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## Does Time Interval from Thawing Multiple Straws of Semen to Insemination Affect Pregnancy Outcome of Lactating Dairy Cows?

J. Fehn

*Kansas State University*, [jo3f@k-state.edu](mailto:jo3f@k-state.edu)

L. G. Mendonça

*Kansas State University, Manhattan*, [mendonca@k-state.edu](mailto:mendonca@k-state.edu)

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## Does Time Interval from Thawing Multiple Straws of Semen to Insemination Affect Pregnancy Outcome of Lactating Dairy Cows?

### Abstract

Technicians must follow guidelines when inseminating cows to avoid impacting the quality of semen and pregnancy per AI (P/AI). The interval from initiation of the thawing process of multiple semen straws until AI is an important aspect to be considered when evaluating the performance of AI technicians. Time interval from thawing to AI can be affected by the skill level of the AI technician, distance from the thawing unit to location of insemination, and labor efficiency. Because modern dairy farms are becoming larger, it is important to evaluate if the interval from thawing to AI is impacting P/AI in large herds. The objective of this study was to investigate whether time interval from handling of semen to AI affects pregnancy outcome of lactating dairy cows housed in a modern dairy farm. Most of the AI occurred within 10 minutes after initiation of the thawing process. Pregnancy outcome was not affected by sequence of AI or time interval from thawing until AI. Technicians responsible to inseminate cows in large dairy farms should strive to deposit semen in the reproductive tract within 10 minutes after starting the thawing process. If the AI technician thaws multiple straws of semen simultaneously, it is important to consider the efficiency of the technician to deposit semen after thawing, and temperature of the thawing unit.

### Keywords

semen straws, thawing procedure, sequence of insemination, frozen semen

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## Does Time Interval from Thawing Multiple Straws of Semen to Insemination Affect Pregnancy Outcome of Lactating Dairy Cows?

*J. Febn and L.G.D. Mendonça*

### Summary

Technicians must follow guidelines when inseminating cows to avoid impacting the quality of semen and pregnancy per AI (P/AI). The interval from initiation of the thawing process of multiple semen straws until AI is an important aspect to be considered when evaluating the performance of AI technicians. Time interval from thawing to AI can be affected by the skill level of the AI technician, distance from the thawing unit to location of insemination, and labor efficiency. Because modern dairy farms are becoming larger, it is important to evaluate if the interval from thawing to AI is impacting P/AI in large herds. The objective of this study was to investigate whether time interval from handling of semen to AI affects pregnancy outcome of lactating dairy cows housed in a modern dairy farm. Most of the AI occurred within 10 minutes after initiation of the thawing process. Pregnancy outcome was not affected by sequence of AI or time interval from thawing until AI. Technicians responsible to inseminate cows in large dairy farms should strive to deposit semen in the reproductive tract within 10 minutes after starting the thawing process. If the AI technician thaws multiple straws of semen simultaneously, it is important to consider the efficiency of the technician to deposit semen after thawing, and temperature of the thawing unit.

### Introduction

Reproductive programs in most U.S. dairy farms rely on artificial insemination (AI). Certain guidelines must be followed by AI technicians in order to avoid issues with sperm viability, which influences fertilization of the oocyte, and consequently, pregnancy per AI (P/AI). To ensure that fertilization capacity of the spermatozoa is optimized, frequent training sessions must be conducted with AI technicians to reinforce the importance of proper AI technique and handling of semen.

The following critical steps should be considered when evaluating procedures performed by AI technicians: handling of frozen and thawed semen straws, temperature of the thawing bath, AI gun preparation, transportation of the loaded AI gun, overall cleanliness of the equipment, and location of semen deposition in the reproductive tract. Specific recommendations for these procedures are normally used to

instruct technicians. Another important aspect to be considered is the time interval from thawing multiple straws to semen deposition, which is affected by the skill level of the AI technician and distance from AI gun preparation to insemination location. In modern dairy farms, several cows are inseminated daily and some facilities require technicians to walk a considerable number of steps from AI gun preparation to insemination location. Therefore, technicians usually prepare several straws of semen at once to optimize their work, which creates another nuance when evaluating the time interval from thawing to AI.

The objective of this study was to evaluate whether time interval from handling multiple straws of semen to AI affects pregnancy outcome of lactating dairy cows housed in a modern dairy farm.

## Experimental Procedures

This study was conducted during summer months in a dairy farm milking 5,100 cows in Wisconsin. Cows were housed in pens of 250 to 300 cows in a cross-ventilated free-stall barn. Estrus detection was performed in 11 pens once daily in the morning based on tail chalk removal. The protocol of the farm consisted of one technician identifying cows in estrus in one or two pens, then preparing the AI guns outside the pens before inseminating the cows. Thawed straws were loaded in AI guns and transported in a portable AI gun warmer to maintain the temperature at 95°F. The technician was encouraged to limit the number of straws thawed simultaneously to 5 when preparing AI guns. Because in several instances more than 5 cows were in estrus in one pen, the technician had to repeat the steps described previously more than once to inseminate all cows in estrus. After inseminating all cows from one or two pens, the technician followed the same procedures in the subsequent pens.

During the study a chronometer was used once weekly for a total of 11 weeks to determine the time interval from initiation of the thawing process of multiple straws of semen until inseminations ( $n = 474$ ). Initiation of the thawing process started when the first straw of semen was placed in the thawing unit. Interval from initiation of thawing until insemination was recorded for each individual cow. The thawing unit automatically kept the water temperature between 95 and 99°F. Straws of semen were kept in the thawing unit for 45 seconds after the last straw was placed in the unit.

Three AI technicians performed inseminations during the study period and a total of 18 different sires were used. In one instance, 2 technicians worked on the same day, but did not inseminate cows in the same pens. Cows were inseminated with either conventional or sex-sorted semen, which comprised 94.5% and 5.5% of the inseminations during the study period, respectively. Technicians were trained to deposit semen in the body of the uterus, immediately after passing the cervix. Pregnancy diagnosis was conducted every other week in cows with unknown pregnancy status and more than 42 days after AI. Data regarding parity (primiparous and multiparous), number of previous inseminations, days in milk at AI, and sire code were extracted from the on-farm management software. Because of the limited instances that more than 5 straws were prepared at one time, sequence of inseminations greater than 5 were grouped in one category to conduct one of the statistical analyses. In addition, services were separated into quartiles by interval from initiation of thawing to AI (quartile 1 = 2:05 to 3:38 minutes;

quartile 2 = 3:39 to 4:42 minutes; quartile 3 = 4:43 to 6:01 minutes; quartile 4 = 6:02 to 11:28 minutes). Inseminations were classified as first-AI or  $\geq$  second-AI. A total of 27 cows were not included in the P/AI analysis because of removal from the herd before pregnancy diagnosis, or cow-related problems on the day of AI (e.g., lameness). Pregnancy outcomes were analyzed by logistic regression using the GLIMMIX procedure in SAS v. 9.4 (SAS Inst., Cary, NC). Models used for statistical analysis included the following variables: parity, number of services, AI technician, and sequence of AI or quartiles of interval from thawing to AI. Interactions between variables and sequence of AI, or variables and quartiles of interval from thawing to AI were tested.

## Results and Discussion

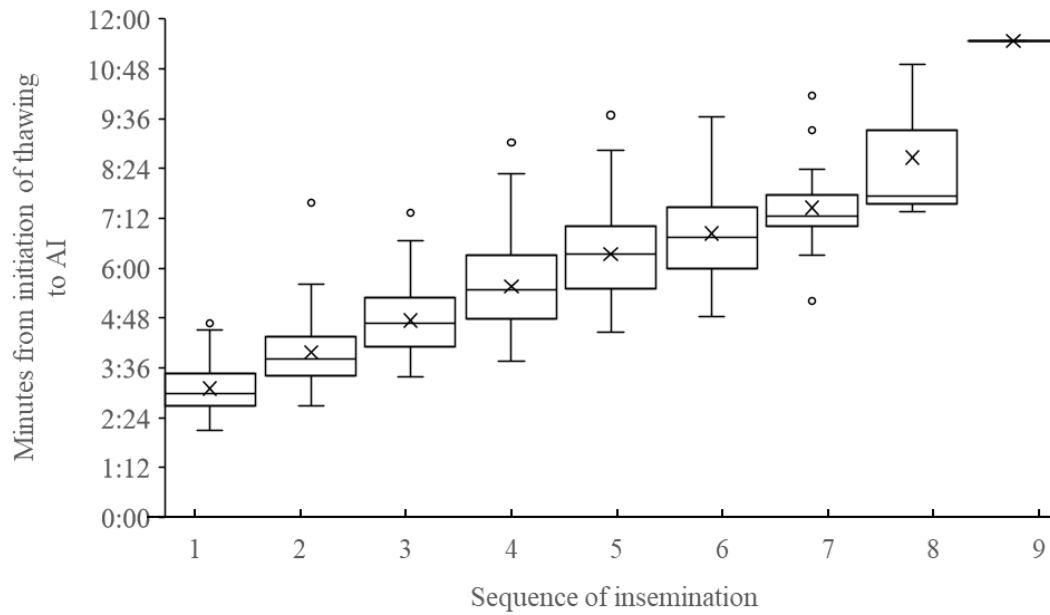
Time interval from initiation of thawing of multiple straws to sequence of AI is depicted in Figure 1. Technicians of this farm were encouraged to limit the number of straws thawed at one time to 5, however, there were instances that 6 to 9 straws were placed in the thawing unit. Despite the increased number of straws thawed at once, most of the inseminations occurred within 10 minutes after initiation of the thawing process. A common recommendation given to technicians is to inseminate cows within 10 minutes after thawing the straws. Although technicians of this farm were able to deposit semen in the uterus within the recommended time when greater than 5 AI guns were prepared at one time, thawing several straws simultaneously is expected to reduce the temperature of the thawing unit and may negatively impact the thawing process.

In the analyses that investigated P/AI by sequence of insemination (Figure 2), P/AI was not affected by sequence of insemination ( $P = 0.30$ ), parity ( $P = 0.90$ ), number of services ( $P = 0.11$ ), technician ( $P = 0.69$ ), or interactions ( $P > 0.28$ ). In the analyses that quartiles defined the interval from thawing to AI, no differences were detected ( $P = 0.49$ ) in P/AI among quartiles (Figure 3). In addition, parity ( $P = 0.98$ ), number of services ( $P = 0.13$ ), technician ( $P = 0.77$ ), and interactions ( $P > 0.36$ ) were not associated with P/AI. The observed findings suggest that interval from initiation of thawing to AI or sequence of AI were not associated with P/AI in this specific farm when inseminations occurred within 12 minutes after starting to thaw the straws. Although P/AI was not affected by the time interval from thawing to AI, we cannot exclude the possibility that thawing multiple straws of semen at the same time can impact temperature of the thawing unit and the thawing process. Straws of semen are usually commercialized in units containing 0.5 mL or 0.25 mL of semen. Because of the increased physical size of 0.5 mL units compared with 0.25 mL units, it is possible that thawing multiple 0.5 mL straws simultaneously causes a greater reduction in the temperature of the thawing unit than thawing multiple 0.25 mL units. Therefore, if several straws of semen will be thawed simultaneously, technicians are encouraged to use 2 thawing units.

Because of the small number of services with sex-sorted semen used during the study period, it was not possible to investigate whether sex-sorted semen was more vulnerable to effects of the time interval from handling of semen to AI than conventional semen. The process to sort semen is expected to have a detrimental effect on the spermatozoa, which results in sex-sorted semen to have reduced P/AI compared with conventional semen. In addition, dose of sex-sorted semen is usually decreased compared with conventional semen. These factors may interfere with how the interval of time from handling of thawed semen until AI affect pregnancy outcomes. Another important

aspect not explored in this study was the effect of sire. Several sires were used during the study period and it was unreasonable to determine whether P/AI was affected by time interval from handling of semen until AI for specific sires.

Findings from this study augment evidence that pregnancy outcomes are not impacted if AI is conducted within 10 minutes after starting the thawing process of multiple semen straws. It is important to consider that this study was conducted during summer and cows were housed in a cross-ventilated barn. Therefore, ambient conditions were controlled, which minimized the impact of weather on semen quality while being handled and transported from the thawing unit to the cow. In scenarios in which multiple straws will be thawed simultaneously, skill level of the AI technician, distance to transport the AI gun, and temperature of the thawing unit when including several straws at once should be assessed to ensure P/AI will not be affected.



**Figure 1. Minutes from initiation of thawing multiple semen straws simultaneously until AI according to sequence of insemination. Top and bottom of the boxplots represent first and third quartiles, respectively. Line through the middle of the boxplot, ‘x’ in the box, and whiskers represent the median value, average, and outliers, respectively.**

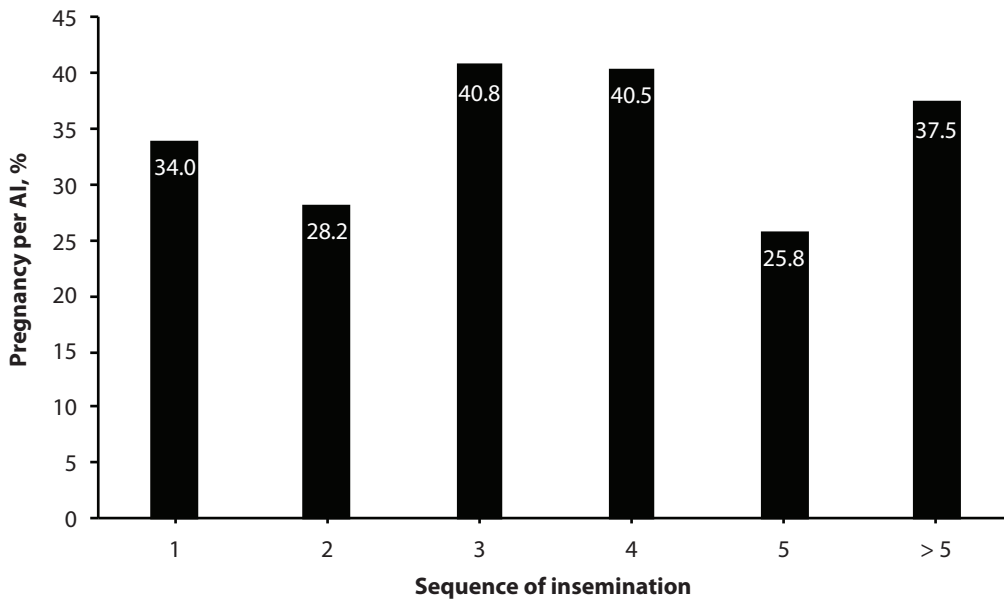


Figure 2. Pregnancy per AI according to sequence of insemination of straws of semen thawed simultaneously. Number of services for each sequence of insemination ranged from 56 to 94. Sequence of AI ( $P = 0.30$ ), parity ( $P = 0.90$ ), number of services ( $P = 0.11$ ), AI technician ( $P = 0.69$ ), and interactions between sequence of AI and forementioned variables ( $P > 0.28$ ).

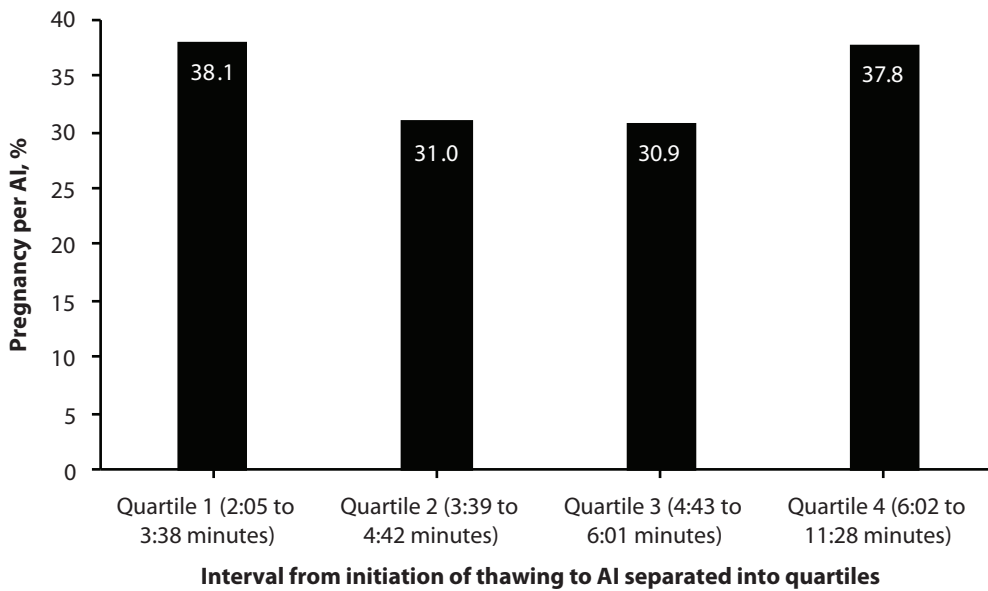


Figure 3. Pregnancy per AI according to interval from initiation of thawing until AI, separated into quartiles from multiple straws of semen thawed simultaneously. Number of services in quartile 1, quartile 2, quartile 3, and quartile 4 are 113, 113, 110, and 111, respectively. Quartiles ( $P = 0.49$ ), parity ( $P = 0.98$ ), number of services ( $P = 0.13$ ), AI technician ( $P = 0.77$ ), and interactions between quartiles and forementioned variables ( $P > 0.36$ ).