POSTPARTUM HORMONAL PROFILES AND UTERINE BACTERIOLOGY IN TWO HIGH PRODUCING ESTONIAN DAIRY HERDS

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ABSTRACT. Postpartum hormonal profiles and uterine bacteriology in two high producing Estonian dairy herds. Early postpartum (6 weeks) ovarian activity, hormonal profiles, uterine infections, were studied in 2 Estonian high producing dairy herds with annual milk production of 7688 (Farm A) and 9425 (Farm B). Ten cows were studied from each farm. All cows had a normal calving performance. Ten ml of jugular vein blood for the hormonal (PGF$_{2\alpha}$-metabolite, progesterone) analyses were withdrawn 3 times per day during first 2 weeks PP and 2 times per day during remaining 4 weeks. 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 levels 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 levels and a second small elevation before the final elimination of bacteria. In farm A, 5 cows out of 10 ovulated during experimental period and in one 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 three cows cystic ovaries were found. No ovulations, but good follicular activity was detected in 4 cows. 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. Based on the present investigations the uterine involution and bacterial elimination in the two selected farms is acceptable but future studies (metabolic) are needed to clarify problems which are associated with delayed resumption of ovarian function.

Key words: Postpartum cow, PGF$_{2\alpha}$, progesterone, milk production, ovarian activity, uterine bacteriology.

Introduction

Average annual milk production in Estonian dairy herds is 5690 kg/year (Animal recording in Estonia 2002). However, we have already some herds, where production of 8000 kg of milk and more have already been achieved. Increasingly, dairy producers and researchers question the economic justification of an increased production for high-producing cows. Several studies have demonstrated that high-producing cows are at increased risk of infectious diseases (Gröhn et al., 1994; Oltenacu, Ekesbo, 1994; Uribe et al., 1995) and decreased reproductive performance (Nebel, McGilliard, 1993; Marti, Funk, 1994). According to official animal records the reasons for culling cows in Estonian herds are foremost fertility problems (25%) (Animal recording in Estonia 2001). 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 such milk production levels in Estonia, farmers have difficulties to solve the problems, especially to cope with new requirements of feeding and management of such cows. No profound and complex scientific investigations have done during recent years to find out what particular postpartum reproductive problems are present in Estonian herds with production levels more than 7000 kg/year. To clarify those problems series of experiments are planned to perform in our high producing herds. It is well reported that hormonal parameters like prostaglandin F$_{2\alpha}$ and progesterone (P$_4$) together with uterine bacteriology investigations can give quite a complex overview about disturbances during postpartum period in dairy cow (Kindahl et al., 1982, 1984; Fredriksson et al., 1985; Bekana et al., 1996a; Kask et al., 1999, 2000b). In the present study experimental groups from two high producing dairy herds were used. Intensive hormonal (PGF$_{2\alpha}$, P$_4$) and microbiological (uterine biopsies) studies were performed during the first 6 weeks PP. If these parameters are deviating in the early postpartum period, measures could be taken to increase reproductive performance of the cows.
**Materials and methods**

**Farms**

Two herds (A and B) were studied. In farm A altogether 352 cows (Estonian Red and Estonian Holstein Friesian) produced an average 7688 kg of milk. Milking in farm A was 2 times per day with machine pipeline system. Cows were kept in tying system, removal of manure was made 2 times a day by an electric scraper. Feeding system was mechanized by food mixer.

Number of animals in farm B was 200 with annual milk production of 9425 kg (Estonian Holstein Friesian breed). Milking 3 times per day with machine pipeline system. Cows were kept in tying system, removal of manure was made 2 times a day by an electric scraper. Feeding system was mechanized by food mixer.

**Animals**

Twenty cows were used in the experiment, 10 from each farms. Cows were chosen, considering having normal pregnancies, normal body condition score (2.5–3) and supposed to calving during one week period. They belonged to Estonian Holstein Friesian breed. Experimental work was done during April – May 2001. Average milk production in used cows 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 hours during day time.

**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. Cultivation was 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).

**Blood sampling**

Starting on second day PP, 10 ml of jugular vein blood for the PGF$_{2\alpha}$-metabolite and progesterone analyses were withdrawn 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 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 after 6 weeks PP. After immediate centrifugation about 5 ml of plasma were removed and stored at –18°C until hormone analyses were performed.

**Hormone analyses**

All plasma samples were analyzed for concentration of 15-ketodihydro-PGF$_{2\alpha}$, according to Granström and 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$_{3\alpha}$ 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 Clinical 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%.

**Statistical analyses**

For comparing the mean milk production between groups Minitab for Windows (Minitab Inc., 1994) and the Two sample T-test was used and 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 was recorded. Nine male and 11 female alive calves were born. Significant difference was found in milk production between both experimental groups (P<0.05).

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, Peptostreptococcus 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 both farms. All animals were free from bacteria 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 Figure 1.

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

Joonis 1. Bakterite emakast elimineerumise aeg farmis A ja B poegimisjärgse kuuenädalase katseperioodi kestel

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 PGF$_{2\alpha}$-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 PGF₂α-metabolite patterns are described in Figures 2 and 3.

**Progestosterone**

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 PGF₂α-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 one 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 one 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 two 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 Figure 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 Figure 3.

**Discussion**

Our attention during the planning of the experiment was to involve into the experiment 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 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 RFM bacterial contamination is 100% (Kaneko et al., 1997, Kask et al., 1999, Kask et al., 2000a). The elimination of bacteria is however very fast – in normal parturition, if the animals get infected, around three weeks (Fredriksson et al., 1985, Bekana et al., 1996b, 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 a day. In farm B it was done 3 times a 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.

Based on progesterone results in the present study out of 10 cows in farm A, 5 cows had their first ovulation during the experimental 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 were the start of ovulations in farm B. In 3 ovulating cows ovulations were detected after day 30 PP. Seven cows no ovulations were seen during the experimental period. 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 essential factor influencing resumption of ovarian activity postpartum (Lamming 1978). 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, then 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 PGF₂α 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.
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.

Joonis 2. Prostaglandiin F2A metaboliti ja progesteroni profiilid poegimisjärgse 6 nädalase katseperioodi kestel farmis A. Nool graafiku ülaosas näitab bakterite esinemise ja ellimineerumise aega emakast. Horisontaaljoon graafiku allosas on märgiks millest ülevalpool olevaid PGF2A väärtusi võib lugeda oluliseks (baastasapind + 2 standardhälvet)
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.

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 two 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 ovulate rather early PP but luteal phase was in normal length.

Conclusions

Based on present investigations the uterine involution and bacterial elimination in the two selected farms is acceptable but future studies (metabolic) are needed to clarify problems which are associated with delayed resumption of ovarian function.

Acknowledgment

This study was financially supported by the Estonian Science Foundation grant No. 4810. Swedish Institute is acknowledged for financial support concerning the laboratory work in Sweden.

References


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Kokkuvõte


Poegimisjärgne hormonaalne profiil ja emaka bakteriooloogia kahes Eesti kõrgetoodangulises lüpsikarjas

Hormoonanalüüside põhjal täheldati kahte tüüpi prostaglandiin \( F_{2\alpha} \)-metaboliidi profiile:

1) kõrgenenud tase esimese 14 poegimisjärgse päeva jooksul, millele järgnes langemine baastasapinnale ja edasi teine väikse tõus, mis langes kokku bakterite lõpliku eliminimuuseumisega emakast;

2) kõrgenenud tase esimese 7 poegimisjärgse päeva jooksul, millele järgnes langemine baastasapinnale ja edasine teine väikne tõus, mis langes kokku bakterite lõpliku eliminimuuseumisega emakast.


Nende uuringutulemustele põhinedes võib öelda, et emakainvolutsioon ja emakainfektsioonide eliminieru-minute on nendes kahes farmis normi piires. Et leida vastuseid munasarja funktsiooni toaattumise venimisele mõlema farmis, on vajalikud edasised uuringud, millesse peab kindlasti lisama detailedse ainevahetuslikud uuringud.