of perinatal mortality (calf death within 24 h of calving) (Berry et al., 2011) since perinatal mortality was higher in male calves (Berry et al., 2011; Hossein-Zadeh et al., 2008; Mee et al., 2008). Nevertheless, analyzing SSR in Iranian Holstein cows, Hossein-Zadeh (2012) observed SSR of 49.6%, which was fairly skewed toward females. This difference could partly be explained by the fact that unlike the study by Hossein-Zadeh (2012), data associated with calves resulted

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