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Assessment of Early Postpartum Reproductive Performance in Two High Producing Estonian Dairy Herds

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Kask K, Kurykin J, Lindjärv R, Kask A, Kindahl H: Assessment of early postpartum reproductive performance in two high producing estonian dairy herds. Acta vet. scand. 2003, 44, 131-143. – Early postpartum (6 weeks) ovarian activity, hormonal profiles, uterine involution, uterine infections, serum electrolytes, glucose, milk acetoacetate and blood urea nitrogen (BUN) levels were studied in 2 Estonian high producing dairy herd with annual milk production of 7688 (Farm A) and 9425 (Farm B). From each farm 10 cows, with normal calving performance were used. Blood samples for the hormonal ($PGF_{2\alpha}$-metabolite, progesterone) analyses were withdrawn. On day 25 PP blood serum samples were taken for the evaluation of metabolic/electrolyte status. On the same day estimation of milk acetoacetate values was done. The ultrasound (US) was started on day 7 PP and was performed every 3rd day until the end of experiment. Uterine content, follicular activity and sizes of the largest follicle and corpus luteum were monitored and measured. Vaginal discharge and uterine tone were recorded during the rectal palpation. Each animal in the study was sampled for bacteriological examination using endometrial biopsies once a week. Two types of $PGF_{2\alpha}$-metabolite patterns were detected: elevated levels during 14 days PP, then decline to the basal level and then a second small elevation at the time of final elimination of the bacteria from the uterus; or elevated levels during first 7 days PP, then decline to the basal level and a second small elevation before the final elimination of bacteria. Endometritis was diagnosed in 5 cows in farm A and in 3 cows in farm B respectively. In farm A, 5 cows out of 10 ovulated during experimental period and in 1 cow cystic ovaries were found. In farm B, 3 cows out of 10 ovulated. In 3 cows cystic ovaries were found. Altogether 40% of cows had their first ovulation during the experimental period. Three cows in farm A and 5 cows in farm B were totally bacteria negative during the experimental period. The most frequent bacteria found were *A. pyogenes, Streptococcus spp., E. coli., F. necrophorum* and *Bacteroides spp.* The highest incidence of bacteriological species was found during the first 3 weeks in both farms. All animals were free from bacteria after 5th week PP in farm A and after 4th week in farm B respectively. Serum electrolytes and glucose levels were found to be within the reference limits for the cows in both farms. No significant difference was found between farms ($p>0.05$). Low phosphorus levels were found in both farms. Significant difference ($p<0.05$) was found in BUN levels between farms. In both farms milk acetoacetate values were staying within the reference range given for the used test (<100 μmol/l). The uterine involution and bacterial elimination in the investigated cows could consider as normal but more profound metabolic studies could be needed to find reasons for later resumption of ovarian activity. Some recommendations to changing feeding regimes and strategies should also be given.

Postpartum cow; milk production; ovarian activity; PGF_{2a}; progesterone; uterine bacteriology; blood electrolytes; glucose; blood urea nitrogen.

Introduction

The main priority of the Estonian agriculture is milk production. During the recent years the farmers and dairy enterprises have done essential investments to increase milk production and quality. Average annual milk production in Estonian dairy herds is 5690 kg/year (*Animal recording in Estonia* 2002). It is less than for example in the Nordic countries. However, we have already some herds, where production is 8000 kg and more have already been achieved. We consider these herds as the perspective herds, which will survive and stay in competition when we will join the EU. In a second order, we should consider also as promising the herds where the annual production exceeding 6000 kg. According to official animal records the reasons for culling cows in Estonian herds are foremost fertility problems (25%) (*Animal recording in Estonia* 2002). A problem is also establishment of a new pregnancy during 90 days postpartum (PP) (*Kask et al.* 1998). This problem has become more and more common in association with increased productivity. According to statistics the average calving interval of Estonian cows is 408 days. As there has never before been such milk production levels in Estonia, farmers have difficulties in solving the problems, especially to coupe with new requirements of feeding and management of such cows. No profound and complex scientific investigations concerning the uterine involution, resumption of ovarian activity and metabolic status, has been done in Estonian herds with production levels more than 7000 kg/year during recent years. The present study will be the first in a series of investigations planned to be performed in coming years in several herds. The objective of the study was to evaluate the PP reproductive performance in 2 high producing Estonian dairy herds. For that 2 groups of cows were selected from both herds. Intensive hormonal ($PGF_{2\alpha}$, progesterone), ultrasonographic (uterine and ovarian ultrasonography) and microbiological (uterine biopsies) studies were performed during the first 6 weeks PP. Once during the experimental period blood glucose, electrolyte levels and acetoacetate values in the milk were investigated to follow the early postpartum metabolic status of the cows. If these parameters are deviating in the early postpartum period, measures could be taken to increase reproductive performance of the cows.

Table 1. Main characteristics of farms used in study.

Farm	No. of cows	Breeds	Annual milk production	Milking	Housing, management
A	352	ER EHF	7688	2× per day machine pipeline	Tying system. Removal of manure 2× a day by an electric scraper, feeding mechanized by food mixer.
B	200	EHF	9425	3× per day, machine pipeline	Tying system. Removal of manure 3× a day by an electric scraper, feeding mechanized by food mixer.

ER = Estonian red breed; EHF = Estonian Holstein Friesian breed.

Materials and methods

Farms

Two herds (A and B) were studied. Overview of the farms are given in Table 1.

Animals

Twenty cows were used in the experiment, 10 from each farms. Cows considered to have normal pregnancies, normal body condition score (2.5) and supposed to calve during one week period were chosen. They belonged to Estonian Holstein Friesian breed. Experimental work was done during April - May 2001. Average milk production in cows used during experiment was 42 kg/day in Farm B and 32 kg/day in Farm A respectively. None of the animals had difficult calving and retained fetal membranes. No treatment was given to the animals either before or after calving. During the last week of the experiment all animals from both farms were at pasture 3 h during daytime.

Collection of uterine biopsies for bacteriological examination

Each animal in the study was sampled for bacteriological examination once a week, starting within 5 days after parturition and continuing for 6 weeks. Endometrial biopsies were aseptically collected according to the techniques and methods described previously by *Fredriksson et al.* (1985), *Bekana et al.* (1994b) and *Kask et al.* (1998). Biopsies were immediately placed in thioglycolate medium for transportation to the laboratory for bacteriological examination. Cultivations were made within 1.5 h after collection. Isolation of the bacterial species was performed at the Department of Infectious Diseases, Unit of Veterinary Microbiology, Estonian Agricultural University, Tartu using standard bacteriological procedures. Plates cultivated aerobically were examined after 24 h and 48 h and plates cultivated anaerobically after 48 h and 168 h. Isolated bacterial strains were identified according to Bergey's Manual of Systematic Bacteriology (*Holt et al.* 1994).

Ultrasonographic and clinical examination

The ultrasound (US) equipment was a real time B-mode linear array scanner (Hondex HS-120, Honda Electronics Co., Ltd., Aichi, Japan)) with 5 MHZ transducer. The standard TV video system was connected to the instrument and the images were recorded on video tape for later analyses. Also prints from a videographic printer were obtained. The US equipment was supplied with image freezer facility and electronic callipers for taking measurements. The US was started on day 7 after parturition and was performed every 3rd day until the end of experiment. For monitoring of the uterine involution, uterine content was recorded according to *Kask et al.* (2000a). Clinical investigations were based on vaginal discharge recording and uterine tone recording during the rectal palpation. Recordings were made according to scoring systems described previously by (*Kask et al.* 2000a). Uterine involution was considered to be completed when the uterus had returned to its normal location in pelvic cavity, restoration of normal uterine form and content and when the difference between previous pregnant and non-pregnant horn was 1 cm or less (*Bekana et al.* 1994a, *Kask et al.* 2000a).

Follicular activity was monitored in the ovaries. Sizes of the largest follicle and corpus luteum (CL) were monitored and measured by freezing the images and using callipers. Based on the size measurements during US and retrospective analysis of videotapes, follicular dynamics were followed. According to *Ginther et al.* (1989), *Knopf et al.* (1989) and *Kask et al.* (2000a, 2000c) follicular wave was defined as an emergence of a group of follicles and was characterized by development of a single large follicle and regression of several subordinates. Ovulation was judged to have occurred if the

largest follicle monitored by US could not be detected at next examination and also confirmed by a subsequent increase in progesterone concentration (*Kask et al.* 2000a,c). Ovulation was postulated to occur 3 days before the first detection of sustained elevation of the plasma progesterone concentration (*Duchens et al.* 1995).

Blood sampling

Starting on the second day PP, 10 ml of jugular vein blood were withdrawn for $PGF_{2\alpha}$-metabolite and progesterone analyses by venipuncture into heparinized Venoject glass tubes (Terumo Europe N. V., Leuven, Belgium) 3 times per day (7 a.m.; 1 and 7 p.m.) during the first 2 weeks PP. Then the sampling was reduced to 2 times per day (7 a.m. and 7 p.m.) and sampling was terminated 6 weeks PP. After immediate centrifugation about 5 ml of plasma were removed and stored at -18 °C until hormone analyses were performed.

On day 25 PP jugular vein samples were taken from each cow into plain Venoject glass tubes (Terumo Europe N. V., Leuven, Belgium) for the evaluation of metabolic/electrolyte status (glucose, magnesium (Mg), calcium (Ca), phosphorus (P), potassium (K), blood urea nitrogen (BUN)). To avoid artefactual changes in these parameters, serum was separated from whole blood by centrifugation within 1.5 h after collection and was used for future analyses.

Detection of ketone bodies in the milk

On the same day as blood samples for the metabolic/electrolyte status analyses were taken, detection of the acetoacetate values in the milk was performed using commercially available milk ketone test (PINK® milk ketone test® Proff Products, Germany). Acetoacetate values >100 μmol/l were considered to be positive.

Hormone analyses

All plasma samples were analyzed for concentration of 15-ketodihydro-$PGF_{2\alpha}$, according to *Granström & Kindahl* (1982). The relative cross-reaction of the antibody raised against 15-ketodihydro-$PGF_{2\alpha}$ were 16% with 15-keto-$PGF_{2\alpha}$, 4% with 13,14-dihydro-$PGF_{2\alpha}$, 0.5% with $PGF_{2\alpha}$ and 1.7% with the corresponding metabolite of PGE_2. The lower limit of detection of the assay was 30 pmol/l for 0.5 ml plasma. All high levels were estimated but for better interpretation, an upper limit was set 3500 pmol/l in figures. The inter-assay coefficient of variation was 14% (at 114 pmol/l) and the intra-assay coefficient of variation varied between 6.6% and 11.7% at different ranges of standard curve.

The duration in days of the PP prostaglandin release was calculated using a skewness method (*Zarco et al.* 1984). All PG-metabolite values were used in the calculation. The higher values were removed from the data set in several cycles which was repeated until no significant elevations were detected. The plasma levels of the $PGF_{2\alpha}$ metabolite were considered to be significantly elevated as long they exceeded the mean basal value plus 2 SD (*Kask et al.* 2000b, 2000c).

Morning plasma samples of each day were analyzed for the content of progesterone (*Duchens et al.* 1995). The assay used was an enhanced luminescence immunoassay (Amerlite®, Kodak Clincal Ltd, Amersham, England). The lowest limit of detection for the assay was 0.2 nmol/l and levels more than 1 nmol/l were considered to be of biological importance. The inter-assay coefficient of variation was below 4%. The intra-assay coefficients of variation calculated were between 4% and 8.1%.

Serum analyses of blood electrolytes, glucose and BUN

Analyses were done within 5 hours after the

Table 2. Characteristics of follicular dynamics and uterine involution length in cows (n=20) of farms A and B during 6 weeks PP

Cow No.	No. of follicular waves	First ovulation (days PP)	Pathology in ovaries	Uterine involution (days PP)
Farm A				
4280	2 (OV)*	14	-	26
1186	2 (OV)*	18	-	29
4488	2 (OV)*	23	-	26
5070	3 (OV)*	32	-	27
836	3 (OV)*	37	-	26
1498	1**	-	Cystic ovaries on day 25 PP	28
1228	4	-	-	26
4278	3	-	-	26
4403	4	-	-	26
4235	3	-	-	28
Farm B				
7557	3 (OV)*	31	-	26
7515	3 (OV)*	31	-	27
7527	3 (OV)*	32	-	27
7581	1**	-	Cystic ovaries on day 23 PP	26
7461	1**	-	Cystic ovaries on day 28 PP	26
7561	1**	-	Cystic ovaries on day 30 PP	32
7523	3	-	-	29
4075	3	-	-	27
7501	4	-	-	27
7201	3	-	-	

(OV)*= Indicates that dominant follicle of the last follicular wave was ovulated. **= dominant follicle developed to cyst.

separation of serum. Equipment used for the analyses was Automatic Serum Photometric Analyzer System Humalyser 815 (Human® Gesellschaft Biochemica und Diagnostice mbh, Wiesbaden, Germany). Obtained values were compared with reference physiological levels for the cows (*Smith* 1996). Values not fitting to given physiological ranges were considered as abnormal.

Statistical analyses

For comparing the mean milk production between the cows in farm A and B Minitab for Windows (Minitab Inc., 1994, USA) and the Two sample T-test was used. Minitab for windows and Two sample T-test was also used for comparing the mean electrolyte, glucose and BUN values between the farms. Differences were considered significant when $p<0.05$.

Results

Calving data and milk production

All chosen 20 cows from both farms showed normal calving performance. The cows calved between 272 - 285 days of pregnancy, which is within normal ranges for Estonian breeds (*Müürsepp et al.* 1981). No assistance during calving process or retained fetal membranes were recorded. Nine male and 11 female alive calves were born. Mean production in experimental groups was 32 kg/day in Farm A and 42 kg/day in Farm B respectively. Significant dif-

ference was found in milk production between experimental groups ($p<0.05$).

Uterine and ovarian ultrasonography

In farm A, according to clinical investigations and US, purulent endometritis was diagnosed in 2 cows which was characterised by thick white purulent discharge during first 4 weeks PP and showing cloudy fluid inside the uterine lumen. In these cows after day 28 clear mucus discharge was observed and no vaginal discharge and uterine content was detected after day 35 PP. Mild catharral endometritis was detected in 3 cows. It was characterised with prolonged flecked pus or cloudy lochial discharge up to day 25 PP. After day 25 clear mucus discharge was detected and no discharge and uterine content was observed after day 30 PP. In 5 cows no signs of endometritis were diagnosed.

In farm B purulent endometritis was diagnosed in 1 cow with presence of uterine content up to day 35 PP with white thick purulent discharge up to day 21 and clear mucus discharge up to day 34. Mild catharral endometritis were recorded in 2 cows with flecked pus or cloudy discharge up to day 20 and no discharge and uterine content after day 28 PP. Diagnosis was also confirmed by uterine bacteriology results. No signs of endometritis were seen in 7 cows. Uterine involution length in individual cows are given in Table 2.

According to ovarian US, follicular activity was detected in all cows in both farms from the start of first US session on day 7 PP. According to US and progesterone results in farm A, 5 cows out of 10 ovulated during experimental period. In 1 cow cystic ovaries were found. Follicular activity but no ovulations were detected during experimental period in 4 cows.

In farm B, 3 cows out of 10 ovulated. In 3 cows cystic ovaries were found. No ovulations, but good follicular activity was detected in 4 cows. In Farm A a short lasting elevation in progesterone levels was seen around 2 weeks PP, which could consider as occurrence of short cycles. Altogether 40% of cows had their first ovulation during the experimental period. More detailed results of ovarian US are given in Table 2.

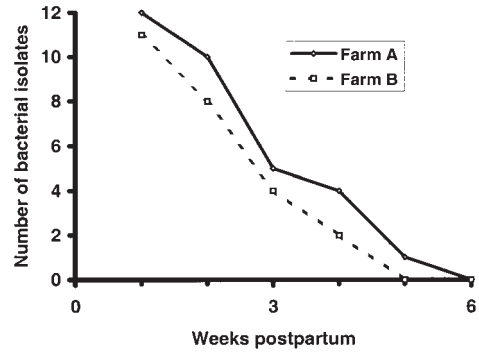

Figure 1. Bacterial elimination from the uterus in farm A and B during 6 weeks PP.

Uterine bacteriology

From 20 animals a total of 120 biopsies were collected, from them 31 were found to be bacteriologically positive and remaining 89 biopsies were negative. Three cows in farm A and 5 cows in farm B were totally negative during the whole 6 week collection period. Out of the 31 positive biopsies, 19 samples showed mixed infections with anaerobic and aerobic bacteria. In 12 samples aerobic (6 samples) and anaerobic (6 samples) organisms in pure cultures were found. The mixed cultures contained mainly *Arcanobacterium pyogenes, Bacteroides spp., Fusobacterium necrophorum, Peptostretococcus indolicus* and *Escherichia coli.* The most frequent aerobic bacteria found were *A. pyogenes, Streptococcus spp*. and *E. coli.* The main anaerobic bacteria found were *F. necrophorum* and *Bacteroides spp.*

The highest incidence of bacteriological species was found during the first 3 weeks in

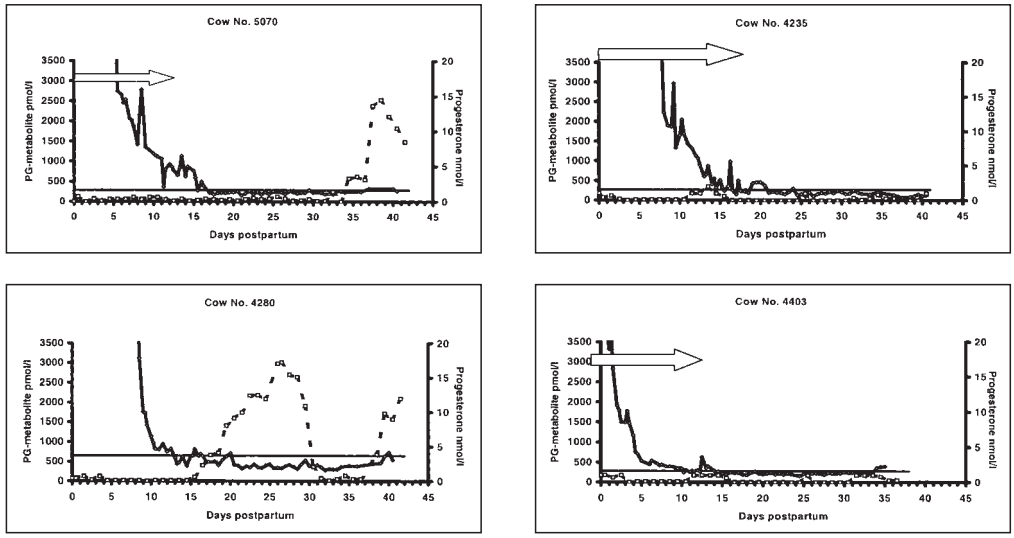

Figure 2. Examples of the PG – metabolite (—) and progesterone (-------) profiles during 6 weeks PP in farm A. Block arrow in graphs denotes the bacterial presence and elimination time. The horizontal line in the graphs denotes the line of significance (mean basal value + 2 SD) for the $PGF_{2\alpha}$ metabolite.

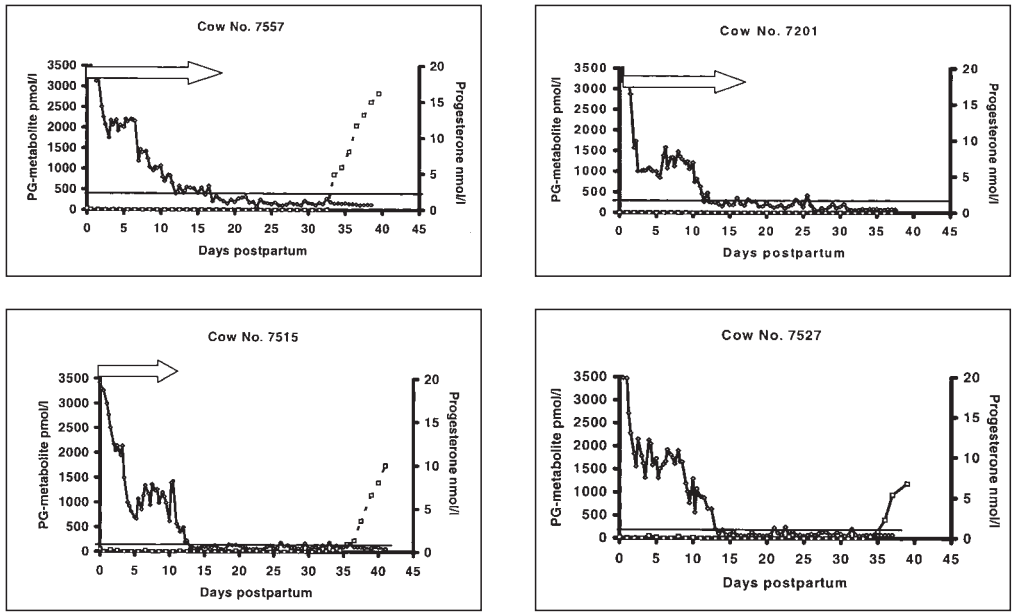

Figure 3. Examples of the PG – metabolite (—) and progesterone (------) profiles during 6 weeks PP in farm B. Block arrow in graphs denotes the bacterial presence and elimination time. The horizontal line in the graphs denotes the line of significance (mean basal value + 2 SD) for the $PGF_{2\alpha}$ metabolite.

Table 3. Average blood electrolyte, glucose and BUN in two herds (SD of the mean is given in parenthesis).

Herd	Ca mmol/L	P mmol/L	Mg mmol/L	Potassium mmol/L	Glucose mmol/L	BUN mmol/L
A	2.3 (± 0.1)	1.1 (± 0.20)	0.9 (± 0.06)	4.3 (± 0.64)	2.9 (± 0.42)	10.0 (± 1.96)
B	2.4 ± 0.09)	1.0 (± 0.16)	0.9 (± 0.06)	4.5 (± 0.57)	3.5 (± 0.19)	14.3 (± 2.69)
Normal physiological value	2.2 - 3.0	1.8 - 2.1	0.7 - 0.9	3.9 - 5.8	2.5 - 3.6	7.0 - 11.0

both farms. Final elimination of bacteria occurred after 5th week PP in farm A and after 4th week in farm B respectively. Elimination of the bacteria in both farms is described in Fig. 1.

15-ketodihydro-PGF$_{2\alpha}$

Generally two types of PGF$_{2\alpha}$-metabolite patterns were detected.

1. Elevated levels during 14 days PP, then decline to the basal levels and then a second small elevation at the time of final elimination of the bacteria from the uterus.
2. Elevated levels during first 7 days PP, then decline to the basal levels and a second small elevation before the final elimination of bacteria.

The second elevations were not seen in the cows who had no bacteria in the uterus. In farm A both patterns of PGF2a-metabolite were seen. In 7 cows first type of pattern was seen and the second type pattern was detected in 3 cows.

In farm B only first type pattern was seen. Generally the values were considered to be significantly elevated as long as they exceeded the mean basal value plus 2 SD (line of significance). Both types of PGF$_{2\alpha}$-metabolite patterns are described in Figs. 2 and 3.

Progesterone

Low levels of progesterone were seen immediately after parturition in all animals in both farms. In farm A the levels remained low in 8 animals during the first 2 weeks PP. This coincides to the presence of high levels of the PGF$_{2\alpha}$-metabolite. Then sustained rise of progesterone (>1 nmol/l) was seen in 4 animals. The average duration of the rise in those particular animals was 12.7 days. Then the levels declined to the low levels and a new rise was seen in 1 animal before the end of the experimental period. This is an indication that these 4 animals had their first ovulation during the first 42 days PP. In 1 cow from this farm, the first sustained rise was seen on day 41 PP and it was continuing when the experiment was finished. Thus 5 animals out of 10 from farm A had ovulated during the experimental period. In 2 animals from these farm (No. 4235 and 4403) a small elevation of progesterone was detected between days 12 and 16 PP. Examples of progesterone patterns in farm A are described in Fig. 2.

In farm B the first sustained release of progesterone (>1 nmol/l) was seen after day 30 PP only in 3 animals and the levels were still elevated at the end of experiment indicating that these 3 cows had their first ovulation during the 42 days experimental period. Some progesterone patterns in Farm B are described in Fig. 3.

Blood electrolytes, glucose and BUN status
Levels of blood electrolytes (Ca, Mg, K) and glucose were found to be in the reference range for the cows in both farms. Except for BUN where a significant difference ($p>0.05$) was found between groups. Exception was phosphorus, which was found to be low in both farms. Average detected values in both farms and reference ranges for the cow are presented in Table 3.

Acetoacetate values in the milk
In all cows in both farms the tested milk acetoacetate values were staying in normal frames given for the used test (<100 μmol/l).

Discussion
Our intention during the planning of the experiment was to involve cows with normal health parameters, condition and normal calving performance. All the cows from both farms used in experiment had normal calving performance. According to *Arthur et al.* (2001) it is important that there should be a normal puerperium for the cow, because the farmers intention is to breed the animal fairly soon after they have given birth. Any extension of the puerperium can have detrimental effect on the future reproductive performance of the individual animal.
The uterus should after parturition undergo involution and restore the function of the endometrial glands. As an easy rule the uterine size is normalized in about 3 weeks, but for the uterine functions it takes about twice that time (*Schirar & Martinet* 1982, *Arthur et al.* 2001). In the present study the uterine size was normalized during 29 days in all cows. From this point of view we can also consider the involution process as normal. The cervical canal is open during the parturition and it is a high risk of bacterial contamination of the uterus (*DeBois* 1961, *Elliott et al.* 1968, *Griffin et al.* 1974, *Fredriksson et al.* 1985, *Bekana et al.* 1996b, *Kask et al.* 1998). The incidence of positive bacterial cultures varies in normal calving cows, but in cases of disturbances in the labour process or retained fetal membranes (RFM) bacterial contamination is 100% (*Bekana et al.* 1994b, *Kaneko et al.* 1997, *Kask et al.* 1999a, *Kask et al.* 2000a). The elimination of bacteria is however fast – around 3 weeks in normal parturition, if the animals get infected, (*Fredriksson et al.* 1985, *Bekana et al.* 1994b, *Bekana et al.* 1996a, *Kask et al.* 1999, 2000a). In the present study similar results have been obtained. Most of the bacteria were eliminated during first 3 weeks PP. Only in 2 cows in farm A elimination time lasted 4 weeks and in 1 cow 5 weeks. In farm B only in 1 cow the elimination lasted 4 weeks PP. In farm B also more totally negative cows were found (5) compared with farm A (3). The reason for that could be the hygiene conditions in farm A where manure was removed twice per day. In farm B it was done 3 times per day. Unhygienic conditions in and around the cow could increase the bacterial contamination of the vestibulum and vagina, from where they can easily migrate to the uterus after parturition (*Bretzlaff et al.* 1982, *Kask et al.* 1998).
The ovaries should regain normal folliculogenesis and cyclicity after parturition (*Savio et al.* 1990). In the dairy cow, one follicle is selected and becomes dominant and the remaining follicles undergo atresia (*Ginther et al.* 1989). The dominant follicle can ovulate and the earliest time is 10-15 days after parturition (approx. 10% of cows). Approximately 60% of the cows have ovulated before 25 days (*Lamming et al.* 1982, *Ginther et al.* 1989, *Knopf et al.* 1989). Alternatively to ovulation, the dominant follicle undergoes atresia and a new follicular wave is initiated. Thus, in these cases ovulation can be much delayed.
In the present study out of 10 cows in farm A, 5 cows had their first ovulation during the exper-

imental period, and in farm B, 3 cows out of 10. In farm A, 2 cows had their first ovulation before day 20 PP. Somewhat delayed was the start of ovulations in farm B. In 3 ovulating cows ovulations were detected after day 30 PP. In 7 cows no ovulations were seen during the experimental period, but good follicular activity was detected. One reason for the late start of cyclicity could be significantly higher milk production in this farm and also the milk production in these particular cows (42 kg/day). Milk production during PP is an essential factor influencing resumption of ovarian activity postpartum (*Lamming* 1978). This could be also the reason for the follicular cysts in 3 cows in Farm B as high milk production is a common factor for development of cysts (*Roberts* 1986, *Ashmawy et al.* 1992).

It has never been seen that cows ovulate as long as the prostaglandin release is dominating (*Kindahl et al.* 1984, *Kindahl et al.* 1992). First, when the prostaglandin metabolite levels are close to baseline or later on in time, the ovulation can occur. It is not known if this is a direct effect of $PGF_{2\alpha}$ or if other products are formed in the uterus concomitant with the prostaglandins, exhibiting this inhibitory effect. Uterine infections are also influencing the time of the first ovulation. As an example from *Fredriksson et al*. (1985), noninfected animals ovulated on average 16 days after parturition as compared to infected animals which ovulated 31 days after parturition. The longer release of $PGF_{2\alpha}$ in infected animals might explain why these animals ovulate later. The similar situation was seen in the present study. No ovulations were detected when $PGF_{2\alpha}$ release was dominating and in the cows, who had infected uterus ovulations occurred later.

In cows with normal parturition and uncomplicated involution, the duration of the prostaglandin release postpartum is negatively correlated with time for completed uterine involution (*Lindell et al.* 1982). In animals with varying degrees of intrauterine infections or with RFM/endometritis a positive correlation is seen instead (*Lindell et al.* 1982, *Fredriksson et al.* 1985, *Bekana et al.* 1996a, *Kask et al.* 1999, 2000b, 2000c). In these infected animals, prostaglandin metabolite levels decreased after parturition similar to the observations in uninfected animals. However, before a final drop in the levels, sustained and pulsatile elevations were seen. The levels return to baseline at the same time as the final elimination of bacteria occurs (*Bekana et al.* 1996a). This implies that an increased release of $PGF_{2\alpha}$ is an indication of the infection/inflammation in the uterus and may also play a role for the elimination of the infection. Similar results were observed in the present study.

An important aspect of ovarian cyclicity in the postpartum period is the high incidence of short oestrous cycles (*Kindahl et al.* 1984, Bekana 1997, Kask et al. 2000a, 2000c). The normal interovulatory interval in the oestrous cycle is 18-24 days, but in the cases of short cycles the interval is 10-11 days (*Kindahl et al.* 1984, *Bekana* 1997). Calculating on the luteal phase instead, the normal is 14 days and in cases of short cycles about 5-8 days. These events are possible to follow using progesterone analyses. There is also a very strong correlation between time of ovulation and occurrence of short oestrous cycles – if the animals are early ovulators the incidence is much increased (*Fredriksson et al.* 1985, *Bekana* 1997, *Kask et al.* 2000b, 2000c). The explanation for occurrence of the short cycles is that at the time of ovulation, the uterus has not regained its normal functions and an uncontrolled prostaglandin release occurs resulting in a premature regression of the corpus luteum function (*Bekana* 1997). Only in 2 cows in farm A short lasting elevation in progesterone levels was seen around 2 week PP, which lasted 5 days (Figure 2). In many studies,

a short oestrous cycle is seen initiating normal ovarian cyclicity. However, none of these cows showed normal oestrous cyclicity during rest of the experimental period. Two cows ovulated rather early PP but luteal phase was in normal length.

Serum electrolytes, glucose and BUN values were in normal ranges for the cows except low P in both herds and elevated BUN level in farm B. The results showed that no serious metabolic dysfunctions was found. Also no elevated level of acetoacetate values in milk was found, indicating that negative energy balance, could not be a problem in these herds. Elevated level of BUN in farm B indicates the protein overfeeding. This will have a bad effect in the long run. High protein content will influence ovarian activity, it is also a contributory factor for development of cystic ovaries which was found in an higher frequency in farm B. High protein content leads to low serum progesterone concentration and to low fertility (*Strang et al.* 1998, *Butler* 1998). High rumen degradable protein causes ammonia overproduction. Elimination of ammonia needs more metabolic energy which can cause a deepening of negative energy balance PP (*Webb et al.* 1999, *Rukkwamsuk et al.* 1998). However, as only 10 cows from both herds were used it is rather difficult to evaluate reproductive performance for the whole herd, but we can get a valuable information what kind a problems associated with reproduction can be present in the herd.

Conclusions

Based on this study the uterine involution and bacterial elimination in the two selected groups could consider as normal but more profound metabolic studies could be needed to find reasons for later resumption of ovarian activity. Some recommendations to changing feeding regimes and strategies should also be given.

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References

Arthur GH, Noakes DE, Pearson H, Parkinson TJ: Veterinary Reproduction and Obstetrics 2001, W. B. Saunders Company Ltd., pp. 193-208.

Ashmawy AA, Vogt DW, Garverick HA, Younquist RS: J. Anim. Breed. Genet. 1992, *2,* 109-129.

Bekana M: Clinical, ultrasonographic, bacteriological and hormonal studies in post-partum cows with particular emphasis on retained fetal membranes. Doctoral thesis. 1996b, Uppsala, 1-38.

Bekana M: Prostaglandin $F_{2\alpha}$ and progesterone profiles in postpartum cows with short luteal phases. Acta vet. scand. 1997, *38,* 323-330.

Bekana M, Ekman T, Kindahl H: Ultrasonography of the bovine postpartum uterus with retained fetal membranes. J. Vet. Med. A 1994a, *41,* 653-662.

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

Bekana M, Odensvik K, Kindahl H: Prostaglandin $F_{2\alpha}$ metabolite and progesterone profiles in postpartum cows with retained foetal membranes. Acta vet. scand. 1996a, *37,* 171-185.

Butler WR: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. Review. J. Dairy Sci. 1998, *81,* 2533-2539.

Bretzlaff KN, Whitmore HL, Spahr SL, Ott RS: Incidence and treatments of postpartum reproductive problems in a dairy herd. Theriogenology. 1982, *17,* 527-537.

DeBois CHW: Endometritis in postpartum cow. Endometritis en vruchtbaarheid bij het rund. Doctoral Thesis. 1961, Utrecht, Holland, 145 pp. (In Dutch).

Duchens M, Kindahl H, Forsberg M, Gustafsson H, Rodriguez-Martinez H: Influence of low plasma progesterone concentrations on the release of prostaglandin $F_{2\alpha}$ during luteolysis and oestrus in heifers. Anim. Reprod. Sci. 1995, *40,* 261-268.

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

Fredriksson G, Kindahl H, Sandstedt K, Edqvist L-E: Intrauterine bacterial findings and release of

$PGF_{2\alpha}$ in the postpartum dairy cow. Zbl. Vet. Med. A 1985, *32,* 368-380.

Ginther OJ, Kastelic JP, Knopf L: Composition and characteristics of follicular waves during the bovine oestrous cycle. Anim. Reprod. Sci. 1989, *20,* 187-200.

Granström E, Kindahl H: Radioimmunoassay of the major plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro- $PGF_{2\alpha}$. Methods Enzymol. 1982, *86,* 320-339.

Griffin JFT, Hartigan PJ, Nunn WR: Nonspecific uterine infection and bovine fertility. 1. Infection patterns and endometritis during the first seven weeks postpartum. Theriogenology 1974, *1,* 91-106.

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

Kaneko K, Kawakami S, Miyoshi M, Abukawa T, Yamanaka S, Mochizuki M, Yoshihara S: Effect of retained placenta on subsequent bacteriological and cytological intrauterine environment and reproduction in Holstein dairy cows. Theriogenology 1997, *48,* 617-624.

Kask K, Gustafsson H, Gunnarsson A, Kindahl H: Induction of parturition with prostaglandin $F_{2\alpha}$ as a possible model to study impaired reproductive performance in the dairy cow. Animal Reproduction Science 2000c, *59,* 129-139.

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 cows in different milking frequencies. Acta vet. scand. 1999, *40,* 287-297.

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

Kask K, Kindahl H, Gustafsson H: Resumption of uterine and ovarian functions following dexamethasone-induced parturition in dairy cows. Acta vet baltica 2000a, 3, 11 - 21.

Kask K, Kindahl H, Magnusson U, Gustafsson H: Uterine bacteriology, prostaglandin $F_{2\alpha}$ metabolite and progesterone profiles, blood granulocyte function and uterine cytology in postpartum cows after dexamethasone-induced parturition. Acta vet baltica 2000b, *3,* 22-30.

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

Kindahl H, Fredriksson G, Madej A, Edqvist L-E: Role of prostaglandins in uterine involution. Proceedings 10th International Congress on Animal Reproduction and Artificial Insemination. Illinois, June 10-14, 1984, Volume IV, XI9-16.

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

Knopf L, Kastelic JP, Schallenberger E, Ginther OJ: Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Dom. Anim. Endocrinol. 1989, *6,* 111-119.

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

Lamming GE, Peters AR, Riley GM, Fisher MW: Endocrine regulation of postpartum function. In: Current Topics in Veterinary Medicine and Animal Science, Karg H., Schallenberger E., (Editors), Martinus Nijhoff Publishers, The Hague, 1982, Vol. 20: 148-172.

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.

Minitab for Windows: Reference manual 1994, Minitab Inc., USA.

Müürsepp I, Valge L, Jalakas M: Veterinary Obsterics and Gynaecology. Veterinaarsünnitusabi ja günekoloogia. 1981, Tallinn, Valgus, p. 298 (in Estonian)

Results of Animal Recording in Estonia 2002. Eesti jõudluskontrolli aastaraamat 2002: Jõudluskontrolli keskus, Kirjastus Elmatar, pp. 52. (In Estonian)

Roberts SJ: Veterinary obstetrics and genital diseases (Theriogenology). 1986, Edwards Brothers, Inc. Ann Arbor, Michigan, USA.

Rukkwamsuk T, Wensing T, Geelen MJH: Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci. 1998, *81,* 2904-2911.

Savio JD, Boland MP, Hynes N, Roche JF: Resumption of follicular activity in the early post-partum

period of dairy cows. J. Reprod. Fert. 1990, *88,* 569-579.

Schirar A, Martinet J: Postpartum ovarian activity and its interaction with the uterus in resuming cyclic activity post partum. In: H. Karg and E. Schallenberger (eds.), Factors influencing Fertility in the Postpartum Cow. Current Topics in Veterinary Medicine and Animal Science 1982, Vol. 20, Martinus Nijhoff Publishers, The Hague, pp. 67-94.

Smith BP: Large Animal Internal Medicine. Mosby - Year Book, Inc. 1996, pp. 2040.

Strang BD, Bertics SJ, Grummer RR, Armentano LE: Effect of long chain fatty acids on triglyceride accumulation, gluconeogenesis, and ureagenesis in bovine hepatocytes. J. Dairy Sci. 1998, *81,* 728-739.

Zarco L, Stabenfeldt GH, Kindahl H, Quirke JF, Granström E: Persistence of luteal activity in the non-pregnant ewe. Anim. Reprod. Sci. 1984, *7,* 245- 267.

Webb R, Garnsworthy PC, Gong JG, Robinson RS, Wathes DC: Conseguences for reproductive function of metabolic adaption to load. In: Metabolic stress in dairy cows. British Society of Animal Science. Occasional Publication. 1999, *24,* 99-112.

Sammanfattning

Beskrivning av reproduktionsmått i den tidiga postpartumperioden i två högproducerande estländska mjölkkobesättningar.

Äggstocksaktivitet, hormonprofiler, uterusinvolution, uterusinfektioner, blodelektrolyter och glykos, mjölkketonkroppar och blodurea studerades under 6 veckor efter förlossningen i två högproducerande estländska mjölkkobesättningar (7688 kg i besättning A, och 9425 kg i besättning B). Tio kor utvaldes från respektive besättning. Djuren visade alla en normal förlossning. Jugularvenblodprover samlades för hormonanalyser (PGF2a-metabolit och progesteron) 3 gånger dagligen under de första 2 veckorna och därefter 2 gånger dagligen i 4 veckor. På dag 25 samlades blodprover för metaboliskt status samt mjölk för mjölkketonkroppar. Ultraljudsundersökningar startade på dag 7 och utfördes var 3:e dag under hela försöksperioden. Livmoderns innehåll visualiserades med ultraljudet och vidare noterades vaginalflöden samt livmoderns kontraktionsgrad vid rektalundersökning. Äggstockarnas aktivitet följdes med ultraljudet och storleken på den största follikeln noterades likväl som gulkroppens storlek. Livmoderns bakteriestatus följdes med livmoderbiopsier. Två typer av prostaglandinfrisättningsmönster sågs hos djuren: förhöjda nivåer under 14 dagar efter förlossningen, nedgång i nivåerna till baslinjen och sedan mindre nivåhöjningar samtidigt som bakterierna slutligen eliminerades från livmodern; det andra mönstret var förhöjda nivåer under endast 7 dagar och en liknande höjning när bakterierna försvann från livmodern. Variga endometriter och milda katarrala endometriter syntes i 2 respektive 3 kor i besättning A. Övriga 5 djur i denna besättning hade inga påvisbara patologiska förändringar i livmodern. För besättning B var motsvarande siffror 1, 2 respektive 7. Data för ovulationer var att i besättning A 5 av 10 ovulerade och 1 ko fick cystor i äggstockarna. Vidare sågs i 4 kor en tydlig follikelaktivitet, men dessa djur ovulerade inte. För besättning B var det endast 3 av 10 som ovulerade; 3 kor med cystor och 4 icke-ovulerande kor, men dessa hade god follikelaktivitet. Tre kor från besättning A och 5 från B var helt fria från bakterier i livmodern. I de bakteriepositiva biopsierna hittades Arcanobacterium pyogenes, Streptococcus spp., Escherichia coli, Fusobacterium necrophorum och Bacteroids spp. De flesta bakteriepositiva proverna hittades under de 3 första veckorna och därefter eliminerades bakterierna helt efter 5:e veckan (besättning A) eller efter 4:e veckan (besättning B). Blodelektrolyter och glykosvärden låg inom referensvärdena och ingen statistisk skillnad förelåg mellan besättningarna. Låga fosfatvärden hittades dock i båda besättningarna. Signifikanta skillnader förelåg i blodurea mellan besättningarna, men mjölkketonkropparna var inom normalvärdena. Utifrån de studerade djuren kan det konstateras att reproduktionsmåtten är acceptabla, men att korrigera foderstater är en viktig åtgärd.

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