The Effect of Uterine Biopsy on Reproductive Performance of Dairy Cattle: A Case-Control Study

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ABSTRACT

The objective of this study was to evaluate the effect of uterine biopsy on the reproductive performance of dairy cows in commercial herds. Fifty four Israeli Holstein cows in four dairy herds were randomly allocated for uterine biopsy before first service and 157 control cows were paired-matched to the biopsy cows. Reproductive performance of biopsy cows was not poorer than that of the control cows. Biopsy cows did not differ significantly from the control cows in the first artificial insemination conception rates, 44.4% and 38.9%, respectively, and for pregnancy rate at 150 Days in Milking (DIM), 55.6% and 45.2%, respectively. Using survival analysis to compare time to pregnancy, biopsy and control cows did not differ significantly in their pregnancy rates until 150 DIM (log-rank test, P =0.246). These results suggest that uterine biopsy done in post involution cows is not a detrimental procedure and does not affect reproductive performance.

Keywords: Cattle; Dairy; Uterus; Biopsy; Reproduction

INTRODUCTION

For years, reproductive performance has been a major economical and management issue in dairy cow farming. While milk yield is on the rise, conception and pregnancy rates have remained low or have even declined worldwide. Reproduction is a major part of veterinary chores and frustrations, an important part of the veterinarian work load in a herd (1), and has a foremost influence on a farm's financial situation. Despite the introduction of different means of heat detection, ultrasonography and the wide implantation of hormonal protocols, there has been no major breakthrough to date in reproductive management. The challenge to reduce the amount of non-pregnant cows being culled, and the attempt to decrease the use of hormones calls for a search for new or renewed ideas. The understanding of the reasons for reproductive failure in some of the cyclic and the non-cyclic cows remains by and large, a mystery. Uterine biopsies are routinely used for diagnosis of reproductive failure in human gynecology and are thought to be the gold standard for endometrial evaluation in infertile women (2). Endometrial biopsies are also routinely used for diagnosis of reproductive failure in equine medicine (3-5). In bovine practice, uterine biopsies were first described by Zurgligen in 1948 and the technique and equipment for endometrial biopsy were described in 1951(6). The value of uterine biopsies as a diagnostic tool in bovine medicine has also been described in the past (7-10). Although uterine biopsy has been used for research purposes over the years (11-15), and has been recommended in some studies as a diagnostic tool for the bovine practitioner (6, 10), it has also been claimed in many studies that uterine biopsies may have a detrimental effect on reproductive parameters in dairy cows (16-20), or that uterine biopsies can harm the cows' health (21) . The objective of this study was to reevaluate the effect of uterine biopsies on the reproductive performance of dairy cows. The purpose of this study became even more stimulating in light of a recent study published in

human medicine which showed improved conception and implantation rates following uterine biopsies (22).

MATERIALS AND METHODS

Animals

Four Israeli Holstein commercial dairy herds, each consisting of 250 to 350 milking cows that calved between January 2002 and March 2003 took part in the trial. Cows were housed in loose barns and fed a total mixed ration (TMR) *ad libitum*. All four farms use computerized dairy herd management systems, including electronic milk meters, cow identification and heat detection systems (SAE Afikim, Israel, Afimilk™). The herds are milked three times per day with an average annual milk production of 10,000-12,000 kg per cow. All herds were visited at least twice weekly during the trial period by the Ambulatory Clinic of The Koret School of Veterinary Medicine, which provided a comprehensive herd-health program in all herds. Clinical, reproduction, production and management data were collected and stored using computerized dairy farm management software (NOA, Israel Cattle Breeders Association) by the herd manager and by the attending veterinarians.

Clinical evaluation

Body condition score (BCS) was evaluated by the attending veterinarian three times per lactation: at 5-14 days postpartum, at 50-60 days from calving and prior to drying off (at 190- 200 days pregnant). All farms used only artificial insemination (AI) performed by trained technicians employed by the Artificial Insemination Cooperative (Sion Co. Israel). Heat detection was based on observation and a computerized pedometry system (SAE Afikim, Israel, Afimilk™). Synchronization protocols were not used during the trial period. Pregnancy diagnosis was performed by transrectal palpation of the uterus 40-50 days after insemination.

Using the brand numbers of the cows, fifty four cows were randomly allocated for uterine biopsy. The average time of biopsy from calving was 66.9 days (ranging from 44 to104 days). One hundred and fifty seven control cows were paired-matched to the biopsy cows using the following criteria: farm, parity, twinning, postpartum uterine diseases (UD) and calving season (summer or winter).

UD included retained placenta and endometritis. Retained placenta was defined as retained fetal membranes at 24 hours or more after parturition. Post-partum endometritis was diagnosed by the veterinarian using both vaginal and rectal examinations at 5-12 days after calving, and was defined as fetid smell, murky, watery, or pussy uterine discharge, combined with a large, thin walled, gas or excess fluid filled uterus. All cows with daily milk yield of less than 30 kg or poor appetite were tested for ketoneuria with a disposable plastic catheter and a ketone test strip (Ketostix®, Bayer, Holland). Cows with urine acetoacetate concentration of ≥ 15 mg/dl were recorded as being ketotic.

Uterine Biopsies

All biopsies were performed by the same investigator using uterine biopsy forceps (Uterus biopsy forceps 54 cm, H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany). The forceps were guided with one hand covered by a sterile latex glove over a rectal sleeve through the vagina into the cervix, and then by the otehr hand in the cow's rectum through the cervix and into the uterus. The biopsy site was directed with one hand in the cow's rectum at the dorsal aspect of the right uterine horn roughly half way between the uterine body and the cranial tip. Special care was taken to include in the sample only the endometrial layer. After each cow the biopsy forceps were soaked in 10% povidone iodine solution (Polydin®, Dr. Fisher, Israel) for 5 minutes and rinsed with sterile saline solution before the next cow, and washed with sterile water and autoclaved at the end of each day of use.

Data and data analysis

Cows' data were collected on the farms' management software (NOA, Israel Cattle Breeders Association). The data were exported to a Microsoft Excel (version 2000, Microsoft, Redmond, WA) spreadsheet. Statistical analysis was done using SPSS 10.0.1 (SPSS Inc., Chicago IL, USA).

The effect of the biopsy on the first AI conception rate and on the rate of non-pregnant cows at 150 DIM (Empty at 150 DIM) was calculated using binary logistic regression models. Uterine biopsy was included in the models as an independent variable and various parameters and disease conditions were tested in the models as possible confounders. These included; length of the dry period, BCS, calving season (summer: April through August; winter: September through March), twin calving, ketosis, milk fever, and displaced abomasums, stillborn calves and induction of calving. Time

from biopsy taking to first AI was calculated and included in the model as a possible confounder. The final models for first AI conception effects and cows empty at 150 DIM were built in two steps. The various possible confounders were tested in a univariate binary logistic model, with entry criteria set at $P<0.10$. To build the final models, the biopsy variable was forced into the models and subsequent covariates were analyzed by forward stepwise procedure with exit criteria set at $P>0.1$. For all analysis, values of $P<0.05$ were considered significant.

Using time to pregnancy (Days open) as time variables, Kaplan-Meier survival analysis was performed to evaluate the effect of uterine biopsy. Cows contributed a maximum of 150 days to the analysis and observations were censored when a cow died, culled or was excluded from breeding.

RESULTS

Control and biopsy cows data

The data of the control cows were comparable and matched the biopsy cows in all criteria except for calving season (Table 1). Despite the fact that a larger portion of biopsy cows calved during the hot and humid Israeli summer, their reproductive performance was not worse than that of the control cows as seen in the binary logistic regression models. No injuries to the uterus or the cows were recorded. Neither did the biopsy cows develop any noticeable adverse reactions following the procedure. Survival analysis compared time to first AI for biopsy and control cows (data not shown). Using the log-rank test (P =0.434) the biopsy cows and control cows did not differ significantly in their rest period.

Table 1: Descriptive statistics of the biopsy and control cows.

Criteria	Biopsy	Control
N	54.0	157.0
Farm: a	24	68
b	9	33
c	13	26
d	8	30
1st lactation (%)	29.6	28.7
2nd lactation (%)	18.5	19.1
≥ 3rd lactation (%)	51.9	52.2
PP Endometritis (%)	24.1	20.4
Retained placenta (%)	20.4	19.1
Ketosis (%)	9.3	4.5
Summer calving (%)	63.0	40.1
BCS 50-60 DIM: mean	2.699	2.714
SD	0.317	0.458
DIM at biopsy	66.9	
range 44 - 104		
Biopsy to AI interval mean	40.5	
Range 5 - 111		
Days to first AI: mean	107.4	104.6
SD	23.7	23.7
1st AI Conception rate (%)	44.4	38.9
Days Open	147.3	150.8
Open at 150 DIM (%)	44.4	54.8

First Insemination conception rate

Factors effecting conception rate in the first insemination were analyzed using a binary logistic regression model (Table 2). Biopsy cows did not differ significantly from the control cows in the first AI conception (P =0.538). Cows with UD had odds ratio (OR) of 0.519 to conceive in the first AI compared with cows without UD, (P =0.034, Table 2). Increasing body condition score at 50-60 days in milk tended to improve first AI conception rate (OR = 2.083 for additional BCS point, P=0.093 Table 2).

Table 2: Summary of binary logistic regression model of the effects of uterine biopsy on conception rates from first insemination.

Factor	β	S.E.	df	Sig.	OR	95% CI for OR
Farm A	-0.358	0.421	1	0.394	0.699	0.306 – 1.594
B	0.083	0.516	1	0.872	1.087	0.395 – 2.988
C	0.939	0.492	1	0.056	2.557	0.976 – 6.700
D (reference)				0.018		
Uterine diseases	-0.655	0.308	1	0.034	0.519	0.284 – 0.951
BCS 50-60 DIM	0.734	0.437	1	0.093	2.083	0.885 – 4.902
Biopsy	0.206	0.335	1	0.538	1.229	0.637 – 2.370

Cows not pregnant at 150 DIM.

Uterine biopsy cows, although not significant, tended to have a higher pregnancy rate at 150 DIM. The OR of biopsy cows to be empty at 150 DIM was 0.614 (P =0.146, Table 3). Significant factors affecting pregnancy rate at 150 DIM were parity and body score at 50-60 days in milk (Table 3). The first and second lactation cows had higher pregnancy rates at 150 DIM than third and above lactation cows (Table 3). Cows with higher body score at 50-60 DIM had also higher pregnancy rate at 150 DIM (OR to be empty =0.280 for additional BCS point, P=0.006, Table 3).

Table 3: Summary of binary logistic regression model of the effects of uterine biopsy on the rates of cows not pregnant at 150 DIM.

Factor	β	S.E.	df	Sig.	OR	95% CI for OR
Biopsy	-0.489	0.336	1	0.146	0.614	0.317 - 1.186
Parity 1	-0.942	0.350	1	0.007	0.390	0.196 - 0.774
2	-1.035	0.386	1	0.007	0.355	0.167 - 0.757
3	(reference)			0.004		
BCS 50-60 DIM	-1.274	0.467	1	0.006	0.280	0.317 - 1.186

Time to pregnancy

Survival analysis compared time to pregnancy for biopsy and control cows. Using the log-rank test (P =0.246, Fig. 1) the biopsy cows and control cows did not differ significantly in their pregnancy rates until 150 DIM.

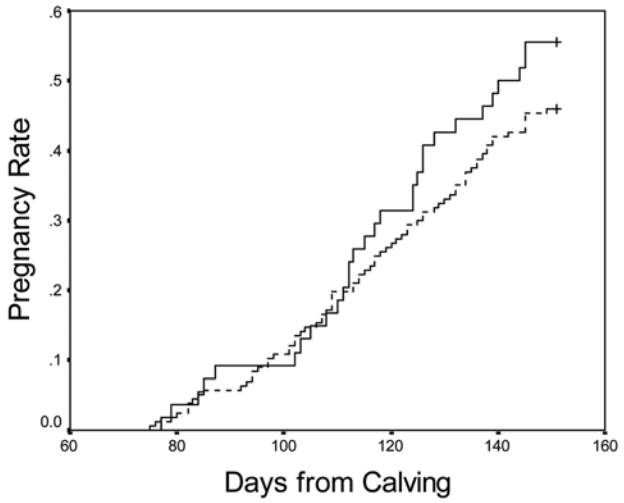

Fig. 1. Kaplan-Meier survival analysis of pregnancy rates of biopsy (–) and control cows (--). Biopsy versus control cows did not differ significantly using the log-rank test (P =0.246).

DISCUSSION

This study shows that performing uterine biopsy can be done with no harm to dairy cows' health or reproductive performance. However the procedure is invasive and has potential risks of damage and contamination and therefore should be performed only by a skillful and cautious operator. One of the most important economical indices on a dairy farm is the average number of days open. In this study, the biopsy cows had fewer days open compared with the control group cows (non-biopsy). The pregnancy rate of the biopsy cows at 150 DIM was higher as well. In the past, most publications claimed that uterine biopsies had a detrimental effect on the future reproductive performance of a cow (16-19). These authors cite Bonnett *et al.* (16), who mentions previous studies in her work (20). Etherington *et al.*, in the cited study, showed that uterine biopsies at 26 DIM resulted in a significant increase in calving to conception intervals. In that study the first AI conception rate was not affected by the biopsies. It is also mentioned in that study that a small group of cows that underwent uterine biopsy at 40 DIM had a shorter calving to conception interval by approximately 19 days. The size of this group was small (n=8) and no statistical analysis was done on the days open (20). Another possible reason for a negative effect of biopsy could be the number of biopsies done on each cow. In our study, only one biopsy was taken from each cow, whereas Etherington *et al.* took two biopsies from each cow, some within a short period of time (on the 26 and on the 40 DIM).

In our study, the time period between calving and the performance of the biopsy was lengthier than that described by Etherington *et al.*, (20) (average of 66.9 DIM). This longer time period may explain the difference in the outcomes. It may be assumed that uterine biopsy performed before full completion of the uterine involution, may harm or slow down complete recovery of the endometrium and future reproductive performance of cows. On the other hand, a uterine biopsy performed after completion of involution, might have a positive effect on conception and maintenance of pregnancy. Positive endometrial response could be time dependent. A similar positive effect of uterine biopsy on fertility was found in human medicine (22). In that study, uterine biopsy was performed in infertile women all of which were treated hormonally and underwent *in vitro* fertilization (IVF) or embryo-transfer (ET). The study showed that a limited and controlled injury to the endometrium in women may provoke a reaction which will lead to a better embryo implantation, pregnancy rate and rate of take-home babies. The mechanism, which is not fully understood, is suggested to be by a massive secretion of different of cytokines and growth factors that are involved both in implantation and in wound healing, or by involvement of histamines that are released by the uterine tissue in response to the biopsy trauma (22).

In the present study the biopsy was done before the first

AI thus there was no previous knowledge regarding existing infertility disorders or future anestrus. The cows were neither hormonally treated nor underwent ET. However, the favorable effect of uterine biopsies in the human study calls for a further investigation of this phenomenon.

CONCLUSION

Endometrial biopsy in dairy cows taken after completion of uterine involution can be used without harm for both the diagnosis and research of infertility. The procedure is not detrimental and does not affect reproductive performance.

ACKNOWLEDGMENTS

This study was supported by the Israeli Milk Board Research Fund. We wish to thank the managers and workers of the dairy farms for their efforts and support.

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