



# Time of insemination relative to reaching activity threshold is associated with pregnancy risk when using sex-sorted semen for lactating Jersey cows

Gabriel D. Bombardelli<sup>a,b</sup>, Henrique F. Soares<sup>a,b</sup>, Ricardo C. Chebel<sup>a,b,\*</sup>

<sup>a</sup> Department of Large Animal Clinical Sciences, University of Florida, Gainesville, Florida, USA

<sup>b</sup> Department of Animal Sciences, University of Florida, Gainesville, Florida, USA

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## ABSTRACT

The objective of this observational experiment was to determine the association between the interval from reaching activity threshold (AT) to artificial insemination (AI) with sex-sorted semen and the probability of pregnancy. Jersey cows ( $n = 678$ ) from a commercial dairy herd were fitted with heat-rumination long-distance collars (SCR Ltd., Netanya, Israel) at approximately  $42 \pm 7$  days postpartum. Cows were presynchronized with three injections of PGF $2\alpha$  given 14 days apart starting on Day  $39 \pm 3$  postpartum. Cows in estrus, based on tail paint removal, after  $50 \pm 3$  days postpartum were inseminated by one of three technicians with semen from one of six sires. Cows were examined for pregnancy at  $31 \pm 3$  and  $66 \pm 3$  days after insemination. Only cows inseminated in estrus and examined for pregnancy at  $31 \pm 3$  days after AI were used in the experiment ( $n = 419$ ). A subsample of cows ( $n = 35$ ) were examined by ultrasound every 8 hours after reaching AT to estimate the interval from reaching AT and ovulation. The mean ( $\pm$  standard error of the mean) interval from reaching AT to AI was  $16.9 \pm 0.5$  hours, whereas the interval from peak activity to AI was  $9.8 \pm 0.5$  hours. The length of high activity among cows diagnosed in estrus was  $17.3 \pm 0.3$  hours. The mean interval from reaching AT to ovulation was  $25.7 \pm 1.2$  hours. There was a quadratic effect of the interval between reaching AT and AI on probabilities of pregnancy at  $31 \pm 3$  ( $P = 0.07$ ) and  $66 \pm 3$  ( $P = 0.15$ ) days after AI. Pregnancy per AI at  $66 \pm 3$  days after AI was higher for cows inseminated between 23 and 41 hours after the onset of estrus ( $\leq 3$  hours = 20.0%, 4–12 hours = 27.1%, 13–22 hours = 39.1%, 23–41 hours = 45.6%, and  $\geq 42$  = 40.0%). Insemination of lactating Jersey cows with sex-sorted semen closer to expected ovulation yielded the highest probability of pregnancy.

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## 1. Introduction

Insemination of dairy heifers with sex-sorted semen has resulted in reduced pregnancy per artificial insemination (P/AI) compared with insemination with conventional semen [1,2]. Consequently, the use of sex-sorted semen in lactating dairy cows has been limited because of the risks of reducing pregnancy rates [3,4]. Reduced sperm count per inseminating dose and damage to the sperm cells during

sorting, consequence of staining, centrifugation, and exposure to pressure and laser beam during processing are important reasons for the reduced fertility after insemination with sex-sorted semen [5,6].

Improved estrus detection accuracy and precise determination of the onset of estrus are critical for the success of AI. Insemination of cows with conventional semen yields the highest probability of pregnancy when inseminations occur 4 to 12 hours after the onset of estrus based on mounting activity [7]. Conversely, P/AI of multiparous cows inseminated with conventional semen was highest when insemination occurred 0 to 12 hours after reaching activity

\* Corresponding author. Tel.: 352-294-4303; fax: 352-392-8289.  
E-mail address: [rcchebel@ufl.edu](mailto:rcchebel@ufl.edu) (R.C. Chebel).

threshold (AT), and P/AI of primiparous cows inseminated with conventional semen was highest when insemination occurred between 13 and 16 hours after reaching AT [8]. Although mount sensors (e.g., HeatWatch) determine precisely the moment of the first mount event during the estrus, they are rarely used in commercial herds because of the challenges in keeping the sensors on the cows. Sensors placed on the legs or necks of cows that diagnose estrus on the basis of changes in the number of steps or activity, respectively, have had great penetration in the US dairy industry in the past 5 years and are more common than mount sensors because of their practicality [9]. The fine-tuning of the time of insemination in relation to the onset of estrus and ovulation may significantly improve the probability of pregnancy of lactating cows inseminated with sex-sorted semen. Postponing the insemination of Jersey heifers and Nellore cows with sex-sorted semen in 6 to 12 hours increased the probability of pregnancy [10,11]. There is a strong correlation between the interval from the first standing event to ovulation and the interval from reaching AT to ovulation and is approximately 28 h [8]. Therefore, it appears that insemination of cattle with sex-sorted semen closer to the expected time of ovulation may improve probability of pregnancy.

The pattern of estrus expression based on activity and the association between interval from onset of estrus to AI and probability of pregnancy of lactating Jersey cows inseminated with sex-sorted semen have not been described. The hypothesis of the current observational experiment was that the interval from reaching AT to AI (ATAI) is associated with probability of pregnancy after AI with sex-sorted semen. The objective of the current observational experiment was to identify the ATAI interval that yields the highest P/AI. Furthermore, the circadian pattern of estrus expression, the length of activity of cows in estrus, and the interval between reaching AT and ovulation were characterized in the current experiment.

## 2. Materials and methods

All procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

### 2.1. Animals, housing, and feeding

Lactating Jersey cows (primiparous = 274, multiparous = 404) from a commercial herd in south central Minnesota were used in this experiment. Cows were fitted with the heat-rumination long-distance collars (SCR Engineers Ltd., Netanya, Israel) 10 days before the end of the voluntary waiting period and 7 days before treatment with PGF<sub>2α</sub>. Cows were housed in a low-profile cross-ventilated barn with 12 pens of two freestall rows each. Cows were fed once a day a total mixed ration balanced to meet the requirements of Jersey cows producing 40 kg of 3.5% fat-corrected milk (FCM) [12].

### 2.2. Characteristics of estrus

DataFlow II (SCR Engineers Ltd.) was used to monitor activity and estrus expression of cows. Data regarding date

and time when cows reached AT, when cows reached peak of activity (AP), and when activity fell below the threshold (ABT) were recorded. Furthermore, the “heat index” (0 = low, 100 = high) generated by DataFlow II (SCR Engineers Ltd.), which takes into consideration a cow’s activity, rumination, and interval from previous estrus and/or AI, was recorded for each cow. Finally, the intervals from AT to AP, from AT to ABT, and from AP to ABT were calculated for each cow.

### 2.3. Reproductive management and pregnancy examinations

Starting at approximately 21 days in milk (DIM), cows received tail paint daily at the same time of the day for detection of estrus. Cows were treated with PGF<sub>2α</sub> (25 mg of dinoprost as tromethamine salt; 5-mL Lutalyse sterile solution; Zoetis Animal Health, Florham Park, NJ, USA) at  $39 \pm 3$ ,  $53 \pm 3$ , and  $67 \pm 3$  DIM. Cows were observed daily in the morning for signs of estrus, which was characterized by removal of tail paint or standing estrus. Secondary signs used to confirm estrus were increased nervousness and activity, walking fence line, and swelling and reddening of the vulva. Cows observed to be in estrus and at greater than 45 DIM were inseminated on the same morning by one of three technicians with semen from one of six sires. The sire used to inseminate cows was predetermined according to mating strategies of the collaborating herd. Exact date and time of AI was recorded by AI technicians. Cows not inseminated by  $79 \pm 3$  DIM were enrolled in the 5-day CoSynch [13] and were removed from the experiment.

Pregnancy was diagnosed by transrectal ultrasonography (5-MHz linear probe; Ibex Lite; EI Medical Imaging, Loveland, CO, USA) of the uterine contents at  $31 \pm 3$  days after AI. Pregnancy was characterized by the presence of fluid, embryo, and heartbeat. Cows diagnosed pregnant were reexamined 5 weeks later,  $66 \pm 3$  days after AI. Pregnancies per AI were calculated by dividing the number of cows diagnosed pregnant at  $31 \pm 3$  and  $66 \pm 3$  days after AI by the number of cows receiving AI. Proportion of cows having pregnancy loss was calculated as the number of cows diagnosed as not pregnant at  $66 \pm 3$  days after AI divided by the number of cows diagnosed pregnant at  $31 \pm 3$  days after AI.

### 2.4. Ultrasonography of the ovaries and determination of ovulation after reaching AT

To estimate the interval from AT to ovulation, a subgroup of cows (primiparous = 22, multiparous = 13) had their ovaries examined by ultrasound (5-MHz linear probe; Ibex Lite; EI Medical Imaging) within 4 hours after reaching AT and every 8 hours thereafter until ovulation was characterized or 120 hours after reaching AT. Ovulation was characterized by the disappearance of a follicle of diameter of 10 mm or greater in two consecutive ultrasound examinations [13]. Time of day when ovulation was considered to have occurred was the interval between the ultrasound in which the ovulatory follicle was last observed and the ultrasound in which ovulation was determined to have occurred.

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