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Uterine Bacteriology, Histology, Resumption of Ovarian Activity and Granulocyte Function of the Postpartum Cow in Different Milking Frequencies

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[1]Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Centre for Reproductive Biology, and [3]Department of Animal Nutrition and Management, Faculty of Agriculture, Landscape Planning and Horticulture, Swedish University of Agricultural Sciences, [2]Swedish Dairy Association, Eskilstuna, and [4]Department of Bacteriology, Swedish National Veterinary Institute, Uppsala, Sweden.

Kask K, Gustafsson H, Magnusson U, Bertilsson J, Gunnarsson A, Kindahl H: Uterine bacteriology, histology, resumption of ovarian activity and granulocyte function of the postpartum cow in different milking frequencies. Acta vet. scand. 1999, 40, 287-297. – The postpartum uterine bacteriology, histology, resumption of ovarian activity and polymorphonuclear granulocyte (PMN) number and function in 18 Swedish dairy cows were studied. Cows were milked either 2x (n = 9) or 3x per day (n = 9). Endometrial biopsy samples for bacteriological and histological investigations were collected during 8 weeks postpartum, starting within one week after calving. Milk samples for progesterone determination were collected twice a week until the cows had shown normal reproductive cyclicity. Blood samples for granulocyte function (phagocytic capacity and total number) were collected from each animal on the same days as when the biopsies were obtained. All animals in both groups were free from bacteria at the latest after 6 weeks port portum and there was no difference regarding bacterial elimination and bacterial species between milking groups. No difference regarding uterine histology between milking groups was seen. In both groups, 8 cows had normal to slight infiltration of leukocytes in the endometrium at the end of sample collection. No changes in granulocyte function could be seen in the 2 milking groups. Resumption of ovarian activity was detected on day 45.6 ± 9.3 (mean ± SD) postpartum in the 2x milking group and 36.6 ± 9.0 (mean ± SD) post-partum in the 3x milking group ($p = 0.05$). Based on our findings, an increased milking frequency from 2 to 3 times a day did not influence the uterine function postpartum.

Key words: post-partum period; uterine histology; polymorphonuclear granulocytes; phagocytic capacity.

Introduction

During the postpartum period (PP) the dairy cow should re-establish normal uterine and ovarian activities. Thus, a better understanding of the patho-physiology of these organs is important since failure to return to normal function may delay the first postpartum ovulation and cause prolongation of the uterine involution (*Lamming et al.* 1981, *Kindahl et al.* 1982, *Lindell et al.* 1982, *Thatcher et al.* 1982, *Thompson et al.* 1987, *Kindahl et al.* 1992).

Several studies have been published regarding microbiology of the postpartum bovine genital tract (*Rasbech* 1950, *De Bois* 1961, *Elliott et al.* 1968, *Griffin et al.* 1974ab, *Studer & Morrow* 1978, *Fredriksson et al.* 1985, *Bekana et al.* 1994, *Bekana et al.* 1996). All studies agree in principle, but they differ about the incidence of

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infected cows and the duration of the infection. The inconsistent results may be due to different managemental and environmental conditions, general hygiene, selection of the material and sampling technique. Recent studies have shown that less aerobic and more anaerobic bacteria have been isolated (*Bekana* 1996).

Prolonged anoestrous in milked and multiple suckled cows involves an extension of one or more of the characteristic PP phases, most commonly a prolonged depression of plasma gonadotropins, especially of luteinizing hormone (LH) (*Lamming et al.* 1982). An increased machine milking frequency might also affect fertility and delay resumption of ovarian function after calving. These effects are mediated over the hypothalamus-pituitary-gonadal axis and in some extent on a higher demand on the metabolic system. Furthermore, it has been shown that the risk of silent heat, ovarian cysts and uterine problems increased with increasing milk production (*Saloniemi et al.* 1986, *Gröhn et al.* 1990, *Gröhn et al.* 1994). It is documented that milk production is generally higher for cows milked 3x versus 2x (*Bertilsson et al.* 1997) and thus the risks mentioned above may increase. Also it is known that incidence of disease is increased around parturition due to decreased host defence mechanisms (*Lloyd* 1983, *Hussain* 1989, *Tian-Quan et al.* 1994, *Lewis* 1997).

There are no studies performed about the influence of increased milking frequency after calving on uterine involution and elimination of bacteria. Since increased milking frequency gives a higher milk production, it should be important to study milking frequency per se in animals under good management conditions and with no negative energy balance.

In this study, 2 groups of cows milked 2 times (2x) and 3 times (3x) daily were investigated. The aims of the study were to evaluate postpartum uterine involution, based on bacteriological findings and postpartum histology of the endometrium, and to study some granulocyte function parameters in postpartum cows in different milking frequencies. Milk progesterone was also used to determine the resumption of ovarian activity in these cows.

Materials and methods

Animals

Experiments were carried out in 18 cows of different age and parity. They were all of the Swedish Red and White (SRB) breed group. The cows belonged to one herd of the Experimental Farm Kungsängen, Swedish University of Agricultural Sciences (SLU) in Uppsala. Cows from this particular farm were divided into 2 milking frequency groups, 2x and 3x per day. The average production in the farm is 9000 kg energy corrected milk (ECM). Cows from the 3x milking group produced 10400 kg ECM (about 15% more than the 2x group). In the present study, 9 cows from each milking group were used. Average time from calving to first insemination in this herd was around 50 days. Average parity and age of the cows used was 2.3 and 4.2 years in the 2x milking group and 2.6 and 4.6 years in the 3x milking group.

The cows were confined to individual stalls without tying but were milked in a milking parlour. They were fed ad libitum with a mixed ration. Ration consisted of grass silage 40%, hay 10% and concentrate 50% (6 kg). Normal calving traits were recorded in all 18 animals. Two cows needed minor assistance during calving, but the parturitions were nonetheless considered normal. During the experimental period cows were not allocated to artificial inseminations.

Bacteriology

Each animal was sampled for bacteriological examination once a week (Wednesday) starting within 10 days after calving and continuing up to 8 weeks postpartum (PP). A total of 125 en-

dometrial biopsies were aseptically collected according to the following methods. The cow was restrained, the faeces were removed from the rectum and the tail was secured. The perineal area and the vulva were washed with soap and water. Next the vulvar area and the vestibulum were disinfected with a mild iodophore. The vulvar lips were parted to introduce an outer protective stainless steel tube as described by *Fredriksson et al.* (1985). The instrument was advanced into the vagina and fixed in the external opening of the cervix. A long guarded biopsy instrument was advanced into the uterus by cervical manipulation (*Messier et al.* 1984, *Fredriksson et al.* 1985, *Bekana et al.* 1994). The instrument was opened and a milled cavity about 2 cm in length, 0.5 cm from the tip, was located with the forefinger of the hand in rectum. This cavity with its sharpened edge, formed a curette to obtain biopsies from the endometrium.

The biopsies were immediately placed in thioglycolate medium for transportation to the laboratory for bacteriological examination. They were cultured within 2 h. Each sample was inoculated on a blood-agar plate and a lactose agar plate for aerobic incubation and on a Fastidious Anaerobe Agarplate for anaerobic incubation in a cabinet (Model 1024/1028, Forma Scientific, AB Nino Lab. Stockholm, Sweden) in an atmosphere of nitrogen/carbon dioxid/hydrogen (80/10/10) in the presence of a palladium catalyst. All plates were incubated at 37°C. The plates for aerobic cultivation were read after 24 and 48 h and the plates for anaerobic cultivation after 48 and 168 h. Isolated bacterial strains were identified according to Bergey's Manual of Systemic Bacteriology (*Holt et al.* 1994) at the Department of Veterinary Microbiology, Section of Clinical Microbiology, Swedish University of Agricultural Sciences and the Department of Bacteriology, Swedish Veterinary Institute.

Histology

From each animal in this experiment, samples were obtained for histological examination, starting within 10 days after calving and continuing up to 8 weeks PP. All endometrial biopsies were collected in the same manner as described in the bacteriology section.

Histological biopsies were placed in 10% formol saline solution. After fixation, tissues were trimmed, embedded in parafin, sectioned at 6μm and stained with haematoxylin and eosin. Investigations were made under microscope for presentation of inflammatory cells in the endometrium. Infiltration of cells was diagnosed as normal to slight (0 to 29 cells per field), medium (30 to 80 cells per field) or dense (>80 cells per field) based on the presence of neutrophils in the biopsy specimen. The diffuse cellular reaction involving also the mononuclear cells was assessed by counting the inflammatory cells in 6 high power fields chosen at random and calculating the average number of cells per high power field (Griffin et al. 1974a).

Blood sampling and blood cell counts

Blood samples were collected in evacuated sampling tubes with heparin (for analysing the phagocytic capacity of polymorphonuclear leukocytes (PMN)) or EDTA tubes (for the total number of white blood cells (WBC) and counting differential counts). The samples were taken by coccygeal venipuncture, starting on the same day as the first biopsy and collection was done once a week and continued until 8 weeks after parturition. For the cell parameters, the analyses were performed within 90 min after the collection.

The total number of WBC was counted in an electronic cellcounter (Sysmex F-800; TOA Medical Electronics, Kobe, Japan) and differential WBC counts were made by microscopic counting of 100 cells from blood smears stained with May Grünewald Giemsa solutions accord-

ing to the standard procedures at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences.

Assessment of the phagocytic capacity of polymorphonuclear granulocytes

The phagocytic capacity was assessed by measuring the chemiluminescence following phagocytosis of opsonized zymosan particles according to *Magnusson & Holst* (1998). Briefly, samples and negative controls were set in triplicates on a micro-titre plate. The samples contained luminol, PBS, pooled allogenic serum and zymosan. Zymosan was omitted in the controls. Zymosan was allowed to be opsonized for 15 min in darkness at room temperature. Then 20 μl blood was added to all wells and the plate was immediately placed into the pre-warmed luminometer (ML 3000, Dynatech Laboratories, In Vitro, Solna, Sweden). The light emission (relative light units, RLU) from the chemiluminescence was monitored at intervals of 45 sec for 60 cycles at a temperature of 38°C.

Progesterone sampling and analysing

Milk samples for progesterone determination were collected twice weekly (Monday and Thursday), commencing the second week after parturition until the cows had shown normal reproductive cyclicity. Thereafter, the frequency of sampling was reduced to once a week until the end of the experimental period. The minimum progesterone value for the detection of ovarian activity was considered to be 6.7 nmol/l (*Laitinen* 1983). Milk sampling was performed within 60 min after the morning milking. Samples from each cow were collected into plastic tubes containing 100 μl of preservative (Bronopol 2% + MTB 0.05%) and stored at 4°C until assay. The content of milk progesterone was determined by RIA (Spectria, Orion Diagnostica, Espoo, Finland). The interassay coefficient was below 14% and the intraassay coefficient was below 7%. Detection limit of the assay was 1 nmol/l.

Detection of ketone bodies in the milk

Milk acetone as a marker of ketone bodies, was determined with a flow injection analysis method (FIA) described by *Marstorp et al.* (1983). A milk acetone value above 0.7 mM was deemed as ketonaemia according to *Gustafsson* (1993).

Statistical analysis

Statistical analysis of the data was performed using the Statistical Analysis System (*SAS Institute Inc.* 1994). Normality of distribution of the data and studies over time were analysed using Univariate procedure. The General Linear Model (GLM) procedure for analysis of variance was used. Individual variables presented in the immunological part of the results are least-square mean values obtained from GLM. For bacteriology and histology, the FREQ procedure for Chi-Square and the Mantel-Haenszel Chi-Square test were used ($p<0.05$ was considered as a significant difference).

Results

The mean milk production in the 2x milking group was 8658 Kg ECM/year and in the 3x milking group 9529 Kg ECM/year. According to statistical analysis a difference in the mean milk production between the groups was found ($p = 0.03$). During the experimental period of the 2 first months after calving, the mean milk production was 36.6 Kg ECM/day in the 2x milking group and 42.6 Kg ECM/day in the 3x milking group. A statistical difference was found between the groups ($p< 0.05$).

All animals in both groups were found negative for milk acetone during the whole experimental period (acetone concentration < 0.7 *mM*).

Table 1. Presence of bacteria in the uterus of cows milked 2 or 3 times per day respectively, during 8 weeks post partum.

Bacteria	Weeks postpartum							
	1	2	3	4	5	6	7	8
Cows milked 2x								
Aerobes								
Actinomyces pyogenes	2	4	3	2	0	0	0	0
Others	0	5	3	2	2	1	0	0
Total	2	9	6	4	2	1	0	0
Anaerobes								
Bacteroides spp.	1	2	1	0	0	0	0	0
Fusob. necrophorum	0	1	0	0	0	0	0	0
Others	1	0	1	1	0	0	0	0
Total	2	3	2	1	0	0	0	0
Aerobes + Anaerobes	4	12	8	5	2	1	0	0
Cows milked 3x								
Aerobes								
Actinomyces pyogenes	0	1	1	1	0	1	0	0
Others	3	2	2	3	2	0	0	0
Total	3	3	3	4	2	1	0	0
Anaerobes								
Bacteroides spp.	1	0	1	0	1	1	0	0
Fusob. necrophorum	0	0	1	1	1	1	0	0
Others	0	1	0	1	0	0	0	0
Total	1	1	2	2	2	2	0	0
Aerobes + Anaerobes	4	4	5	6	4	3	0	0

Bacteriology

The results of bacteriological examinations are presented in Table 1 and in Fig. 1. From 18 animals a total of 125 biopsies were collected, of which 37 were found to be bacteriologically positive and the remaining 88 biopsies were negative. Altogether 19 samples are missing due to difficulties of collection. From the positive samples, 12 showed mixed infections and 19 showed aerobic bacterial growth in pure cultures. Obligatory anaerobic organisms in pure cultures were not detected. The mixed cultures contained mainly *Actinomyces pyogenes, Bacteroides* spp. and *Fusobacterium necrophorum.* Totally 58 isolates were identified from the 37 bacteriological positive biopsies, amongst them being 40 aerobic and 18 obligate anaerobic bacteria. The most frequent among aerobic pathogens were *A. pyogenes, Escherichia coli* and *Streptococcus* spp. The major obligate anaerobic bacteria isolated were *Bacteroides* spp., *F. necrophorum* and *Peptostreptococcus assacharolyticus/indolicus.*

In 6 cases it was demonstrated growth of mixed colonies on the plates and these findings were considered as undefined bacteria. Contamination of the sample during collection might have taken place.

The highest incidence of bacterial growth was found during the first 4 weeks after parturition, and the largest number of bacteria was detected during 2 and 3 weeks after parturition. Around second week a sharp increase of bacterial isolates was found in the 2x milking group, which

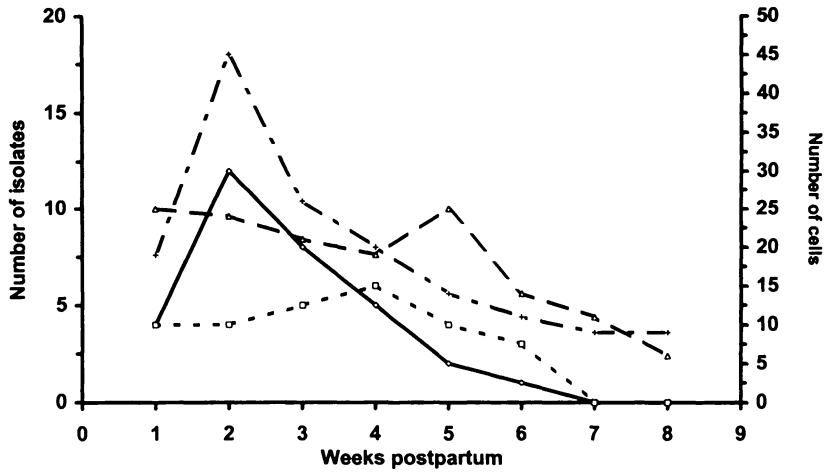

Figure 1. Presence of intrauterine bacteria in cows milked 2x per day (— ◊); 3x per day (- - - □) and arithmetic means of inflammatory cell numbers (right Y-axis) in endometrium 2x milking group (– – – △) and 3x milking group (– – – +) during 8 weeks post-partum ($p>0.05$).

was followed by a subsequent decrease. After 5-6 weeks of collection all animals were free from bacteria. During the 8 weeks of collection, altogether 4 negative animals were found. Elimination of bacteria according to milking groups can be studied in Fig.1. The total elimination of bacterial isolates is shown in Table 1. No significant difference between milking groups according to bacterial elimination was found ($p>0.05$).

Histology

A total of 113 endometrial biopsies were collected for histological examination. Due to difficulties to obtain biopsies, a total of 31 biopsies were missing. The highest incidence of cell infiltration occurred in weeks 2, 3, 4, and 5 after parturition in both milking groups. A peak in cell numbers was detected in the 3x milking group around second week PP, which was followed by a subsequent decrease and no futher peaks were detected. Dense infiltration in the 2x milking group was not detected during the 8 weeks of collection. Two dense infiltration cases were detected in the 3x milking group, but in that group the incidence of medium infiltration was somewhat lower. Generally, no significant difference between milking groups according to presence of inflammatory cells in uterine tissue biopsies was found ($p>0.05$). The real cell numbers according to milking groups are presented in Fig. 1.

Progesterone

The first elevation of progesterone considered to be of luteal origin (more than 6.7 nmol/l) was detected on day 45.6 ± 9.3 (mean ± SD) PP in the 2x milking group and on day 36.6 ± 9.0 (mean ± SD) PP in the 3x milking group. The difference in these mean values showed a tendency to be significantly different ($p = 0.05$).

Changes in number of PMN leukocytes in whole blood and phagocytic capacity

The ranges for the concentration of PMN leukocytes in the blood of cows milked 2x per day

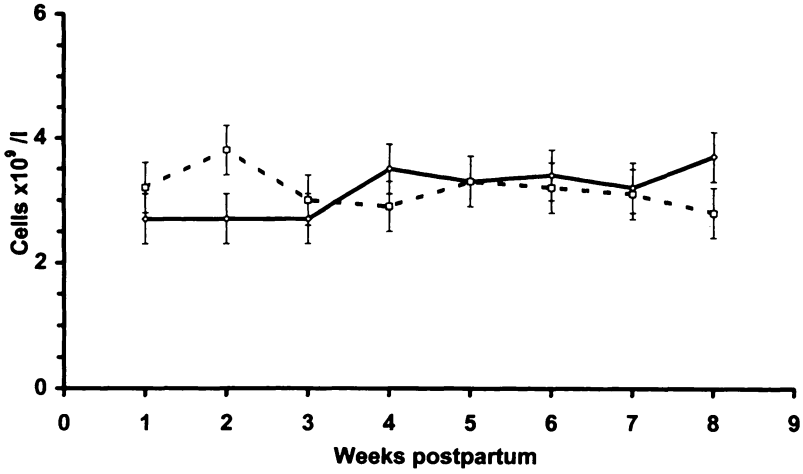

Figure 2. Number of PMN granulocytes in blood during 8 weeks post-partum in cows milked 2x per day (— ◊) and 3x per day (- - - □);(Least-squares means + S.E.M.).

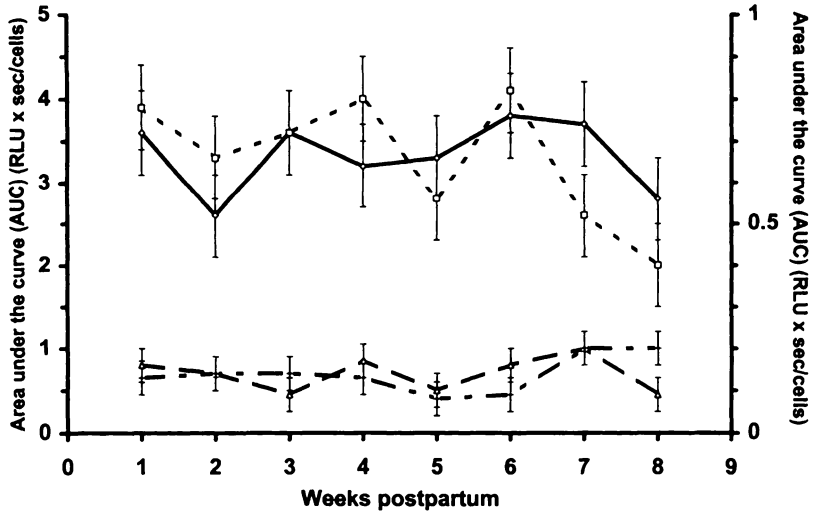

Figure 3. The light emission from spontaneous chemiluminescence (right Y-axis) in whole blood assay in cows milked 2x per day (- - - △) and 3x per day (- - - +) during 8 weeks post-partum, and chemiluminescence following phagocytosis of opsonized zymosan particles in cows milked 2x per day (— ◊) and 3x per day (- - - □) during 8 weeks post-partum; (Least-squares means + S.E.M.).

during the experiment were 2.7-3.7 x 10^9/l and 2.8-3.8 x 10^9/l in cows milked 3x per day respectively. No significant difference was found ($p>0.05$, Fig. 2).

There was no significant difference in spontaneous metabolic burst, associated mainly with the formation of reactive oxygen metabolites during the phagocytic process, and induced phagocytic capacity between the 2x and 3x per day milking groups during the experimental period ($p>0.05$). Results are presented in Fig. 3.

Discussion

Early in the post-partum period there is a fall in plasma levels of oestradiol-17β leading to restoration of pituitary sensitivity to GnRH, which is dependent on the frequency of mammary stimulation (*Lamming* 1978, *Lamming et al.* 1982). Frequent suckling or an intense suckling stimulus delay the natural manifestation of the inherent neuroendocrine rhythm controlling GnRH release. The increased milking frequency could also improve the milk let down mechanism as such leading to the similar results as in case of suckling when the resumption of ovarian activity is delayed due to suppressive effect in the hypothalamus-pituitary-gonadal axis.

It is documented that increased milk production per cow during recent years has been playing a major role in the increased incidence of treatment of silent heat, ovarian cysts and also postpartum metritis (*Erb et al.* 1985, *Saloniemi et al.* 1986, *Butler & Smith* 1989). Most of the studies have concentrated on the interaction between milk yield and postpartum ovarian activity (*Lamming* 1978, *Ferguson* 1991, *Ratnayake et al.* 1998). It is known that increased milking frequency will increase the milk production by 6-25% but at the same time feed intake does not increase to the same extent, indicating that milking frequency can affect metabolism (*Amose et al.* 1985, *Allen et al.* 1986, *Gisi et al.* 1986). The changed metabolism may lead to a decrease in the general resistance of the cow. The early postpartum period is the time when all these factors are capable of influencing the reproductive performance of the cow.

The present study, was focusing mainly on the reproductive patho-physiology of the postpartum cow such as uterine infections and general immunological parameters of the cows studied. However, compared with similar studies conducted previously (*Fredriksson et al.* 1985, *Bekana et al.* 1994, *Bekana et al.* 1996), no principal differences according to the incidence of uterine infections and presence of bacterial isolates were found. The most frequently isolated species were *A. pyogenes* together with *Bacteroides* spp. and *F. necrophorum*. Also *E. coli* and *Streptococcus* spp. were found. A peak of the numbers of bacterial isolates in the 2x milking group was detected on 2nd week PP and it was followed by a subsequent decrease. This increase could be considered as a part of the normal uterine involution with elimination of bacteria during the first 25 days PP. After 5 weeks postpartum all cows except one in the 3x milking group were free from infections, and no infected cows in the 2 groups were detected later than 6 weeks postpartum. In both milking groups, cases of undefined cultures were detected. These cases may be considered as possible contamination during sample collection, and prolonged the estimated elimination time for an additional week in 4 cows.

The histological investigations indicated that uterine infections are not a problem in cows with normal calving. In this context we agree with previous histological studies (*Hartigan et al.* 1972, *Griffin et al.* 1974a, *Kask et al.* 1998), that cows eliminating bacteria early postpartum, had a normal endometrium prior to day 50 postpartum or even earlier, provided that *A. pyogenes* and *F. necrophorum* infection was not

present. In cows milked 3x times, an increase of inflammatory cell numbers were detected on 2nd week PP. We consider this change also to be associated with the normal uterine involution. Based on this study, it seems that increased milking frequency did not influence the bacterial content or elimination or resumption of uterine activity.

It is well-known that incidence of diseases increases around and after parturition due to the decrease of host defence mechanisms (*Lloyd* 1983, *Hussain* 1989, *Tian-Quan et al.* 1994, *Lewis* 1997). It is also documented that the number of PMN leukocytes and phagocytic capacity on whole blood PMN leukocytes differ from the uterine secretion (*Vandeplassche & Bouters* 1983, *Romaniukowa* 1984, *Zerbe* 1994). In the present study only the blood PMN leukocyte phagocytic capacity was investigated, but no significant changes were detected during collection time in the 2 animal groups. On the other hand, all cows used may be considered as healthy animals because no serious uterine infections, udder infections and other diseases were detected. This suggests that increased milking frequency in cows with normal puerperium will not influence the general immunological status.

Only a tendency to a difference was found between groups according to days of first progesterone rise PP ($P = 0.05$). In the studies by *Ratnayake* (1996), no difference between the 2 milking groups was found, but longer interval was found in the time from 1st artificial insemination to conception in the 3x milking group. This parameter, however, was not followed in the present study. All animals in the present study belonged to an experimental farm, where feeding and managemental care are outstanding. Managemental problems, such as poor feeding of the cow, may play a major role in the incidence of negative energy balance after calving which may lead to delayed resumption of ovarian activity. No animals with negative energy balance were found in the present study based on the milk acetone analyses. Under these circumstances it is obviously not a drawback to milk the cow 3 times a day and that the ovulations are earlier as compared to 2 times milking frequency.

Conclusion

Results from the present study indicate that no remarkable changes were found in occurrence of uterine infections and granulocyte function parameters between cows in 2x and 3x milking groups. Thus, the increase in milking frequency from 2x to 3x is not influencing the reproductive performance in the postpartum cow.

Acknowledgments

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References

Allen DB, DePeters EJ, Laben, RC: Three times a day milking: effects on milk production, reproductive efficiency and udder health. J. Dairy Sci. 1986, *69,* 1441-1446.

Amose HE, Kiser T, Lowenstein M: Influence of milking frequency on productive and reproductive efficiencies of dairy cows. J. Dairy Sci. 1985, *68,* 732-739.

Bekana M: Clinical, ultrasonographic, bacteriological and hormonal studies in post-partum cows with particular emphasis on retained fetal membranes. Uppsala, Sweden. 1996. PhD Thesis.

Bekana M, Jonsson P, Ekman T, Kindahl H: Intrauterine bacterial findings in post-partum cows with retained foetal membranes. J. Vet. Med. A. 1994, *41,* 663-670.

Bekana M, Jonsson P, Kindahl H: Intrauterine bacterial findings and hormonal profiles in post-partum cows with normal puerperium. Acta vet. scand. 1996, *37,* 251-263.

Bertilsson J, Berglund B, Ratnayake G, Svennersten-Sjaunja K, Wiktorsson H: Optimising lactation

cycles for the high-yielding dairy cow. A European perspective. Livestock Production Sciences, 1997, *50,* 5-13.

Butler WR, Smith RD: Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci., 1989, *72,* 767-783.

De Bois CHW: Endometritis en vruchtbaarheid bij het rund. (Endometritis and fertility in the cow). Utrecht, The Netherlands. 1961. PhD Thesis.

Elliott L, McMahon KJ, Gier HT, Marion GB: Uterus of the cow after parturition: bacterial content. Am. J. Vet. Res., 1968, *29,* 77-81.

Erb HN, Smith RD, Oltenacu PA: Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in holstein cows. J. Dairy Sci., 1985, *68,* 3337-3349.

Fergusson DJ: Nutrition and reproduction in dairy cows.Veterinary Clinics of North America. Food Animal Practice, 1991, 7, No. 2: 483-507.

Fredriksson G, Kindahl H, Sandstedt K, Edqvist L-E: Intrauterine bacterial findings and release of $PGF_{2\alpha}$ in the post-partum dairy cow. Zbl. Vet. Med. A., 1985, *32,* 368-380.

Gisi DD, DePeters EJ, Pelissier CL: Three times daily milking of cows in California dairy herds. J. Dairy Sci., 1986, 69: 863-868.

Griffin JFT, Hartigan PJ, Nunn WR: Non-specific uterine infections and bovine fertility. 1. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology, 1974a, *1,* 91-106.

Griffin JFT, Murphy JA, Nunn WR, Hartigan PJ: Repetitive in vivo sampling of the bovine uterus under field conditions. Br. Vet. J., 1974b, *130,* 259-264.

Gröhn YT, Erb HN, McCulloch CE: Epidemiology of reproductive disorders in dairy cattle: associations among host characteristics, disease and production. Prev. Vet. Med., 1990, *8,* 25-39.

Gröhn YT, Hertl JA, Harman JL: Effect of early lactation milk yield on reproductive disorders in dairy cows. Am. J. vet. Res., 1994, 55, No. 11: 1521-1527.

Gustafsson AH: Acetone and urea concentration in milk as indicators of the nutritional status and the composition of the diet of dairy cows. Uppsala, Sweden. 1993. PhD Thesis.

Hartigan PJ, Murphy JA, Nunn WR, Griffin JFT: An investigation into the causes of reproductive failure in dairy cows. I. Gross and microscopic observations on the genitalia of slaughtered non-pregnant cows. Irish vet. J., 1972, *26,* 225-228.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST: Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co, Baltimore. 1994, 9th ed., 299 pp.

Hussain AM: Bovine uterine defence mechanisms: a review. J. Vet. Med. B, 1989, *36,* 641-651.

Kask K, Kindahl H, Gustafsson H: Bacteriological and histological investigations of the postpartum bovine uterus in two Estonian dairy herds. Acta vet. scand. 1998, *39,* 423-432.

Kindahl H, Edqvist L-E, Larsson K, Malmqvist Å: Influence of prostaglandins on ovarian function post-partum. In: Karg H, Schallenberger E (Eds.): Current Topics in Veterinary Medicine and Animal Science, 1982, Vol. 20, Martinus Nijhoff Publishers. The Hague/Boston/London: 173-196.

Kindahl H, Odensvik K, Aiumlamai S, Fredriksson G: Utero-ovarian relationships during the bovine post-partum period. Anim. Reprod. Sci., 1992, *28,* 363-369.

Laitinen J: Oestrus confirmation, pregnancy diagnosis and postpartum ovarian follow-up of the Finnish dairy cows by milk progesterone assay. University of Kuopio, Finland. 1983. PhD Thesis. 110 pp.

Lamming GE: Reproduction during lactation. In: Crighton DB, Foxcroft GR, Hayness NB, Lamming GE (Ed.). Control of Ovulation 1978, 335-353.

Lamming GE, Peters AR, Riley GM, Fisher MW: Endocrine regulation of postpartum function. In: Karg H, Schallenberger E (Eds.): Current Topics in Veterinary Medicine and Animal Science, 1982, Vol. 20. Martinus Nijhoff Publishers. The Hague/Boston/London: 148-173.

Lamming GE, Wathes DC, Peters AR: Endocrine patterns of the post-partum cow. J. Reprod. Fert., Suppl., 1981, *30,* 155-170.

Lewis GS: Uterine health and disorders. J. Dairy Sci., 1997, *80,* 984-994.

Lindell J-O, Kindahl H, Jansson L, Edqvist L-E: Post-partum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. Theriogenology, 1982, *17,* 237-245.

Lloyd S: Effects of pregnancy and lactation upon infection. Vet. Immunol. Immunopathol., 1983, *4,* 153-176.

Magnusson U, Holst H: Assaying granulocyte phagocytosis by chemiluminescence: effect of storage, time and temperature of blood samples. J. Vet. Med. B. 1998, *45,* 217-222.

Marstorp P, Anfält T, Andersson L: Determination of

oxidized ketone bodies in milk by flow injection analysis. Anal. Chim. Acta, 1983, *149,* 281-289.

Messier S, Higgins R, Couture Y, Morin M: Comparison of swabbing and biopsy for studying the flora of the bovine uterus. Can. Vet. J., 1984, *25,* 283-288.

Ratnayake DRTG: Management and reproduction in dairy cows. Effects of increased milking frequency and extended calving interval. Uppsala, Sweden. 1996. Licentiate Thesis. 28 pp.

Rasbech NO: Den normale involutio uteri hos koen. (Normal uterine involution in the cow). Nord. VetMed., 1950, *2,* 655-687.

Ratnayake DRTG, Berglund B, Bertilsson J, Forsberg M, Gustafsson H: Fertility in dairy cows managed for calving intervals of 12, 15 or 18 months. Acta vet. scand., 1998, *39,* 215-228.

Romaniukowa K: Phagocytosis in the uterus. Proc: 10th Int. Cong. Anim. Reprod. Artif. Insem., 1984, 4-XI, 33-38.

Saloniemi H, Gröhn YT, Syväjärvi J: An epidemiological and genetic study on registered diseases in Finnish Ayrshire cattle. II. Reproductive disorders. Acta vet. scand., 1986, *27,* 196-208.

SAS Institute Inc.: SAS/STAST®, Guide for personal computers, Version 6 edn. SAS Institute Inc., Cary, NC, 1994.

Studer E, Morrow DA: Post-partum evaluation of bovine reproductive potential: comparison of findings from genital tract examinations per rectum, uterine culture, and endometrial biopsy. J. Am. Vet. Med. Ass., 1978, *172,* 489-494.

Thatcher WW, Guilbault LA, Collier RJ, Lewis GS, Drost M, Knickerbocker J, Foster DB, Wilcox CJ: The impact of antepartum physiology on postpartum performance in cows. In: Karg H, Schallenberger E (Eds.): Current Topics in Veterinary Medicine and Animal Science, 1982, Vol. 20, Martinus Nijhoff Publishers. The Hague/Boston/London: 3-24.

Thompson FN, Page RD, Cook CB, Caudle AB: Prostaglandin $F_{2\alpha}$ metabolite levels in normal and uterine-infected post-partum cows. Vet. Res. Comm., 1987, *11,* 503-507.

Tian-Quan C, Weston PG, Lund LA, Brodie B, McKenna DJ, Wagner WC: Association between neutrophil function and periparturient disorders in cows. Am. J. Vet. Res., 1994, *55,* 934-943.

Vandeplassche M, Bouters R: Phagocytosis in the blood and uterine exudate of mares, cows and sows. Proc. 3rd Intern. Symp. Vet. Lab. Diagnostic Ames, 1983, I, 83-89.

Zerbe H: Functional and phenotypic investigations with bovine neutrophilic granulocytes from blood and uterus. Hannover, Tierärztliche Hochschule Germany. 1994, 62-64.

Sammanfattning

Bakteriologiska och histologiska förändringar i livmodern, samt återupptagandet av äggstocksaktivitet i postpartalperioden hos mjölkkor med olika mjölkningsfrekvenser

I postpartalperioden undersöktes bakteriologiska och histologiska förandringar, normalisering av äggstocksfunktioner, samt granulocyternas (PMN) antal och funktion hos 18 kor av svensk röd och vit boskap. Korna mjölkades antingen 2 gånger dagligen (n = 9) eller 3 gånger dagligen (n = 9). För att studera bakteriologi och histologi i livmoder togs endometriebiopsier varje vecka under 8 veckor, med start inom en vecka efter kalvningen. Mjölkprover samlades 2 gånger i veckan och analyserades med avseende på progesteroninnehåll tills korna uppvisade normal äggstockscyklicitet. Samtidigt med biopsierna togs blodprov för studier av granulocytfunktion (totalantal och fagocytoskapacitet). Korna var fria från bakterier i livmodern senast 6 veckor efter kalning och det fanns ingen skillnad i bakterieeliminationen eller bakteriearter mellan de 2 mjölkningsfrekvens-gruppena. Vidare kunde ingen skillnad i livmoderhistologi påvisas. I både grupperna hade 8 djur en normal eller mycket obetydlig leukocytinfiltration vid försökets slut. Granulocyternas funktion och antal var lika i de båda grupperna. Återupptagandet av äggstockarnas funktion (höga progesteronvärden) syntes 45.6 ± 9.3 (medelvärde ± SD) dagar efter kalvingen i gruppen som mjölkades 2 gånger dagligen. Motsvarande siffror för gruppen som mjölkades 3 gånger dagligen var 36.6 ± 9.0 dagar. Denna skillnad tenderar att vara signifikant (p = 0.05). Slutsatsen från försöket är att en ökad mjölkningsfrekvens från 2 till 3 gånger dagligen inte påverkar livmoderns eller äggstockarnas funktioner i en negativ riktning.

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