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

K. Kask, J. Kurõkin, R. Lindjärv, A. Kask, H. Kindahl

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 Ten ml of jugular vein blood for the hormonal ($PGF_{2\alpha}$-metabolite, progesterone) analyses were withdrawn 3 times per day during first 2 weeks PP and 2 times per day during remaining 4 weeks.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 5^{th} week PP in farm A and after 4^{th} 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 coupe 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 $PGF_{2\alpha}$ 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%.

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, 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 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.

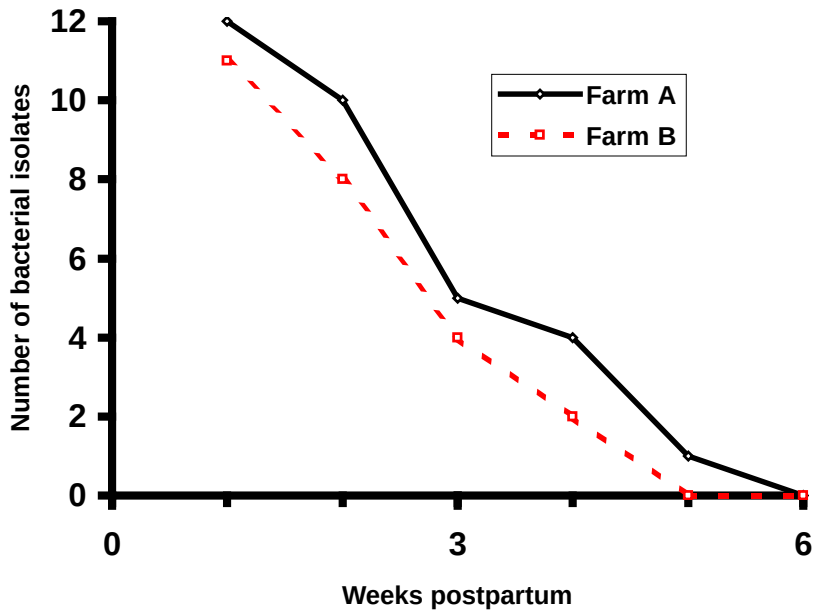

Figure 1. Bacterial elimination from the uterus in farm A and B during 6 weeks PP
Joonis 1. *Bakterite emakast elimineerumise aeg farmis A ja B poegimisjärgse kuuenädalase katseperioodi kestel*

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 $PGF_{2\alpha}$-metabolite patterns are described in Figures 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 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 $PGF_{2\alpha}$ 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.

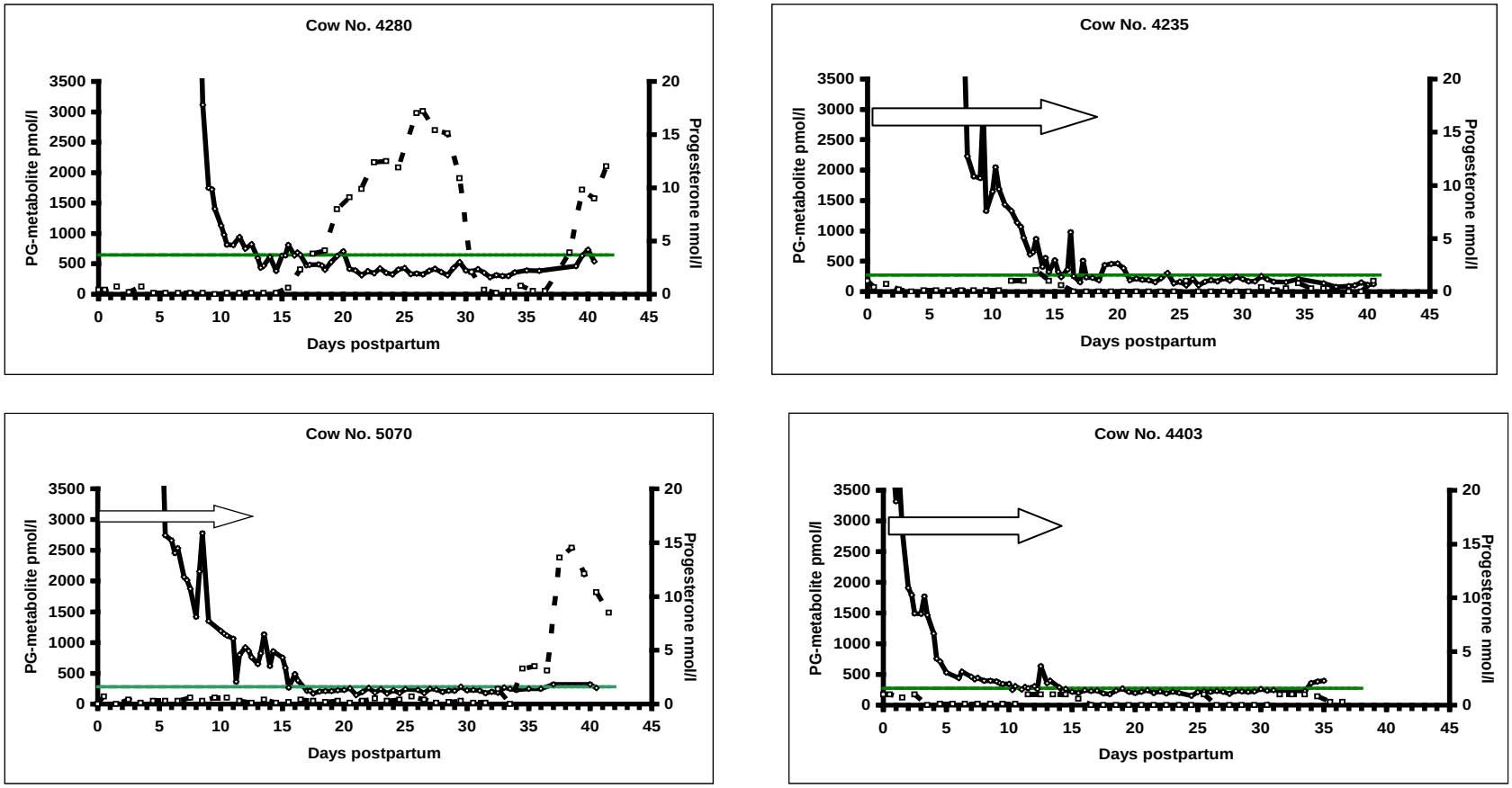

Figure 2. Examples of the PG – metabolite and progesterone profiles during 6 weeks PP in farm A. Block arrow in graphs denotes the bacterial presence and elimination time. The horizontal line in the graphs denotes the line of significance (mean basal value + 2 SD) for the $PGF_{2\alpha}$ metabolite

Joonis 2. *Prostaglandiin F2A metaboliidi ja progesterooni 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)*

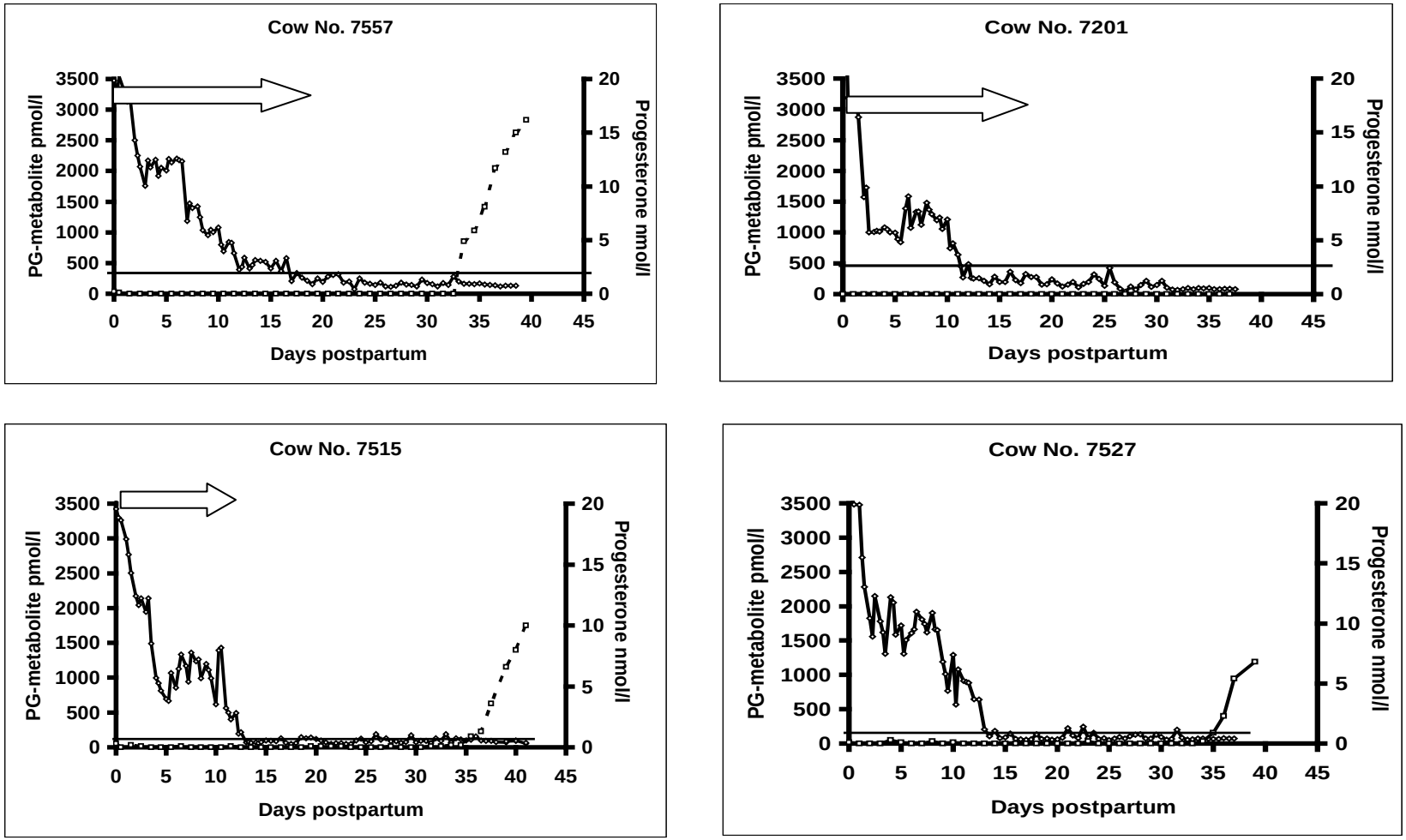

Figure 3. Examples of the PG – metabolite (**-**) and progesterone (- - -) profiles during 6 weeks PP in farm B. Block arrow in graphs denotes the bacterial presence and elimination time. The horizontal line in the graphs denotes the line of significance (mean basal value + 2 SD) for the $PGF_{2\alpha}$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

Joonis 3. *Prostaglandiin F2A metaboliidi ja progesterooni 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)*

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.

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Poegimisjärgne hormonaalne profiil ja emaka bakterioloogia kahes Eesti kõrgetoodangulises lüpsikarjas

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Kokkuvõte

Varajase poegimisjärgse aja (esimesed 42 poegimisjärgset päeva) uuringud tehti kahes Eesti kõrgetoodangulises lüpsikarjas. Aastatoodang farmis A oli 7688 kg ja farmis B 9425 kg. Mõlemas farmis komplekteeriti üks 10 lõpptiinest lehmast koosnev katserühm. Lehmade valiku kriteeriumiks seati hea toitumuse hinne (2,5–3), tiinuse normaalne kulgemine, poegimine kindla ajaperioodi kestel (1 nädal) ja poegimise normaalne kulgemine (mitte abistatud poegimine ja päramiste eemaldumine 12 tunni kestel). Uuriti hormonaalseid parameetreid (prostaglandiin $F_{2\alpha}$, progesteroon) ja emaka infektsioonide esinemist. Emakas esinevate bakterite ja nende elimineerumise aja kindlakstegemiseks võeti emaka endomeetriumist koeproovid (biopsiad). Biopsiad võeti üks kord nädalas kuue nädala jooksul pärast poegimist. Protseduuri teostamiseks kasutati standardset veise emaka biotoomi koos kattesilindriga, vältimaks instrumendi saastumist looma tupes ja tupeesikus. Kogutud biopsiad asetati tioglükolaadi lahusesse (spetsiaalne lahus, mis soodustab just anaeroobsete bakterite paremat säilimist) ja transporditi laborisse külvide tegemiseks ja hilisemaks bakterite määramiseks. Kõik bakterioloogilised analüüsid tehti EPMÜ loomaarstiteaduskonna mikrobioloogia laboris. Aeroobsete bakterite määramine toimus 24 ja 48 tundi ning anaeroobsete bakterite määramine 48 ja 168 tundi pärast külvide tegemist. Alates teisest poegimisjärgsest päevast võeti hormoonanalüüside tarvis vereproovid kägiveenist järgneva skeemi alusel: esimese 14 poegimisjärgse päeva kestel 3 korda päevas (kell 07:00, 13:00 ja 19:00), alates 15. poegimisjärgsest päevast kuni 42. päevani 2 korda päevas (kell 07:00 ja 19:00). Pärast 42. päeva vereproovide võtmine lõpetati. Vere kogus igal võtmisel oli 10 ml ja vereproovid koguti hepariniseeritud katsutitessse. Vahetult pärast proovide võtmist tsentrifuugiti proovid ja eraldatud vereplasma külmutati hilisemate analüüside tegemiseks. Kõik hormoonanalüüsid tehti Rootsi Põllumajandusteaduste Ülikooli loomaarstiteaduskonna laborites Uppsalas. Farmis A oli 3 lehmal 10-st ja farmis B 5 lehmal 10-st emakas täiesti bakterivaba kogu katseperioodi kestel. Kõige levinumad bakterid, mida täheldati mõlema farmi katserühmas, olid *Arcanobakterium pyogenes, Streptococcus spp*., *Escherichia coli*, *Fusobakterium nekrophorum* ja *Bakteroides spp*. Suurimat bakterite kontsentratsiooni täheldati esimese kolme poegimisjärgse nädala kestel mõlemas katserühmas. Bakterite elimineerumine emakast lõppes farmis A pärast 5. poegimisjärgset nädalat ja farmis B pärast 4. nädalat. Bakterite elimineerumine on ära toodud artiklis (joonis 1).

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 väike tõus, mis langes kokku bakterite lõpliku elimineerumisega emakast;
2) kõrgenenud tase esimese 7 poegimisjärgse päeva jooksul, millele järgnes langemine baastasapinnale ja edasine teine väikene tõus, mis langes kokku bakterite lõpliku elimineerimisega emakast.

Prostaglandiin $F_{2\alpha}$ – metaboliidi profiilide näited mõlema katserühma kohta on ära toodud artiklis joonistel 2 ja 3. Progesterooni analüüside andmetel toimusid ovulatsioonid ja seega munasarjafunktsiooni taastumine katseperioodi kestel farmis A 5 lehmal 10-st. Ovulatsioone ei täheldatud 4 lehmal. Farmi B katserühmast täheldati 3 lehmal 10-st ovulatsiooni toimumist katseperioodi kestel. Kokkuvõtvalt oli katseperioodi lõpuks normaalne munasarjafunktsioon taastunud ainult 40% katses kasutatud lehmadest. Progesterooni profiile mõlema katserühma kohta on võimalik jälgida joonistel 2 ja 3.

Nendele uuringutulemustele põhinedes võib öelda, et emakainvolutsioon ja emakainfektsioonide elimineerumine on nendes kahes farmis normi piires. Et leida vastuseid munasarjafunktsiooni taastumise venimisele mõlemas farmis, on vajalikud edasised uuringud, millesse peab kindlasti lisama detailsed ainevahetuslikud uuringud.