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Relationship between the timing of insemination based on estrus detected by the automatic activity monitoring system and conception rates using sex-sorted semen in Holstein dairy cattle

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Abstract. We investigated the optimal timing of artificial insemination (AI) for achieving pregnancy according to the onset/end of estrus detected by an accelerometer system in Holstein cattle. The conception rates of conventional semen were used as a reference. The conception rate from AI of sex-sorted semen was higher at −4 to 4 h (57.1%) from the end of estrus than those at −12 to −4 h (37.7%) and 12–20 h (30.3%), whereas AI at 4–12 h showed an intermediate conception rate (47.4%). Conversely, conception rates were similar in AI performed between 0 and 32 h from the onset of estrus. Regarding conventional semen, the interval from the onset and end of estrus did not affect conception rates. The present results suggest that the time of the end of estrus is the better indicator of optimal AI timing for sex-sorted semen than the onset of estrus.

Key words: Accelerometer, Automated activity monitoring system, Dairy cattle, Sex-sorted semen

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Sex-sorted semen is now commercially available and used worldwide. The application of sex-sorted semen in dairy farming contributes to accelerated genetic improvement and efficient herd expansion by producing heifers [1]. Nevertheless, one of the challenges with the employment of sex-sorted semen is the timing of artificial insemination (AI) to achieve maximum pregnancy. A previous study indicated that the optimal AI timing for sex-sorted semen was later and shorter than that for conventional semen [2]. This delayed optimal AI timing of sex-sorted semen is believed to be attributable to the shorter lifespan of sperm in the female reproductive tract due to damage induced by the sorting process [2, 3].

Although AI is usually performed based on the time of visual estrus detection or the start of estrus detected by automatic monitoring systems (*e.g.*, accelerometers and pressure sensors), the timing of ovulation is important to achieve successful fertilization. This is because sperm lifespan after injection into the genital tract is limited [4], and the sperm needs to fertilize an oocyte before its lifespan ends. It has been reported that the interval from the onset and end of estrus to ovulation is 27–30 h [5–9] and 12–19 h [5, 7, 8], respectively, and the duration of estrus (from the onset to the end of estrus) is 7–16 h [5, 6, 8, 10]. The optimal AI timing for high pregnancy rates was 5–16 h from the onset of estrus using conventional semen [11] and 23–41 h [12] and 16–24 h [13] using sex-sorted semen. One of the challenges in considering the optimal AI timing is the large variation in the interval from the onset of estrus to ovulation [9]. When estrus continues for a certain period (*e.g.*, 24 h) from the first AI, the second AI is commonly decided by herdsmen [14, 15] to ensure that the animal is inseminated at the optimal timing in relation to ovulation; thus, the time of the end of estrus is occasionally used as a reference for the second AI implementation in the field. Accelerometer systems that continuously monitor individual cow activity can detect the onset and end of estrus of individuals based on temporal changes in activity intensity [16]. Valenza *et al.* [8] indicated that the interval from the onset of estrus to ovulation extended in parallel with the period from the onset to the end of estrus (estrus duration) detected by Heatime® (SCR Engineers Ltd., Netanya, Israel), an automatic activity monitoring system that uses an accelerometer. This finding suggests that the time of the end of estrus detected by this system can estimate the time of ovulation more accurately than the onset of estrus. Therefore, we hypothesized that the end of estrus could be an indicator of optimal AI timing using sex-sorted semen. This knowledge can provide a new reference for evaluating optimal AI timing and contribute to a better AI strategy in the field.

This study aimed to examine the optimal AI timing for sex-sorted semen in relation to estrus, detected by an accelerometer system. Specifically, we hypothesized that the time of the end of estrus could be an indicator of optimal AI timing and compared the conception rates of AI using sex-sorted and conventional semen at different intervals from the onset and end of estrus. Conventional semen was used as a reference and tested for conception rates.

First, we confirmed the distribution of the estrus duration. The mean estrus duration of all animals was 14.5 ± 3.8 h (ranging between 8 and 28 h, n = 567), and the upper quartile (19.6 ± 2.2 h) was approximately twice as long as the lower quartile (9.9 ± 1.5 h).

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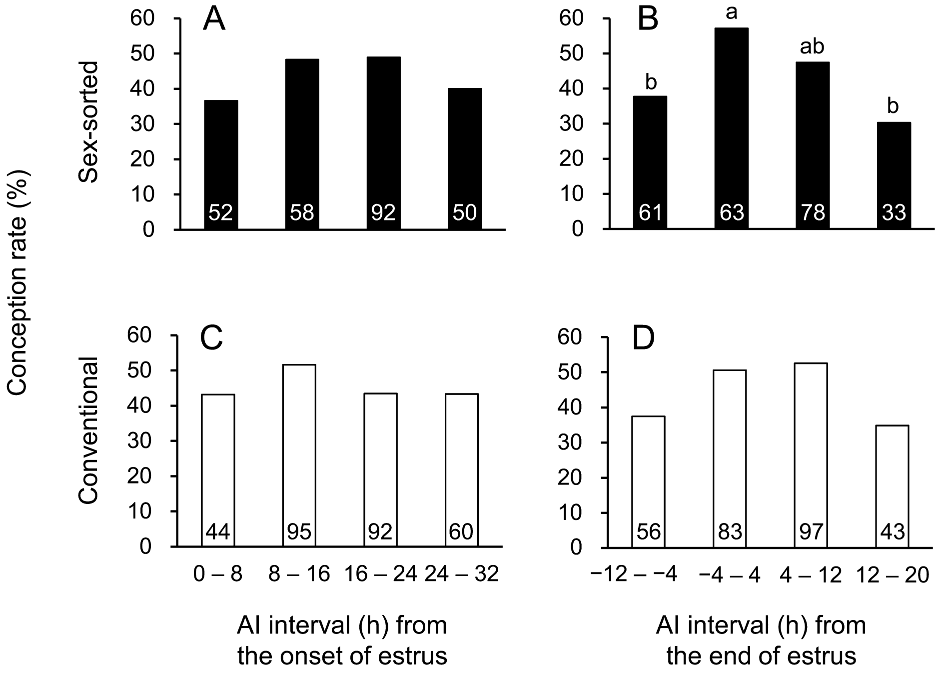

Fig. 1. Relationship between artificial insemination (AI) timing in relation to the onset/end of estrus and conception rates. The numbers within the bars represent the number of animals included in each AI-timing group. ^{ab} Different letters indicate significant differences ($P < 0.05$).

We examined the relationship between AI timing and estrus and conception rates using sex-sorted semen. The results of the logistic regression analysis showed that the AI interval from the onset of estrus did not affect conception rates ($P = 0.42$; Fig. 1A). Conversely, the AI interval from the end of estrus indeed affected conception rates, that is, AI performed at −4 to 4 h from the end of estrus showed higher conception rates (57.1%) than those at −12 to −4 h (37.7%) and 12–20 h (30.3%) ($P < 0.05$, respectively; Fig. 1B). This finding was consistent with the previous finding that pregnancy rates were similar between a control group inseminated based on the time estrus was detected using the Heatime® system and a delayed AI group inseminated approximately 12 h later than the control group in Holstein heifers [17]. In contrast, other studies using the Jersey breed indicated that pregnancy rates were highest at 23–41 h [12] and 16–24 h [13] from the onset of estrus detected by the accelerometer and a pressure sensor system, respectively. The reason why the time of the onset of estrus did not indicate the optimal AI timing in the present study was the potentially large variation in the interval from the onset of estrus to ovulation [9] compared with that from the end of estrus to ovulation. The large variation in estrus duration from 8 to 28 h observed in the present study, which is consistent with previous findings [8, 16], supports this explanation. Accordingly, the present findings suggest that the end of estrus can be a better indicator of the optimal AI timing than the onset of estrus.

We subsequently examined the relationship between AI timing and conception rate using conventional semen. The obtained results found that conception rates did not differ significantly between the different AI intervals from the onset of estrus ($P = 0.63$; Fig. 1C) and the end of estrus ($P = 0.10$; Fig. 1D). Although AI with conventional semen showed similar conception rates between −12 to −4 h (37.5%) and −4 to 4 h (50.6%) from the end of estrus ($P = 0.13$), AI with sex-sorted semen showed a lower conception rate at −12 to −4 h than that at −4 to 4 h. This finding was consistent with previous findings indicating delayed optimal AI timing for sex-sorted semen compared with conventional semen in a timed AI protocol [2, 3]. Meanwhile, another study using an ear tag accelerometer system [18] indicated that pregnancy rate of conventional semen was higher in the earlier inseminations relative to the time of peak activity intensity; however, those of sex-sorted semen were not affected by AI timing relative to the defined time of estrus. Different patterns in AI timing and conception rates between the present and previous studies may be attributable to differences in the estrus defined by neck- and ear-mounted accelerometers or tested animals; heifers were used in the previous study [18] while cows were mainly used in the present study. Additionally, the conception rate of AI with sex-sorted semen was lower at 12–20 h from the end of estrus than that at −4 to 4 h. The interval from the end of estrus to ovulation, as detected by the system, has been reported to be 12 h [8] and 16 h [7]. Therefore, the decreased conception rate with AI at 12–20 h from the end of estrus in the present study may be attributable to the increased proportion of animals that had experienced ovulation. Meanwhile, the adverse effects of delayed AI, at 12–20 h from the end of estrus, on conception rates were not detected in conventional semen. One of the potential explanations for these different patterns between sex-sorted and conventional semen is that sex-sorted sperms damaged by the sorting process are more sensitive to the postovulatory alteration in oviductal physiology, including inflammation and hormonal balance [19, 20], than conventional sperms.

The optimal AI timing based on the time of estrus is considered to differ depending on the type of data used for defining estrus (*e.g.,* standing behaviors, acceleration data, *etc.*). However, the duration of estrus detected by the Heatime® system has been demonstrated to be positively correlated with the duration of standing and mounting behaviors when this system was used with an activity threshold standard deviations setting of ≥ 5 [7]. The duration of estrus detected by the system corresponds to 16–27 h before ovulation, and the duration of standing and mounting activities corresponds to 23–33 h before ovulation [7]. The optimal AI timing should be investigated in future studies for other automatic monitoring systems that can theoretically detect the end of estrus in real time, such as systems using combined data of body temperature and activities (acceleration data) [21].

In conclusion, the conception rate of AI with sex-sorted semen was higher at −4 to 4 h than those at −12 to −4 h and 12–20 h from the end of estrus, whereas AI at 4–12 h showed an intermediate conception rate. Based on the present results, we conclude that the optimal AI timing for sex-sorted semen is between −4 and 12 h from the end of estrus, as detected by the system. This information potentially contributes to the decision of the necessity of second AI after a certain period of time from the first AI within the same estrus and provides a reference for assessing whether AI timing is optimal in individual cases so that herders can review their AI strategy. Accordingly, the present study provides another method of utilizing accelerometer devices, in addition to the conventional application, by focusing on the time of the end of estrus.

Methods

Data were collected from 602 artificially inseminated Holstein cows (n = 556, 1–3 parity) and heifers (n = 46). The AI was performed between December 2016 and January 2019. The animals were from two commercial farms (354 and 248 heads from farms A and B, respectively) in Hokkaido, Japan, housed in free-stall barns and grazed during daytime and/or nighttime from June to October. The average milk production of the farms was 12,000 kg/305 days in farm A and 8,500 kg/305 days in farm B. Estrus was spontaneous or induced by an intravaginal progesterone-releasing device (CIDR®1900; Zoetis, Parsippany, NJ, USA) or a single administration of prostaglandin $F_{2\alpha}$ analog (cloprostenol; Dalmazin®, Kyoritsu Seiyaku Corp., Tokyo, Japan or Resipron®-C, Aska Animal Health Co., Ltd., Tokyo, Japan). Gonadotropin-releasing hormone agonist was not administered at the time of AI. Semen was selected for AI by farm staff based on the condition of the animal; conventional semen was used (10 heifers and 313 cows), and sex-sorted semen was mainly used for young animals with presumed high fertility (36 heifers and 243 cows). Sex-sorted semen (from 12 bulls) and conventional semen (from 40 bulls) from frozen seminal straws were purchased from two commercial companies. Pregnancy was confirmed by ultrasonographic examination, rectal palpation, or pregnancy-associated glycoprotein examination of milk approximately 30 days after AI.

The method for measuring activities and the criteria for identifying estrus using the system have been reported elsewhere [16]. The system quantified all animal movements and movement intensities in 2-h time blocks. To identify increases in activity, the average of 8-h period activity levels was compared with a normal baseline activity level established according to the previous 7-day average activity level. The system identified an animal in the preovulatory follicular phase when the current average activity level was more than 5-fold the standard deviation above baseline [16]. The sensitivity and positive predictive value of estrus detection using the system with the standard deviations of activity threshold of ≥ 5 were reported to be 90% and 83%, respectively [7]. Data for the identified estrus period were obtained using software (farm A: Time for Cows [Lely Holding N.V., Maassluis, NL]; farm B: DataFlow II [SCR Engineers Ltd.]).

The time at which the activity index exceeded the threshold level, selected by the system for the first time, was defined as the onset of estrus, and the time at which the activity index was below the threshold level for the first time was defined as the end of estrus. In three animals, the activity index fluctuated before the end of estrus, was below the threshold level for 2 h, and increased again above the threshold level. In these cases, the end of estrus was defined as the time when the activity index was below the threshold level for the second time. The period from the onset to the end of estrus was defined as the duration of estrus. Data of AI for animals showing a short estrus duration of ≤ 6 h (n = 35) were excluded from the analysis according to a previous report [16] that indicated an increased false-positive rate for the preovulatory phase when the estrus duration determined by this system was <6 h. Accordingly, a total of 567 AI data were used for the analysis.

AI was performed between −21 and 46 h from the onset of estrus. AIs performed before 0 h (n = 8) and 32 h (n = 16) from the onset of estrus were excluded from the analysis because these exceptionally early and late inseminations were not of interest in the present analysis. Accordingly, 543 data points inseminated between 0 and 32 h from the onset of estrus were used for the analysis, and the interval from the onset of estrus to AI was categorized into four groups: 0–8, 8–16, 16–24, and 24–32 h from the onset of estrus to AI. To elucidate the optimal AI timing, we divided the groups into 8-h blocks according to previous findings that the optimal "time window" for AI to achieve pregnancy is approximately 8 h (6–12 h) [10, 11, 22] when conventional semen is used. AI was performed between −35 and 28 h from the end of estrus. Early and late AIs performed before −12 h (n = 38) and 20 h (n = 15) from the end of estrus were excluded from the analysis. Data from 514 AIs performed between −12 and 20 h from the end of estrus were used, and the interval from the end of estrus to AI was categorized into four groups based on the time of ovulation (12 h after the end of estrus) [8]: −12 to −4, −4 to 4, 4–12, and 12–20 h from the end of estrus to AI.

Statistical analyses were performed using JMP Pro 15.2.0 (SAS Institute, Cary, NC, USA). To investigate the relationship between AI timing and estrus and conception rates, logistic regression analysis was employed to compensate for the limitation of retrospective data in the present study by removing the influence of confounding factors [8]. To test the effect of AI timing in relation to the onset and end of estrus and the effect of different semen types (sex-sorted and conventional), a total of four models were created. The initial models contained the following categorical explanatory variables: farms (A *vs.* B), parity (0 *vs.* 1–3), AI interval from the onset/end of estrus (four categories), and the interaction between all these variables. The explanatory variables for the final model were determined using a backward elimination procedure. All variables with $P > 0.10$ were removed from the initial models, except for AI timing, which remained in all models. The procedure found that AI timing was the only explanatory variable in all final models. Odds ratios were estimated in the final models and $P < 0.05$ was considered to indicate a significant difference between the levels of the AI timing. Data included in the text are expressed as mean ± standard deviation.

Conflict of interests: The authors declare that there are no conflict of interest.

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