Journal of Agricultural Science and Technology A 6 (2016) 64-68
doi: 10.17265/2161-6256/2016.01.007

Effect of Delayed Insemination on Holstein Cows' Reproductive Performance

Charlotte Gaillard, Jakob Sehested and Mogens Vestergaard
Department of Animal Science, Aarhus University, DK-8830 Tjele, Denmark

Abstract: Holstein cows' fertility has decreased in the last decade, creating a need for new management methods to improve the reproductive performance which in this case was defined by pregnancy rates and number of artificial inseminations (AI) per pregnancy. Previous studies showed that deliberately delaying rebreeding until after peak lactation can improve reproductive performance compared with the traditional rebreeding in early lactation. The objective was to compare the reproductive performance of cows in consecutive lactations of different lengths. The reproductive performance (pregnancy rate and number of insemination to pregnancy) of 62 Holstein cows involved in a 16 months extended lactation trial was recorded and compared with the reproductive performance of the previous and following 10 months lactation of the same cows. It was hypothesized that a late rebreeding (at eight months, 16 months lactation) will improve the reproductive performance compared with an early rebreeding (at two months, 10 months lactation) in the previous lactation, and it will have no negative effect on the reproductive performance in the following lactation with an early rebreeding. The results showed that the pregnancy rates were similar for the 16 months lactation and the previous and following 10 months lactations, while the number of AI to pregnancy was increased in the 10 months lactation, following by the 16 months lactation. The use of the double AI technique did not improve pregnancy rates compared with a single AI per estrus, and induced confusion, as how to report the herd reproductive performance. To conclude, the reproductive performance was similar between the heifers, primiparous and multiparous cows, and between 10 months and 16 months lactations.

Key words: Extended lactation, pregnancy rate, double insemination.

1. Introduction

During the last decades, Holstein cow's milk production has increased and fertility has decreased [1, 2]. More artificial inseminations (AI) in successive estruses are now needed to obtain pregnancy which is then involuntarily delayed [3]. This increase of reproductive failure and number of AI needed per pregnancy might be partly due to a decrease of estrus behavior, which makes it difficult to detect estrus and to inseminate the cow at the right stage [4]. This also indicates a negative correlation between estrus duration and genetic selection for milk yield [5]. Thus, new management methods to improve reproductive performance are needed. Delayed insemination might be of interest. A study found that deliberately delaying rebreeding until after peak lactation improved pregnancy rates compared with rebreeding in early lactation [6]. In that case, the first AI occurred in a period of more positive or less negative energy balance compared with the early lactation period [7, 8]. Furthermore, the return to estrus had become regular compared to the early lactation period [7], which facilitated estrus detection. The use of delayed AI also increased the number of cows' expressing estrus behavior at time of AI [9]. Nevertheless, the effect of delayed AI on the reproductive performance of the following lactation has not been studied up to now. Thus, the objective of the present study was to compare the reproductive performance of cows in consecutive lactations of different lengths, i.e., insemination at different time from calving. The reproductive performance was defined by the number of AI per pregnancy and the pregnancy rates. It was hypothesized that the pregnancy rates will be higher for the 16 months lactation compared with the

Correponding author: Charlotte Gaillard, Ph.D., research field: dairy science.

previous 10 months lactation, and that the extended lactation will not have a negative effect on the reproductive performance in the following 10 months lactation.

2. Material and Methods

2.1 Facilities and Animals

A 16 months extended lactation (EL) trial was performed at the Danish Cattle Research Centre at Aarhus University, Foulum. The 62 Holstein cows involved in the experiment were housed in one group pen on slatted concrete floors with cubicles equipped with mattresses and sawdust bedding. The cows had access to water and an automatic milking system (AMS; DeLaval AB, Tumba, Sweden) and were fed partially mixed rations with complementary concentrates supplied during visits in the AMS. More information on the setting of the experiment during the extended lactation, details about the rations and milk production can be found in the study of Gaillard et al. [10, 11].

The number of AI per pregnancy and the pregnancy rates at first and second AI were recorded for each of the 62 cows in EL. The reproductive performance of the previous lactation (PL) of these 62 cows, which was a shorter lactation of 10 months, was observed, as well as the reproductive performance of 38 of 62 cows during the 10 months lactation following EL (FL). The number of heifers, primiparous and multiparous cows per lactation group (PL, EL, FL) is detailed in Table 1. For the 10 months and 16 months lactations, the AI was initiated at the first estrus after 60 d and 220 d in milk, respectively.

2.2 Double Insemination Technique

In this data set, it was observed that some heifers (6 among 17), primiparous (13 lactations among 47) and cows (51 lactations among 98) were inseminated two times around a given estrus day, with on average 2 d between these two inseminations. This double AI technique could increase the pregnancy rates, so the

Table 1 Effect of insemination time on the number of AI per pregnancy and pregnancy rate (%) for Holstein cows.

Parameter	Lactation group			SEM	*P* value
	PL	EL	FL		
Total number of animals	62	62	38	-	-
Heifers	17	-	-	-	-
Primiparous	30	17	0	-	-
Multiparous	15	45	38	-	-
Number of cows with double AI technique	25	25	21	-	-
Calving interval[1] (d)	406[b]	526[a]	370[b]	11	< 0.001
Number of insemination per pregnancy					
Estrus_AI[2]	1.90[ab]	1.80[a]	2.60[b]	0.20	0.03
Total AI[3]	2.40[ab]	2.30[a]	3.40[b]	0.20	0.03
Pregnancy rates					
Estrus_PR_1 (%)[4]	50	58	53	-	0.66
Total PR_1 (%)[5]	35	40	21	-	0.13
Estrus_PR_2 (%)[4]	52	42	28	-	0.27
Total PR_2 (%)[5]	70	46	53	-	0.79

PL: previous 10 months lactation; EL: 16 months extended lactation; FL: 10 months following extended lactation; SEM: standard error of the mean.

[1]Calving interval = lactation period + dry period.

[2]Estrus_AI: number of insemination at estrus, not counting the double AI at estrus.

[3]Total AI: total number of AI per pregnancy, counting the double AI at estrus.

[4]Estrus_PR_1 or PR_2: pregnancy rates at first and second AI (with the first AI defined as estrus_AI).

[5]Total PR_1 or PR_2: pregnancy rates at first and second AI (with the first AI defined as total AI).

technique (single or double) was included as a factor in the analysis of the reproductive performance (Table 2). The herdsmen's decision to inseminate a cow twice was based upon if the cow stayed in heat the days following the first AI. There were no other criteria used to decide when to apply this double AI technique. The number of cows assigned to single or double AI per lactation group is given in Table 1. Two variables were used to define the number of AI per pregnancy: the total number of AI per pregnancy (total_AI) which counts the double AI at estrus, and the number of estrus inseminated (estrus_AI) does not counts the double AI at estrus and only counts the number of estrus where AI was used. The same codes were used to define the pregnancy rates (PR) at first and second AI, depending on the definition of the first AI (total_PR or estrus_PR).

2.3 Statistical Analysis

The statistical analysis was made using R version 3.0.0 [12]. The factors taken into account were the age of the cows (parity), the lactation type and group, and the AI technique. The parity was defined by three levels: heifers (parity 0), primiaprous (parity 1) and multiparous (parity 2+). A variable "lactation type" was defined with three levels, "0" for the non-lactating heifers inseminated, EL and the normal 10 months lactations (NL) grouping into the PL and FL (Table 2). As there was no interaction between the factors (parity, lactation group, lactation type) presented in Table 2, a simple linear mixed-effects model was used to test the effects of one factor at a time on the total_AI, estrus_AI, and on the length of the calving interval. A chi-square test was used to determine the effect of parity, lactation group, lactation type and AI technique on the pregnancy rates at first and second AI.

3. Results and Discussion

3.1 Number of Inseminations

The number of AI per pregnancy was different among PL, EL and FL group (P = 0.03). The results show that the number of AI per pregnancy was slightly lower for EL compared with PL (-0.1 for estrus_AI or total AI), however this difference was not significant (P = 0.9). This is partly in accordance with Larsson and Berglund [13] who found that delaying AI until after the peak yield decreased significantly the number of AI per pregnancy. The number of AI per pregnancy increased during the FL compared with EL (+0.8 for estrus_AI or +1.1 for total_AI). The number of AI per pregnancy was similar when comparing the lactation type (0, EL, NL) (P = 0.20) which is in accordance with Christiansen et al. [14]. The parity had no effect on the number of AI per pregnancy (P = 0.38 for estrus_AI, and P = 0.18 for total_AI). These results indicate that even though the number of AI increased in FL, it is not different from the number of AI in PL, so there is no negative effect of EL on the number of AI in FL.

3.2 Pregnancy Rates

There was no difference in pregnancy rates at first and second AI between the lactation groups (Table 1). This result is in accordance with Bertilsson et al. [15] and Gaillard et al. [16], who found no differences in pregnancy rates between a 16 months and a 10 months

Table 2 Factors and levels studied.

Factor	Levels
Parity	Heifers (0), primiparous (1), multiparous (2+)
Lactation group	Extended lactation (EL), lactation preceding extended lactation (PL), lactation following extended lactation (FL)
Lactation type	Non-lactating heifer (0), normal 10 months lactation (NL) (includes PL and FL), 16 months extended lactation (EL)
AI technique	Single, double

Table 3 Effect of the double insemination technique on the pregnancy rates at first and second AI.

Insemination	Number of lactations	Pregnancy rate at first AI (%)	Pregnancy rate at second AI (%)
Double insemination	71	45	23
Single insemination	91	60	64
P value	-	0.05	< 0.01

The *P* value compare the pregnancy rates between the cows receiving the double AI and those receiving one AI at estrus.

lactation. Nevertheless, other studies, like Kolver et al. [17], Larsson and Berglund [13] and Schindler et al. [6], found an improved pregnancy rate with use of extended lactation. The estrus_PR results in this paper are similar to those of Schindler et al. [6]: the pregnancy rate at first AI increased from 50% to 58% for PL to EL, respectively, and decreased from 58% to 53% for EL to FL, respectively.

3.3 Double Insemination

The lactation group had no effect on the pregnancy rates. Higher pregnancy rates were observed for the 91 lactations where the cows received a single AI at estrus, compared with the 71 lactations where the cows received a double AI at estrus (60% vs. 45% pregnant at first AI, $P = 0.05$, and 64% vs. 23% pregnant at second AI, $P < 0.01$) (Table 3). The double AI technique did not improve the pregnancy rates, which is in accordance with Wilcox and Pfau [18] and Stevenson et al. [19], who found no benefits of the double AI technique (+2.5% but non-significant result and +1.4% with $P = 0.3$, respectively). Trimberger and Davis [20] observed a slight increase in pregnancy rate (+1.5%) while using the double insemination technique, but did not recommend it as it is "impractical" for a too small benefit. The results in this paper suggest that the pregnancy failure does not arise from an estrus detection problem *per se* but from another unidentified fertility problem which might be a failure to ovulate or to an inappropriate pattern of ovarian cyclicity [21].

4. Conclusions

To summarize, during a 16 months extended lactation, the number of AI per pregnancy and the pregnancy rates after first and second AI were not different from those of the previous 10 months lactation. The number of AI per pregnancy slightly increased for a 16 months lactation. However, the number of AI of the 10 months lactation following an extended lactation was not different from that of the previous 10 months lactation. The double AI technique did not improve pregnancy rates compared with the use of one AI per estrus, and its use induced confusion as how to report the herd reproductive performance. To conclude, the reproductive performance was not influenced by delayed rebreeding, and the extended lactation had no negative effect on the reproductive performance in the following lactation. Nevertheless, further investigations with larger herds should be carried out to better assess the possible improvement in pregnancy rates with the use of extended lactation.

Acknowledgments

The authors wish to thank the Danish Council for strategic research, the Programme Commission on Health, Food and Welfare and Aarhus University for the financial support for this experiment. The staff at the Danish Cattle Research Centre is acknowledged for their highly committed effort in recording the reproductive data.

References

[1] Butler, W. R. 2000. "Nutritional Interactions with Reproductive Performance in Dairy Cattle." *Anim. Reprod. Sci.* 60: 449-57.

[2] Gilmore, H. S., Young, F. J., Patterson, D. C., Wylie, A. R. G., Law, R. A., Kilpatrick, D. J., Elliott, C. T., and Mayne, C. S. 2011. "An Evaluation of the Effect of Altering Nutrition and Nutritional Strategies in Early Lactation on Reproductive Performance and Estrous Behavior of High-Yielding Holstein-Friesian Dairy

Cows." *J. Dairy Sci.* 94 (7): 3510-26.

[3] Borman, J. M., Macmillan, K. L., and Fahey, J. 2004. "The Potential for Extended Lactations in Victorian Dairying: A Review." *Australian J. Experimental Agri.* 44 (6): 507-19.

[4] Kerbrat, S., and Disenhaus, C. 2004. "A Proposition for an Updated Behavioural Characterization of the Oestrus Period in Dairy Cows." *Appl. Anim. Behav. Sci.* 87 (3-4): 223-38.

[5] Lopez, H., Satter, L. D., and Wiltbank, M. C. 2004. "Relationship between Level of Milk Production and Estrous Behavior of Lactating Dairy Cows." *Anim. Reprod. Sci.* 81 (3-4): 209-23.

[6] Schindler, H., Eger, S., Davidson, M., Ochowski, D., Schermerhorn, E. C., and Foote, R. H. 1991. "Factors Affecting Response of Groups of Dairy Cows Managed for Different Calving Conception Intervals." *Theriogenology* 36 (3): 495-503.

[7] Butler, W. R., Everett, R. W., and Coppock, C. E. 1981. "The Relationships between Energy Balance, Milk Production and Ovulation in Postpartum Holstein Cows." *J. Anim. Sci.* 53 (3): 742-8.

[8] Walsh, S. W., Williams, E. J., and Evans, A. C. 2011. "A Review of the Causes of Poor Fertility in High Milk Producing Dairy Cows." *Anim. Reprod. Sci.* 123 (3-4): 127-38.

[9] Gaillard, C., Barbu, H., Sørensen, M. T., Sehested, J., Callesen, H., and Vestergaard, M. 2016. "Milk Yield and Estrous Behavior during Eight Consecutive Estruses in Holstein Cows Fed Standardized or High Energy Diets and Grouped according to Live Weight Changes in Early Lactation." *J. Dairy Sci.* 99 (4): 3134-43.

[10] Gaillard, C., Vestergaard, M., Weisbjerg, M. R., and Sehested, J. 2016. "Effects of Live Weight Adjusted Feeding Strategy on Plasma Indicators of Energy Balance in Holstein Cows Managed for Extended Lactation." *Animal* 10 (4): 633-42.

[11] Gaillard, C., Friggens, N. C., Taghipoor, M., Weisbjerg, M. R., Lehmann, J. O., and Sehested, J. 2016. "Effects of an Individual Weight-Adjusted Feeding Strategy in Early Lactation on Milk Production of Holstein Cows during Extended Lactation." *J. Dairy Sci.* 99 (3): 2221-36.

[12] R Development Core Team. 2014. *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

[13] Larsson, B., and Berglund, B. 2000. "Reproductive Performance in Cows with Extended Calving Interval." *Reprod. Domest. Anim.* 35 (6): 277-80.

[14] Christiansen, H. C., Danfær, A., and Sehested, J. 2005. "The Cow's Reaction to Differences in Planned Calving Interval and Energy." In *Organic Milk-Feeding and Management of High Self-Sufficiency*, edited by Kristensen, T. Tjele: Forskningscenter for Økologisk Jordbrug, 16. (in Danish)

[15] Bertilsson, J., Berglund, B., Ratnayake, G., Svennersten-Sjaunja, K., and Wiktorsson, H. 1997. "Optimising Lactation Cycles for the High-Yielding Dairy Cow: A European Perspective." *Livest. Prod. Sci.* 50 (1-2): 5-13.

[16] Gaillard, C., Martin, O., Blavy, P., Friggens, N. C., Sehested, J., and Phuong, H. N. 2016. "Prediction of the Productive and Reproductive Lifetime Performance of Holstein Cows Managed for Different Lactation Durations, Using A Model of Lifetime Nutrient Partitioning." *J. Dairy Sci.* 99: 1-10. http://dx.doi.org/10.3168/jds.2016-11051. (in Press)

[17] Kolver, E. S., Roche, J. R., Burke, C. R., Kay, J. K., and Aspin, P. W. 2007. "Extending Lactation in Pasture-Based Dairy Cows: Part I, Genotype and Diet Effect on Milk and Reproduction." *J. Dairy Sci.* 90 (12): 5518-30.

[18] Wilcox, C. J., and Pfau, K. O. 1958. "Effect of Two Services during Estrus on the Conception Rate of Dairy Cows." *J. Dairy Sci.* 41: 997.

[19] Stevenson, J. S., Call, E. P., Scoby, R. K., and Phatak, A. P. 1990. "Double Insemination and Gonadotropin-Releasing Hormone Treatment of Repeat-Breeding Dairy Cattle." *J. Dairy Sci.* 73 (7): 1766-72.

[20] Trimberger, G. W., and Davis, H. P. 1943. "Conception Rate in Dairy Cattle by Artificial Insemination at Various Stages of Estrus." *Neb. Agr. Exp. Sta. Res. Bull.* 129: 1.

[21] Royal, M., Mann, G. E., and Flint, A. P. 2000. "Strategies for Reversing the Trend towards Subfertility in Dairy Cattle." *Vet. J.* 160 (1): 53-60.