J. Reprod. Engineer. 2016; 18: 1–3.
http://sreprod.jp/Contents.htm

Journal of REPRODUCTION ENGINEERING JRE

= Research Note =

Time of insemination affects the sex ratio of Japanese Black cow offspring

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ABSTRACT

This study was conducted to assess whether varying the time of artificial insemination (AI) after detection of estrus influences the sex ratio of Japanese Black cows offspring. Data were collected from 1,829 inseminated cows, of which 753 gave birth to calves, over a period of two and a half years. The animals were divided in two groups: the early AI group (3 h after estrus detection) and late AI group (27 h after estrus detection). All cows were palpated 3 h after estrus detection. If the dominant follicle was large, the cows were inseminated (early AI). The cows with a small dominant follicle were palpated again 27 h after estrus detection and inseminated (late AI group). Although no difference was observed in the calving rates between the two groups, the proportion of male calves in the early AI group was significantly higher ($P < 0.05$) than that in the late AI group (56.9% vs. 41.1%, respectively). Our results indicate that delayed AI decreases the proportion of male calves.

Key words: Artificial insemination, estrus, insemination time, offspring, sex ratio

INTRODUCTION

Varying the timing of artificial insemination (AI) might influence the outcome of sex ratio of cattle offspring. If so, this could be economically beneficial and used for livestock production. Multiple efforts have been made to influence the sex of calves by varying the time of insemination [5,10]. Several studies have reported that early inseminations after the onset of estrus can result in a higher proportion of female calves, whereas delayed insemination result in a higher proportion of male calves. This effect on the sex ratio might be due to differences in the timing of sperm capacitation and survival time of the X and Y chromosome-bearing spermatozoa in the female reproductive tract [5,13]. Some studies provide other explanations to account for the effects of insemination time on the sex ratio of offspring. Differences between the results of these studies might exist because of other factors such as genotype, climate, quality of semen, parity number, postpartum time, and region [3,5,10]. Pursley *et al.* [8] reported that both early and late inseminations resulted in a higher percentage of females. Moreover, a large study in dairy cattle showed that there were no effects of parity, herd, or time of insemination on sex ratio [3]. Numerous factors have been suggested to be associated with the variation in the sex ratio of offspring, but these conflicting reports make it difficult to assess which factors might contribute to an altered sex ratio in cattle. In this study, data from one herd were evaluated, and the effect of time of insemination on the sex ratio of Japanese Black cow offspring was assessed.

Submitted: November 22, 2015
Accepted: March 23, 2016
Advance Publication: March 26, 2015

Table 1. Variation of the calving rates and the proportion of males calves according to the interval between the detection of estrus and artificial insemination (AI)

Interval between estrus and AI	Cows calved/total inseminated cows (%)	Calved males/total calved animals (%)
3 h	573/1,389 (41.3)	326/573 (56.9)[a]
27 h	180/440 (40.9)	74/180 (41.1)[b]
Total	753/1,829 (41.2)	400/753 (53.1)

[a,b] Values with different superscripts in the same column are significantly different ($P < 0.05$).

MATERIALS AND METHODS

Animals and AI

Data were collected from a herd consisting of approximately 350 Japanese Black cows over a period of two and a half years (between 2011 and 2013). Parity of the animals varied between one and eight. The cows were housed in a free stall barn with a sawdust floor and were fed a diet of hay and concentrates according to their production level [1].

Freeze–thawed semen derived from 25 Japanese Black bulls with proven fertility was used for insemination. Estrus detection was conducted daily from 11:00 to 11:30. The cows were classified into insemination groups based on the status of the dominant follicle, which was examined by rectal palpation before insemination, at 3 h after the detection of estrus. Based on the status of follicular development, if the dominant follicle was large and expected to ovulate, the cows were immediately inseminated (early AI group). However, if the dominant follicle was small and not expected to ovulate, the insemination was postponed. Cows were examined again 27 h after estrus detection. If the dominant follicle developed and the cow was expected to ovulate, insemination was performed (late AI group). However, if the dominant follicle did not develop and the cow was not expected to ovulate, or had already ovulated, the cow was excluded from the study. Estrus detection, all rectal palpations, and AIs were performed by one experienced technician, and no hormonal treatment was given to the cows, either before or after insemination. Ovulation was confirmed by rectal palpation 24 h after AI.

Statistical analysis

The total number of inseminated cows was 1,829 and the number of cows that delivered calves was 753. Calving rates and proportion of male calves were evaluated in two groups: the early insemination group (3 h after the detection of estrus) and the late insemination group (27 h after the detection of estrus). Data were then submitted to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (SAS for Windows, version 9.1, SAS Institute Japan, Tokyo, Japan). The statistical model included the type of insemination, season, sire, time of insemination and the two-way interactions. The differences with a probability value (P) of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

Table 1 summarizes the number of inseminations, calving results and the proportion of male calves in the early and late insemination groups. No significant time × season and time × sire interactions were observed in calving rates and proportion of male calves. There was no significant difference between the calving rates obtained for the two groups. However, the proportion of male calves in the early insemination group was significantly higher ($P < 0.05$) than that in the late insemination group (56.9% vs 41.1%, respectively).

Many studies have assessed the sex ratio of calves with respect to the timing of AI during visually observed estrus, mounting behavior and intravaginal conductivity [5,10,12]. Controlling the sex ratio by varying the time of AI with respect to the onset of the estrus can be an effective technique in animal husbandry because of the low associated costs. The variations observed in the sex ratios of offspring because of varying the timing of AI are thought to have arisen owing to differences in the motility, capacitation time, and survival time of the X and Y chromosome-bearing spermatozoa [5,13]. In cows, estrus lasts for a period of 18 h on average and ovulation follows 10–12 h later; thus, the total time from the onset of estrus to ovulation is 30–32 h [5,9]. The recommended period for AI of cows is between 12 and 18 h after the onset of estrus [4,6]. It has been reported that late AI decreased fertility, and the highest conception rates for AI occurred between 4 and 12 h after the onset of

standing activity [2]. In this study, no difference was observed between the early (3 h) and late (27 h) insemination groups regarding the calving rates of inseminated cows, although these rates were lower than those reported by a previous study carried out in Japanese Black cows [11]. Moreover, the present data showed that delayed AI decreased the proportion of male calves. Contradictory to these results, it has been demonstrated that AI immediately after the detection of estrus resulted in more female calves, whereas AI performed later resulted in more male calves [5]. Another study reported that both early and late inseminations resulted in a higher percentage of females [8]. Rorie *et al*. [10] suggested that insemination at approximately 20 h or 10 h before expected ovulation did not alter the sex ratio of calves. During the present study, insemination was postponed if at the time of rectal examination, the follicle was not expected to ovulate, and insemination was then performed at 27 h. All dominant follicles ovulated within 24h after AI in each groups. Therefore, dominant follicle ovulated 3 to 27 h after estrus detection in early insemination group, and ovulated 27 to 51 h after estrus detection in late insemination group. This indicates that estrus period was long and ovulation was delayed in late insemination group. In humans, Martin [6] suggested that shorter follicular phases are associated with shorter periods of penetrable mucus and higher proportions of Y spermatozoa reaching the uterus. Conversely, longer phases are associated with longer periods of penetrable mucus and lower proportions of Y spermatozoa in the uterus. A similar phenomenon may be expected cows.

The disparity between results obtained from various studies is thought to have arisen due to methodological differences, especially the method of estrus detection, the use of different AI protocols, and the variability between males and ejaculates [5]. In conclusion, our results indicate that lasting estrus and delayed AI biased the sex ratio toward more females in Japanese Black cow offspring.

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