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Intrauterine Bacterial Findings and Hormonal Profiles in Post-partum Cows with Normal Puerperium

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Bekana, M., P. Jonsson and H. Kindahl: Intrauterine bacterial findings and hormonal profiles in post-partum cows with normal puerperium. Acta vet. scand. 1996, 37, 251-263. – The post-partum intrauterine bacterial flora, prostaglandin release, uterine involution and resumption of ovarian activity were studied in 9 Swedish dairy cows during the first 8-week period. Uterine involution was monitored by transrectal examinations of the reproductive tract 3 times weekly. Bacteriological examination was performed from twice weekly uterine biopsies. The main $PGF_{2\alpha}$ metabolite (15-ketodihydro-$PGF_{2\alpha}$) was monitored from twice daily blood plasma samples, while morning samples were used for progesterone determinations. The cows were assigned to 2 groups: Group I (n = 7) with an uncomplicated puerperal period and Group II (n = 2) with signs of intrauterine infections. A total of 143 biopsies were collected, of which 129 (90.2%) were found to be bacteriologically negative. Thirteen (9.1%) of the remaining 14 biopsies were bacteriologically positive, while one (0.7%) was probably a contamination on a single occasion. The 13 bacteriologically positive biopsies belonged to the Group II cows from which 31 isolates contained 6 different genera of facultative and obligate anaerobic bacteria. *Actinomyces pyogenes* along with *Bacteroides sp.* and *Fusobacterium necrophorum* were found to predominate in a mixed flora. The bacteria were rapidly eliminated and disappeared completely from the uteri towards the end of the third week post-partum. The average number of days required for completion of uterine involution was 21.8 ± 3.0 for all animals. The plasma levels of the $PGF_{2\alpha}$ metabolite were significantly elevated for the first 12-18, and 18 and 27 days in Group I and Group II, respectively. There was no significant relationship between the duration of $PGF_{2\alpha}$ release and the time required for completion of uterine involution ($p>0.05$). Progesterone analysis showed resumption of ovarian activity and subsequent ovulation in 4 of the 9 cows 44-55 days post-partum. Thus, intrauterine infections are not commonly seen in cows with normal calving and comparison between the duration of $PGF_{2\alpha}$ release and the time required for completion of uterine involution showed insignificant correlation. However, the longer duration of $PGF_{2\alpha}$ release recorded in the 2 cows with intrauterine infections are related to the increased frequency of infections.

prostaglandin; progesterone; uterine biopsy; uterine involution.

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Introduction

The post-partum (pp) period is a crucial event in the life of the dairy cow, during which the animal should re-establish normal uterine and ovarian activities. Understanding the physiology of these organs is thus imperative to the reproductive management, as failure of either one or both to return to normal function may delay the first pp ovulation *(Lamming et al.* 1981, *Kindahl et al.* 1982, *Lindell et al.* 1982, *Thatcher et al.* 1982, *Thompson et al.* 1987, *Kindahl et al.* 1992). Moreover, an inadequate luteal phase may occur during this period due to short corpus luteum lifespan *(Kindahl et al.* 1982, *Hunter* 1991). Consequently, the measurements of the main $PGF_{2\alpha}$ metabolite, 15-ketodihydro-$PGF_{2\alpha}$ and progesterone can be considered to be functional indices of the organs, respectively *(Kindahl et al.* 1982, *Lindell et al.* 1982, *Madej et al.* 1984, *Fredriksson et al.* 1985).

The levels of 15-ketodihydro-$PGF_{2\alpha}$ are elevated at parturition and remain elevated for 7-28 days pp *(Edqvist et al.* 1978, *Kindahl et al.* 1982, *Lindell et al.* 1982, *Bosu et al.* 1984, *Fredriksson et al.* 1985). The levels can be considered to be significantly elevated when they exceed the mean basal value plus 2 standard deviations *(Bekana et al.* 1996). In cows with uncomplicated pp, the levels are inversely correlated to the time required for the completion of uterine involution *(Lindell et al.* 1982, *Bolinder et al.* 1988). On the other hand, more prolonged elevation of the $PGF_{2\alpha}$ metabolite levels has been observed and is positively correlated to the length of uterine involution in cows with intrauterine infections *(Lindell et al.* 1982, *Fredriksson et al.* 1985). In all cases, cyclical ovarian activity does not recur until the concentrations of the $PGF_{2\alpha}$ metabolite are basal or very close to baseline levels *(Kindahl et al.* 1984, *Madej et al.* 1986, *Bekana et al.* 1996). The prolonged elevation of the 15-ketodihydro-$PGF_{2\alpha}$ levels is linked to an increased frequency of uterine infections *(Fredriksson et al.* 1985, *Slama et al.* 1994, *Bekana et al.* 1996) and is considered to be a plausible indication of an inflammatory process in the uterus, and the degree of endometrial damage and repair in cows with retained foetal membranes *(Kindahl et al.* 1984, 1986, *Fredriksson et al.* 1988).

The prevalence of pp uterine infections in cows with normal calving is suggested to be about 50% *(De Bois* 1961, *Fredriksson et al.* 1985) or 85-90% *(Elliott et al.* 1968, *Griffin et al.* 1974a, 1974b, *Hussain et al.* 1990) during the first 2-week period. The risk of uterine infection increases to 100% in cows with abnormal calving, for instance, retained foetal membranes *(Ruder et al.* 1981, *Olson et al.* 1984, *Fredriksson et al.* 1985, *Bolinder et al.* 1988, *Bekana et al.* 1994b) and/or dystocia *(Markusfeld* 1993). In our recent studies, *Actinomyces pyogenes,* along with *Bacteroides levii* and *Fusobacterium necrophorum,* have been reported to be the predominating pathogens in association with the long duration of $PGF_{2\alpha}$ release found in cows with retained foetal membranes *(Bekana et al.* 1996).

The aim of the present study was to determine the presence of facultative and obligate anaerobic bacteria isolated from the pp uterus of cows with normal puerperium. Furthermore, to study the utero-ovarian relationship by analysing the main metabolite of $PGF_{2\alpha}$ and progesterone.

Materials and methods

Animals

Experiments were carried out in 3 second calving and 6 primiparous Swedish dairy cows (3-4 years of age). They were of 4 breed groups, Swedish Red and White (SRB), Swedish Friesian (SLB), crosses between these 2 (SRB×SLB, SLB×SRB) and Swedish Jersey (SJB). The first 3 cows belonged to the Depart-

Table 1. Post-partum uterine characteristics in the nine dairy cows.

Cow No.	Calving Traits			Involution period (d)	First ovulation (d)	Second ovulation (d)	Levels of PG-metabolite at 2 SD (d)
	AG	PA	CA				
1	5	2	F-alive	24	47	56	316 (15)
2	5	2	M-alive	27	–	-	255 (12)
3	5	2	M-alive	20	–	-	281 (14)
4*	3	1	F-dead	25	44	52	251 (18)
5	3	1	M-alive	21	42	51	272 (15)
6*	3	1	F-alive	18	–	-	269 (27)
7	3	1	M-alive	22	–	-	265 (15)
8	3	1	F-alive	20	53	-	274 (17)
9	3	1	M-alive	19	–	–	240 (18)
Mean + SD				21.8 ± 3.0			269.2 ± 21.7 (16.8 ± 4.3)

AG = age; PA = parity number; CA = calving; M = male; F = female; d = days post-partum; PG metabolite at 2 SD = levels of 15-ketodihydro-$PGF_{2\alpha}$ at the line of significance (basal levels + 2 standard deviations) and numbers in parentheses show the number of days post-partum during which the levels are considered to be significantly elevated; – = no ovulation; * = the 2 cows with intrauterine infections.

Table 2. Bacteriological positive examination of endometrial biopsies (n = 13), their number and percentage of the total isolates in the 2 cows with intrauterine infections during the 8-week post-partum period.

Bacteria	Weeks post-partum								No. of isolates	Percent of total
	1	2	3	4	5	6	7	8		
Facultative anaerobic										
Actinmyces pyogenes	2; 1*	2; 2	0; 2	0	0	0	0	0	9	29.0
Streptococcus uberis/dysgalactiae	0	1; 1	2; 0	0	0	0	0	0	4	12.9
Escherichia coli	2; 0	1; 0	0	0	0	0	0	0	3	9.7
Total	5	7	4	0	0	0	0	0	16	51.6
Obligate anaerobic										
Bacteroides sp.	2; 0	1; 2	1; 2	0	0	0	0	0	8	25.8
Fusobacterium necrophorum	0	0; 1	0; 2	0; 1	0	0	0	0	4	12.9
Peptostr. assacharolyticus	1; 0	1; 0	1; 0	0	0	0	0	0	3	9.7
Total	3	5	6	1	0	0	0	0	15	48.4
Facultative + Obligate	8	12	10	1	0	0	0	0	31	100

* Figures before and after the semicolon = cow nos. 4 and 6, respectively.

ment of Animal Nutrition and Management, from where they were brought to the clinic at the Department of Obstetrics and Gynaecology within 2-5 days after calving. The latter 6 animals were bought from farmers and transported to the clinic about 6 weeks prior to parturition. Upon arrival at the clinic, the cows were cared for according to the animal care guide and fed

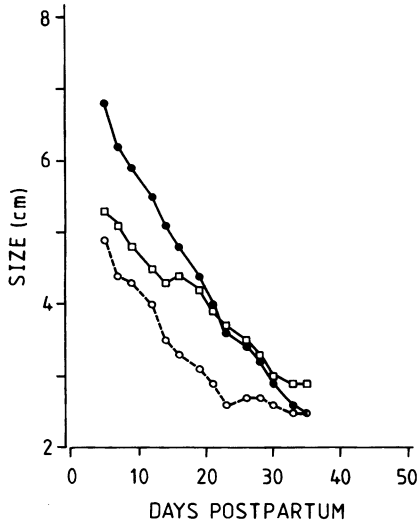

Figure 1. Ultrasonographical measurements of the cervix (□—□) and the 2 uterine horns (left horn pregnant (●—●) right horn non-pregnant (○ - - - ○) in cow no. 2.

according to Swedish standards *(Eriksson et al.* 1972).

Clinical examinations

The cows were closely observed and examined clinically 3 times a week (Tuesday, Thursday and Saturday) as described earlier *(Bekana et al.* 1994a). The date of calving was designated day 0 and the subsequent duration of the pp uterine involution was estimated. Rectal palpation and ultrasound scanning of the cervix, the uterus and the ovaries were performed after inspection of the vulva and the perineal region. The animals were subjected to vaginoscopy when deemed necessary for determination of the type and nature of vaginal discharge.

On the basis of rectal palpation, the position, tone, size and symmetry of the uterus were used as indicators of uterine involution in all animals. The involution was recorded as completed if the uterus had returned to its normal location in the pelvic cavity, and to a normal uterine form and consistency, and if a normal and almost equal size of the 2 uterine horns was restored *(Morrow et al.* 1968, *Lindell et al.* 1982, *Bekana et al.* 1994a). In the second method, employing ultrasonography, measurements of the diameter of the cervix and of the uterine horns were made according to earlier studies *(Bekana et al.* 1994a) and involution was considered to be completed in the second calving cows when 80% reduction of the uterine size was attained. Consequently, the day of completion of uterine involution was judged in the 3 cows by considering the average values obtained with the 2 methods. The day of ovulation was judged from rectal examination and ultrasound scanning of the uterus and the ovaries, and ovulation was assumed to have occurred about 2 days before detection of progesterone concentrations >1.0 nmol/l.

Bacteriology

Each animal in this experiment was sampled for bacteriological examination twice a week (Monday and Friday) from the first week to the end of the eighth week pp. A total of 143 endometrial biopsies were aseptically collected according to the methods and techniques described in earlier studies *(Bekana et al.* 1994b). Biopsies were immediately placed in a thioglycolate medium for transportation to the laboratory for bacteriological examination. The samples were cultured within one h. Plates cultivated aerobically were examined after 24 h and 48 h and plates cultivated anaerobically after 48 h and 168 h. The subsequent identification of bacterial species was performed according to Bergey's Manual of Systemic Bacteriology *(Holt et al.* 1994) at the Department of Veterinary Microbiology, Section of Clinical Microbiology, Swedish University of Agricultural Sciences.

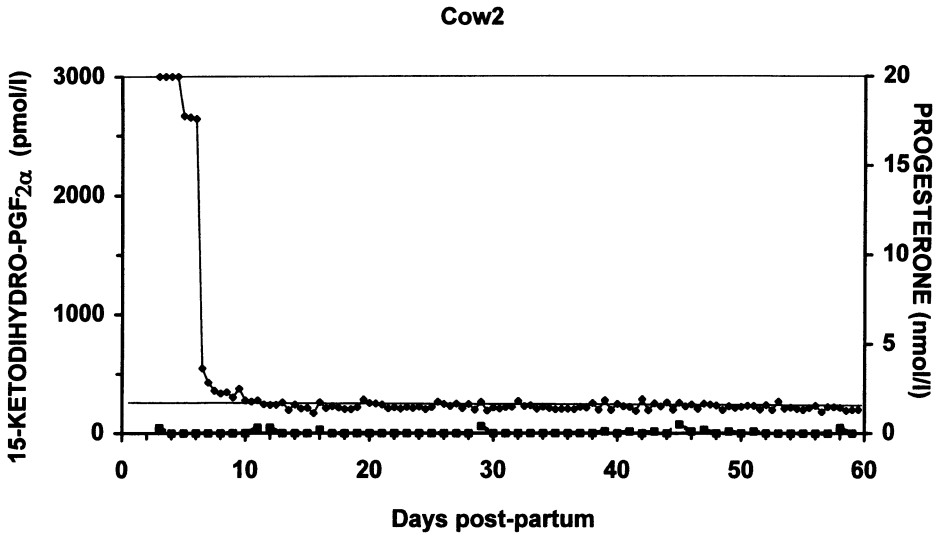

Figure 2. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (■■■) during the first 60 days post-partum in cow no. 2. The horizontal line denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels.

Blood sampling and hormone analysis

Jugular vein blood samples were drawn by venipuncture into heparinized vacutainer tubes twice a day (8 a.m. and 3 p.m.). Sampling started 1 to 5 days pp and continued until 60 days pp. As part of another study, sequential samples were collected every 3 h in the ovulating cows for the next 10 consecutive days to describe the first oestrous cycle. Plasma was removed after immediate centrifugation, and stored in plastic tubes at –20°C until assayed for 15-ketodihydro-$PGF_{2\alpha}$ and progesterone content.

15-Ketodihydro-$PGF_{2\alpha}$ analysis

All plasma samples were analyzed for concentrations of 15-ketodihydro-$PGF_{2\alpha}$ according to *Granström & Kindahl* (1982). The relative cross-reactions 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. In the figures, all the high levels have been estimated, but for practical reasons an upper limit was set at 3000 pmol/l. 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 the standard curve. The duration in days of the pp prostaglandin release was calculated using a skewness method *(Zarco et al.* 1984). The levels were considered to be significantly elevated as long as they exceeded the mean basal value + 2 SD according to *Bekana et al.* (1996).

Progesterone analysis

Progesterone determinations were made from morning plasma samples according to *Forsberg et al.* (1993). The assay is an enhanced lumines-

cence immunoassay (Amerlite®; Kodak Clinical Diagnostic Ltd; Amersham, England). The lowest limit of detection for the assay was 0.2 nmol/l, but levels >1 nmol/l were considered to be of biological importance. The inter-assay coefficient of variation was below 4%. The intra-assay coefficient of variation calculated from quality control samples was between 4% and 8.1%.

Statistical analysis

Statgraphics package (STSC, Inc., 2155 East Jefferson Street, Rockville, Maryland 20852, U.S.A.) and regression analysis were used for comparison and correlation test of the pp duration in days of the $PGF_{2\alpha}$ release and the time required for completion of uterine involution, respectively. Mean values are presented with standard deviation (SD).

Results

Table 1 summarizes recorded clinical data including calving traits, the completion of uterine involution, the number of days pp to the first ovulation, time to the second ovulation and the duration of the pp prostaglandin $F_{2\alpha}$ release at the line of significance. All cows delivered single live calves without assistance after 274-281 days of gestation. An exception to this was cow no. 4 that had dystocia and delivered a dead calf. Seven of the 9 cows were healthy from a clinical point of view and no bacteria were isolated from the uteri throughout the experiment. These cows were designated Group I. The cow with dystocia (no. 4) along with one other cow (no. 6) developed signs of uterine infections as determined by vaginoscopy and transrectal examination. The diagnosis was later confirmed through isolation of pathogenic microorganisms.

In Group I, all cows had normal lochial discharge the first 10-14 days. In Group II, the 2 cows (nos. 4 and 6) showed signs of intrauterine infections, as judged from observations of sanguino purulent or whitish purulent vaginal discharge at vaginoscopy concomitantly with accumulation of a cloudy fluid inside the uterine lumen and thickness of the uterine and/or endometrial walls, particularly during the end of the second week pp at ultrasound scanning. The diagnosis was further confirmed by isolation of pathogens after cultivation of endometrial biopsies (Table 2). Furthermore, mastitis was diagnosed in both cows in Group II and in one in Group I (no. 7) during the end of second week pp. In all cases, the animals were treated intramammarily with antibiotics for 3 consecutive days.

The average number of days required for completion of uterine involution was 21.9 ± 2.8 for Group I cows while for the 2 cows in Group II, involution was completed on day 25 and 18 pp, respectively. The process of uterine involution was associated with a progressive decrease in vaginal discharge and in cervical and uterine diameter. The largest initial diameter of 6.8 and 4.9 cm was found in the previously pregnant and non-pregnant horn, respectively, for instance, cow no. 2. The size decreased to 4.8 and 3.3 cm, respectively, at 16 days pp and continued to decrease to 3.4 and 2.7 cm when the 80% reduction of the uterine size was attained on day 26 post-partum (Fig. 1). The uterine involution was judged by rectal palpation to be completed on day 28 pp. Generally, a similar reduction in the diameter of the cervix and the uterine horns was found in the two remaining second calving cows (nos. 1 and 3). For those cows, the involution was considered to be completed on days 23 and 21, and on days 25 and 19 at rectal palpation and ultrasound scanning, respectively. The average value of the 2 methods is shown in Table 1 for the 3 second calving cows. Alternatively, the completion of uterine involution was estimated only by rectal palpation in the 6 primiparous heifers, as the size of the uterine

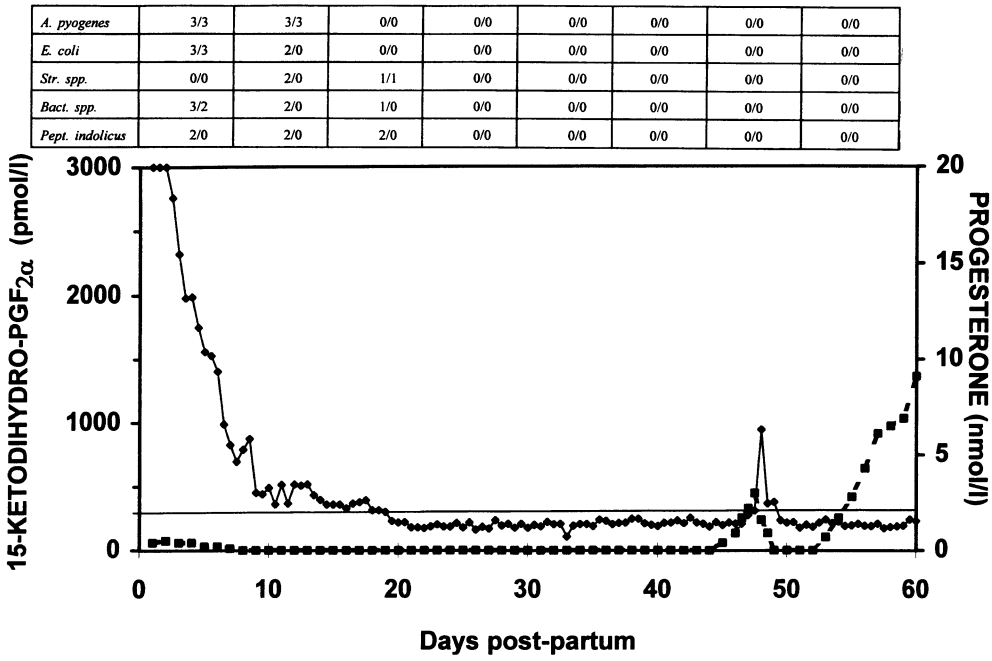

Figure 3. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (■■■) during the first 60 days post-partum in cow no. 4. In the figure, the horizontal line denotes the line of significance (mean basal value + 2 sd) for the prostaglandin metabolite levels. The table at the top of the graph indicates the type and number of isolated bacterial species in relation to hormonal profiles during the 8-week period. The table consists of 9 columns. The first contains a list of identified organisms; columns 2-9 represent twice-weekly collection of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system for bacterial presence; 3 = heavy; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

horns is easily compared and judged using this method.

Appreciable follicular development (> 7mm) was seen on ultrasound scanning as early as 7-11 days pp. In Group I, 3 animals (nos. 1, 5 and 8) had their first pp ovulation on days 47, 42 and 53, respectively. The first 2 cows had further second ovulations 9 days after the first ovulation. In Group II, 1 cow (no. 4) had her first ovulation on day 44 followed by a second ovulation on day 52 pp.

Bacteriology

A total of 143 biopsies were collected, 129 (90.2%) of which were found to be bacteriologically negative. Of the remaining 14 biopsies, bacteria were identified in 13 (9.1%) and the remaining one (0.7%) was considered to be a contamination of *A. pyogenes* on day 18 pp in cow no. 2. Accordingly, the cow was classified as one of the cows with a non-infected uterus during the study period. All the 13 bacteriologically positive biopsies were found in cows nos. 4 and 6 (Group II). The bacterial species recorded, their number and percentage of the total isolates are presented in Table 2. From the bacteriologically positive biopsies, a total of 31 isolates containing 6 different genera of facul-

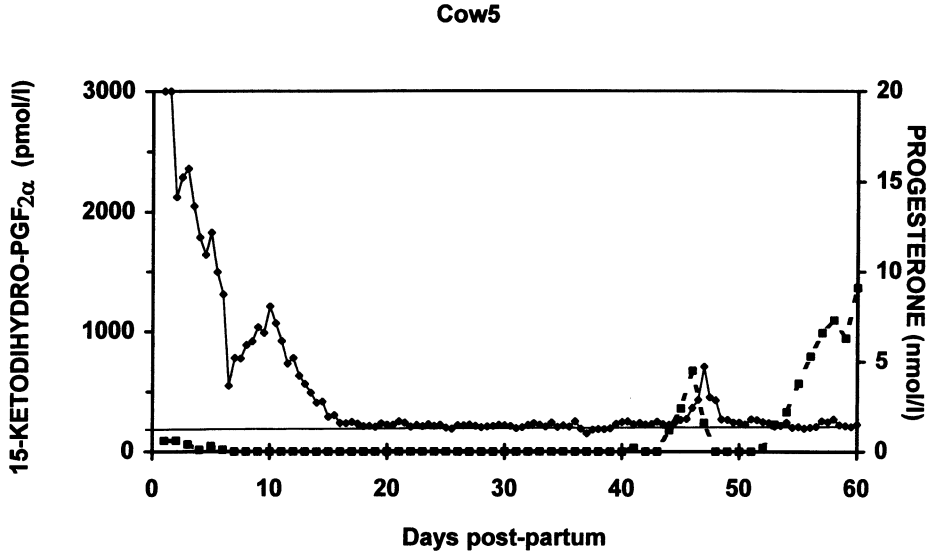

Figure 4. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (■■■) during the first 60 days post-partum in cow no. 5. The horizontal line denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels.

tative and/or obligate anaerobic bacteria were identified in a mixed flora. A pure culture was found in cow no. 6 in a single case *(F. necrophorum* on day 22 pp). Facultative anaerobic bacteria such as *A. pyogenes, Streptococcus uberis* and *Escherichia coli* accounted for 29%, 12.9% and 9.7%, respectively, of the total isolates that yielded both facultative and obligate anaerobic bacteria. The obligate anaerobic bacteria comprised *Bacteroides* sp., *F. necrophorum* and *Peptostreptococcus assacharolyticus/indolicus* which accounted for 25.8%, 12.9% and 9.7%, respectively, of the total isolates. In all cases, the predominating species were *A. pyogenes* along with *Bacteroides sp.* and *F. necrophorum* (Table 2). Generally, a higher prevalence of bacterial growth was found during the second week. Thereafter, the trend was towards a fall in the number of microorganisms and they disappeared completely from the uteri between day 19-22 post-calving.

15-Ketodihydro-$PGF_{2\alpha}$

The overall results relating to hormonal findings are presented in Figs. 2-5. In most cows, the levels of 15-ketodihydro-$PGF_{2\alpha}$ were found to be elevated (higher than 3000 pmol/l) immediately after parturition. The levels then decreased dramatically during the first 10 days, reaching the line of significance (240-316 pmol/l) between 12 and 18, and 18 and 27 days in Groups I and II, respectively. The longest duration of $PGF_{2\alpha}$ release (27 days) was seen in cow no. 6. Her prostaglandin metabolite levels decreased sharply during the first 11 days, but increased again from 435 to 1542 pmol/l on day 12 pp. The high levels of 15-ketodihydro-$PGF_{2\alpha}$ were maintained for 12 additional days concomitantly with the increased frequency of intrauterine infections (Fig. 5). It then declined to the line of significance (269 pmol/l) on day 27 pp. An increase in the concentration of 15-ketodihydro-$PGF_{2\alpha}$ was seen again after the re-

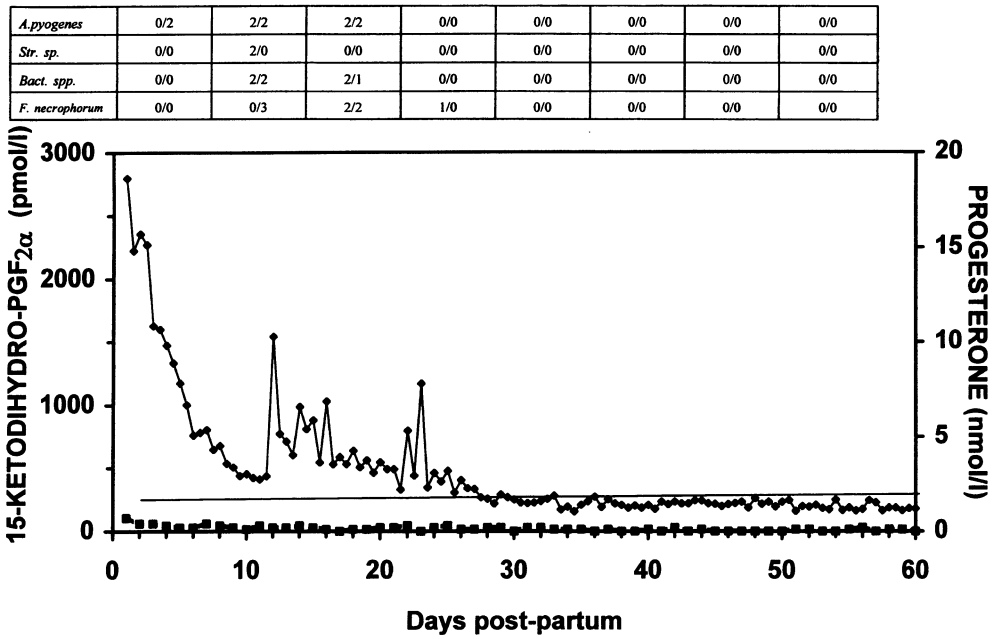

Figure 5. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (■■■) during the first 60 days post-partum in cow no. 6. The horizontal line denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of the graph indicates the type and number of isolated bacterial species in relation to hormonal profiles during the 8-week period. The table consists of 9 columns. The first lists identified organisms; columns 2-9 represent twice-weekly collection of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system for bacterial presence; 3 = heavy; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

turn to baseline in the 4 ovulating cows in association with luteolysis (Figs. 3 and 4).

Progesterone

Low levels of progesterone, <0.7 nmol/l, were found after parturition in all the cows. In the 4 ovulating cows, the low levels were maintained until day 43-54 pp. As can be seen from Figs. 3 and 4, the first rise in progesterone concentrations to >1.0 nmol/l was seen between 44 and 55 days pp and maintained until the time of luteolysis. The levels then started to rise steadily again after the second ovulation. Cow no. 8 had the first ovulation on day 53 followed by a 6-day luteal phase, during which the highest level of progesterone, 4 nmol/l, decreased to 0.3 on day 60 pp. Thus, the sampling was terminated before the second ovulation. Cow no. 4 had the first ovulation on day 44 pp followed by a short-lasting elevation (0.4-3.0 nmol/l). The levels then fell to 0 on day 49 and increased again after the second ovulation which occurred on day 52 pp (Fig. 4). Thereafter, a steady rise in progesterone levels was seen until the last day of the experiment. In the remaining 5 non-ovulating cows, the low levels of <0.7 nmol/l were maintained during the whole 60-day pp period (Figs. 2 and 5).

Discussion

All animals studied calved after 274-281 days of gestation which is within the normal range for Swedish dairy cows *(Berglund & Philipsson* 1987). The average number of days required for completion of uterine involution in all the cows studied was 21.8 ± 3.0. This is similar to the previously reported time of 18-37 days *(Fredriksson et al.* 1985) for Swedish breeds and slightly shorter than the one reported by *Larsson et al.* (1984). The somewhat prolonged interval which was noticed in the cow with dystocia, and the long duration of $PGF_{2\alpha}$ release found in the other cow with intrauterine infections, as compared to the cows with normal calving, may be attributed to the frequent observation of abnormal vaginal discharge. These findings, combined with the thickening of the endometrial and/or uterine walls observed at ultrasound scanning concomitantly with several pathogenic microorganisms isolated in mixed culture, are plausible indications of the inflammatory reaction, possibly leading to longer periods of uterine involution and/or $PGF_{2\alpha}$ release, as has been reported earlier *(Lindell et al.* 1982, *Fredriksson et al.* 1985, *Bekana et al.* 1994b, *Bekana et al.* 1996).

The results from the bacteriological examination of biopsies of endometrial tissue in the present study indicate that intrauterine infections are not commonly found in cows with normal calving. In only 2 of the 9 studied cows were intrauterine infections recorded and a high growth rate of bacteria was noticed at the second week pp. They then declined sharply and disappeared completely from the uteri towards the end of the third week. Thus, our results confirm the accuracy of the technique used showing that contamination during the sampling procedure is very rare. This is in accordance with the previous reports *(Messier et al.* 1984, *Bekana et al.* 1994b) that better depiction of the bacterial status and a more accurate interpretation of the results are obtained when uterine biopsy is used as sampling method, as compared to the swabbing technique *(Griffin et al.* 1974a, 1974b). The low prevalence of uterine infections found in the present study is in line with the previous report of *Fredriksson et al.* (1985) who showed negative bacteriological results in 14 of the 27 cows with a normal puerperium. Similar negative bacteriological findings have been reported *(Spore et al.* 1970) on 26 endometrial aspirates, except for one instance of suspected contamination and for 51 of the 52 segments of fallopian tube from pp women. Thus, the results from this study do not support the contention that up to 90% of the cows with normal calving have uterine infections during the early pp period *(Elliott et al.* 1968, *Griffin et al.* 1974a, 1974b, *Hussain et al.* 1990). Poor management in individual barns could of course increase the frequency of uterine infections.

In the present study, the predominating pathogens in the 2 cows with intrauterine infections were *A. pyogenes* along with *Bacteroides sp.* and *F. necrophorum* in a mixed flora. These results are very similar to the findings in cows with retained foetal membranes in that these 3 organisms were found together and classified as pathogens of primary importance *(Bekana et al.* 1994b). Conversely, other studies do not consider the role of obligate anaerobic bacteria and have suggested *E. coli* to be the predominant pathogen *(De Bois* 1961, *Bretzlaff et al.* 1982, *Bosu et al.* 1984, *Peter and Bosu* 1987, *Hussain et al.* 1990). The present findings also confirm and extend findings in the studies of *Ruder et al.* (1981) and *Olson et al.* (1984) in that these 3 major genera of bacteria were found together and most likely affect the pp uterus synergistically.

The levels of the $PGF_{2\alpha}$ metabolite found here were high immediately after parturition and remained high for 12 to 18, and 18 and 27 days in

cows with and without intrauterine infections, respectively. This is in agreement with the previously reported duration of 7 to 28 days *(Edqvist et al.* 1978, *Lindell et al.* 1982, *Bosu et al.* 1984, *Fredriksson et al.* 1985, *Madej et al.* 1986, *Bolinder et al.* 1988). The longer duration of $PGF_{2\alpha}$ release found in the 2 cows with intrauterine infections are influenced by moderate to heavy growth of a uterine bacterial flora. Comparison between the pp duration of $PGF_{2\alpha}$ release and the time required for completion of uterine involution was insignificantly correlated ($p>0.05$) in all the cows. For the 2 cows with intrauterine infections, no regression analysis was calculated because of their limited number. The longer duration of $PGF_{2\alpha}$ release in the bacteriologically positive group indicated that the infective process may stimulate the synthesis and release of the compound *(Fredriksson et al.* 1985, *Bolinder et al.* 1988, *Kindahl et al.* 1992, *Bekana et al.* 1996).

Determination of plasma progesterone in the present study showed the first pp ovulation between 42 and 53 days, after the high levels of $PGF_{2\alpha}$ metabolite returned to baseline levels. Thus, it seems that the high pp prostaglandin levels play a key role in preventing a too early return to cyclicity. After the first ovulations, the low concentrations of plasma progesterone started to rise progressively, as has been described earlier *(Kindahl et al.* 1982, *Lindell et al.* 1982, *Madej et al.* 1984).

In summary, intrauterine infections are not commonly seen in cows with normal calving and comparison between the post-partum duration of $PGF_{2\alpha}$ release and the time required for the completion of uterine involution showed insignificant correlation. However, the longer duration of $PGF_{2\alpha}$ release recorded in the 2 cows with intrauterine infections compared to those without may be attributed to the frequent early observation of abnormal vaginal discharge, the thickening of the uterine and/or endometrial wall found during transrectal examination and the isolation of pathogens, predominantly *A. pyogenes,* along with *Bacteroides sp.* and *F. necrophorum.* This confirms and extends the previous findings that these organisms most likely affect the post-partum uterus synergistically.

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Sammanfattning

Förekomst av intrauterina bakterier och hormonprofiler hos postpartala mjölkkor med ett normalt puerperium

Den postpartala intrauterina bakteriefloran, prostaglandinfrisättning, uterusinvolution och återupptagande av äggstocksaktiviteten studerades hos 9 svenska mjölkkor under den första 8 veckorsperioden efter kalvningen. Uterusinvolutionen följdes med rektalpalpation av genitalorganen 3 gånger i veckan och uterusbiopsier togs 2 gånger i veckan för bakterieodling. Huvudmetaboliten av prostaglandin (PG) $F_{2\alpha}$ (15-ketodihydro-$PGF_{2\alpha}$) och progesteron analyserades i blodplasma samlat två respektive en gånger dagligen. Sju av djuren visade ett okomplicerat puerperium (grupp I) och två hade tecken på intrauterin infektion (grupp II). Totalt samlades 143 biopsier av vilka 129 (90.2%) var bakteriologiskt negativa. Tretton (9.1%) av de resterande 14 biopsierna var bakteriologiskt positiva, medan en (0.7%) sannolikt var en kontamination vid ett enstaka tillfälle. De 13 bakteriologiskt positiva biopsierna härrörde från grupp II och 31 olika isolat från 6 olika genus av fakultativt och obligat anaeroba bakterier påvisades. *Actinomyces pyogenes* tillsammans med *Bacteroides sp.* och *Fusobacterium necrophorum* dominerade i blandflora. Bakterierna eliminerades snabbt och försvann från livmodern i slutet av tredje veckan efter kalvningen. Det tog i medeltal 21.8 ± 3.0 dagar för alla djur att avsluta uterusinvolutionen. Plasmanivåerna av 3.0 dagar för alla djur prostaglandinmetaboliten var signifikant förhöjda under 12 till 18 dagar för de sju djuren med okomplicerat puerperium respektive 18 och 27 dagar för de två med infektioner. Det kunde inte påvisas något samband mellan prostaglandinfrisättning och tiden för avslutad uterusinvolution ($p>0.05$). Progesteronanalyserna visade att äggstockarna återupptog sin aktivitet och ovulationer inträffade hos 4 av de 9 djuren 44-55 dagar efter kalvningen. Det kan konkluderas från dessa studier att intrauterina infektioner inte är vanligt förekommande hos kor med normal kalvning. Det kunde inte påvisas något samband mellan prostaglandinfrisättning och tiden för avslutad uterusinvolution, men den längre prostaglandinfrisättningen uppmätt hos de 2 korna med intrauterin infektion tyder på att prostaglandiner har betydelse i infektionens förlopp.

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