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Prostaglandin $F_{2\alpha}$ Metabolite and Progesterone Profiles in Post-partum Cows with Retained Foetal Membranes

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Bekana, M., K. Odensvik and H. Kindahl: Prostaglandin $F_{2\alpha}$ metabolite and progesterone profiles in post-partum cows with retained foetal membranes. Acta vet. scand. 1996, 37, 171-185. – Post-partum prostaglandin release and resumption of cyclical ovarian activities were studied in 11 Swedish dairy cows with retained foetal membranes (RFM), leaving the RFM untreated. The main $PGF_{2\alpha}$ metabolite, 15-ketodihydro-$PGF_{2\alpha}$, was measured in blood plasma collected twice daily during the first 50-60 days after delivery. Progesterone was monitored from all morning samples to evaluate the resumption of ovarian activity. The plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ were arbitrarily considered to be significantly elevated between 6-24 days when they exceeded the mean basal value + 2 standard deviations. Comparison between this duration in days of the post-partum $PGF_{2\alpha}$ release and the time required for the completion of uterine involution, placental shedding and last day of post-partum clinical signs showed no significant relations. However, prior to a final decrease below a line of significance of 233-590 pmol/l, pronounced sustained and pulsatile release of $PGF_{2\alpha}$ occurred in relation to the increased frequency of the bacteriological findings. These additional periods of $PGF_{2\alpha}$ release were described as the "total" duration of post-partum release, and were found to be positively correlated with the time required for uterine involution from the stand point of rectal palpation ($p<0.05$), while a tendency towards a positive relationship existed for the last day post-partum of clinical signs ($p = 0.11$). Progesterone analysis revealed resumption of ovarian activity and the first ovulation occurred between 19-29 days in 70% of the cows. The levels of the $PGF_{2\alpha}$ metabolite were again high at the time of luteolysis, thus terminating the luteal phase in the ovulating animals. Thus, it is seen that non-removal of the RFM or the resultant intrauterine infection do not prolong the duration of the immediate post-partum release of $PGF_{2\alpha}$ as compared to normal animals. However, a second release is associated with the increased frequency of uterine infections, indicating that $PGF_{2\alpha}$ may play a role for the early elimination of the infections.

intrauterine infection; luteolysis.

Introduction

The post-partum (pp) uterine involution and resumption of ovarian activity in the dairy cow are major events from a practical and economic point of view. Evidence in the literature (e.g. *Lamming et al.* 1981, *Kindahl et al.* 1982, *Lindell et al.* 1982a, *Thatcher et al.* 1982, *Larsson et al.* 1984, *Matton et al.* 1987, *Eldon* 1991, *Kindahl et al.* 1992) suggests that the progesterone and/or $PGF_{2\alpha}$ profiles provide basic information on the pathophysiological changes prior to or after the reestablishment of the oestrous cycle. Moreover, the bovine uterus and placenta

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are considered to be the main source of prostaglandin during early pp (*Lindell et al.* 1982b, *Guilbault et al.* 1984, *Kankofer et al.* 1994). As a result, retained foetal membranes and the subsequent development of endometritis have been associated with a disturbed synthesis and metabolism of $PGF_{2\alpha}$ in the uterus (*Lindell et al.* 1982a, *Thatcher et al.* 1982, *Bosu et al.* 1984, *Chassagne & Barnouin* 1992), reflecting the degree of endometrial damage or repair (*Kindahl et al.* 1984, 1986, *Fredriksson et al.* 1988). In cows with retained foetal membranes, a prolonged duration of high plasma levels of the main $PGF_{2\alpha}$ metabolite (15-ketodihydro-$PGF_{2\alpha}$) was observed (*Lindell et al.* 1982a, *Fredriksson et al.* 1985, *Matton et al.* 1987, *Slama et al.* 1994). From these studies it can be concluded that the duration and possibly the magnitude of the release of $PGF_{2\alpha}$ are positively correlated to the time required for completion of uterine involution, and cyclical ovarian activity does not resume until the concentration of the $PGF_{2\alpha}$ metabolite is again at a basal level. On the other hand, studies in cows without retained foetal membranes and/or without a subsequent intrauterine infection showed that there is instead a negative correlation between the duration of $PGF_{2\alpha}$ release and the time required for completion of uterine involution (*Lindell et al.* 1982a, *Fredriksson et al.* 1985, *Bolinder et al.* 1988, *Del Vecchio et al.* 1994). The purpose of the present study was to determine, in detail, the release of $PGF_{2\alpha}$ by measuring its main metabolite as an indicator of uterine function, and of progesterone to monitor the ovarian activity in relation to retained foetal membranes (RFM) in dairy cows, leaving the RFM untreated.

Materials and methods

Animals

The study was carried out in 11 pp dairy cows with RFM belonging to the Swedish Red and White Breed (SRB) and the Swedish Friesian Breed (SLB). The cows were primiparous to fifth calvers (ages 3 to 7 years). They all calved after 267 to 283 days of gestation. Three of the cows (nos. 1, 2 and 3) had minor assistance. The term "minor assistance" was applied to the external manual traction of the front legs by their respective owners, to speed up the act of delivery. The cows were housed in individual pens and fed good quality of hay and water ad libitum, as well as 8 kg of commercially prepared concentrate daily according to the Swedish standard for the pp cows (*Eriksson et al.* 1972). The foetal membrane was defined as retained if it was not expelled within the first 24 hours after delivery, and the cows received no treatment. Endometritis was diagnosed according to the previous description (*Bekana et al.* 1994a) on the basis of the observation of significant quantity of abnormal vaginal discharge with concomitant accumulation of cloudy fluid inside the uterine lumen and thickening of the endometrial and uterine walls found at transrectal examination. Furthermore, bacteriological results were used to confirm the diagnosis. For details, see *Bekana et al.* (1994b). Last day of post-partum clinical signs was defined as the day when no vaginal discharge could be observed.

Clinical examination

The cows were closely observed and examined clinically 3 times weekly (Tuesday, Thursday and Saturday) as described in earlier studies (*Bekana et al.* 1994a). All cows were subjected to vaginoscopy when deemed necessary for determination of the type and nature of vaginal discharge. Following the inspection of the vulva and the perineal area, rectal palpation and ultrasound scanning of the cervix, the uterus, and the ovaries were performed.

Uterine position, tone, size, and symmetry were used as indicators of uterine involution on the basis of rectal palpation. The involution was re-

Table 1. Summary of clinical examinations and ovarian activities in the 11 cows during the 8-week post-partum period

	Weeks post-partum							
Post-partum events	1	2	3	4	5	6	7	8
Time to placental shedding (d)	4^{11} $5^{7,8}$ 6^{4} 7^{6}	$8^{2,9}$ 9^{10} $10^{1,5}$						
Involution at: rectal palpation				24^{4} 26^{1} 27^{7}	29^{6} $30^{5,8}$ $32^{10,11}$ 35^{9}	38^{2}		
80% reduction (d)				24^{4} 26^{10} $28^{1,8}$	29^{5} 30^{11} $31^{7,9}$ 32^{6} 33^{2}			
1st OV (d)			19^{6} 20^{4}	23^{1} 24^{7} 27^{5}	$29^{2,10}$			
2nd OV (d)				38^{4}	31^{6} 43^{2}		44^{1} 46^{7}	52^{10}
3rd OV (d)								51^{6}
P_4 >1 nmol/l (d)			20^{6}	23^{4} $26^{1,7}$	29^{5} 31^{10} 32^{2}			

Superscript = cow number; d = days post-partum; 1st, 2nd and 3rd OV (d) = time from parturition to first, second and third ovulation in days, respectively; P_4 >1 nmol/l = days post-partum when progesterone level is higher than 1 nmol/l. Cow no. 3 was slaughtered on day 39 after calving.

corded as completed, if the uterus had returned to its normal location in the pelvic cavity and normal uterine form and consistency, as well as normal and almost equal size of the 2 uterine horns, were restored (*Morrow et al.* 1968, *Lindell et al.* 1982a, *Odensvik & Fredriksson* 1993). Using a second method employing ultrasonography, the involution was considered as completed when 80% reduction of the uterine size was attained according to our earlier description (*Bekana et al.* 1994a). The day of ovulation was judged from the occurrence of post-oestrous bleeding (one day after ovulation) or if the largest follicle, being monitored by ultrasound, could not be detected at the next examination. The subsequent sustained increase in the plasma level of progesterone was used to confirm ovulation. For a summary of the clinical examinations, such as the interval to placental shedding, completion of uterine involution as well as the occurrence of ovulation, see table 1.

Bacteriology

A total of 161 endometrial biopsies were collected for bacteriological examination twice weekly (Monday and Friday) during the first 8-week period according to the methods and techniques described earlier (*Fredriksson et al.* 1985, *Bekana et al.* 1994b). Each biopsy was placed immediately in a sterile glass tube containing thioglycolate medium for transportation to the Department of Veterinary Microbiology, Section of Clinical Microbiology, Swedish University of Agricultural Sciences. Standard bacteriological procedures were used to identify

E. coli	-/3	2/0	0/0	0/0	0/0	0/0	0/0	0/0
A. pyogenes	-/0	0/3	3/2	2/2	2/3	1/1	2/1	0/0
Bact. spp.	-/?	?/3	3/2	2/2	2/1	1/2	2/0	0/0
F. necrophorum	-/?	?/3	3/2	3/3	2/3	1/2	2/2	0/0
Peptostr. sp.	-/?	?/2	2/1	0/0	0/0	0/0	0/0	0/0

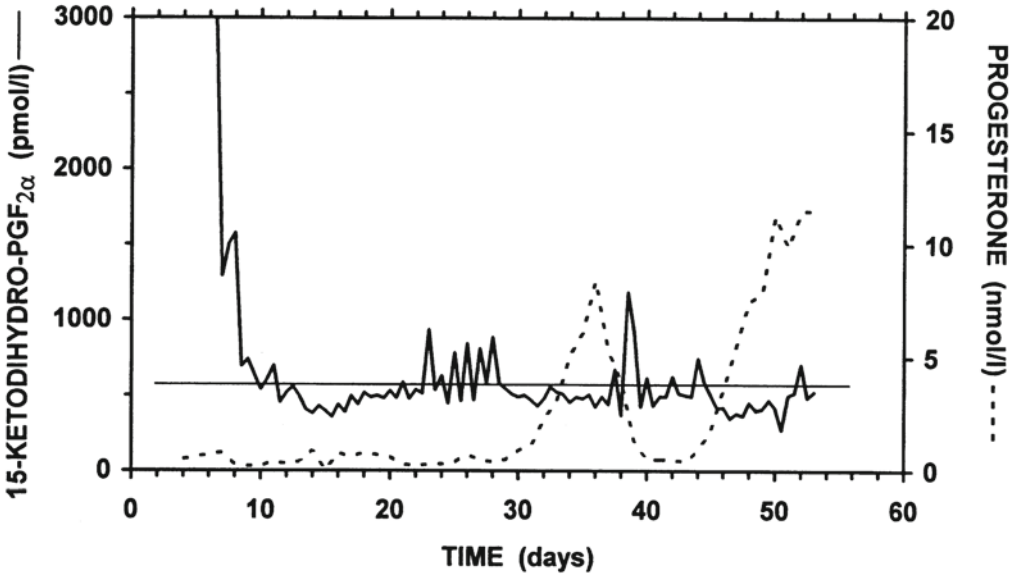

Figure 1. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 2. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; - = missing; ? = unidentified bacteria due to overgrowth of *Proteus spp.;* 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

pathogenic bacteria according to Bergey's Manual of Systemic Bacteriology (*Holt et al.* 1994). Organisms of primary pathogenic importance in the pp uterine flora in relation to hormonal profiles are presented in figures 1-6.

Blood sampling

Ten ml of jugular vein blood samples were withdrawn by venipuncture into heparinized Vacutainer glass tubes (Becton-Dickinson, Rutherford, USA) twice a day (8 a.m. and 3 p.m.). After immediate centrifugation, about 5 ml of plasma were removed and stored in plastic tubes at -20°C until hormone analysis was performed. The sampling started at the beginning of or in the middle of the first week pp and continued until a total of 100 samples had been collected from each animal. An exception was

E. coli	-/3	2/0	1/1	1/0	0/0	0/0	0/0	0/0
A. pyogenes	-/3	1/0	0/0	0/0	0/0	0/0	0/0	0/0
Bact. spp.	-/?	?/2	1/1	0/0	0/0	0/0	0/0	0/0
F. necrophorum	-/?	?/2	1/1	0/0	0/0	0/0	0/0	0/0

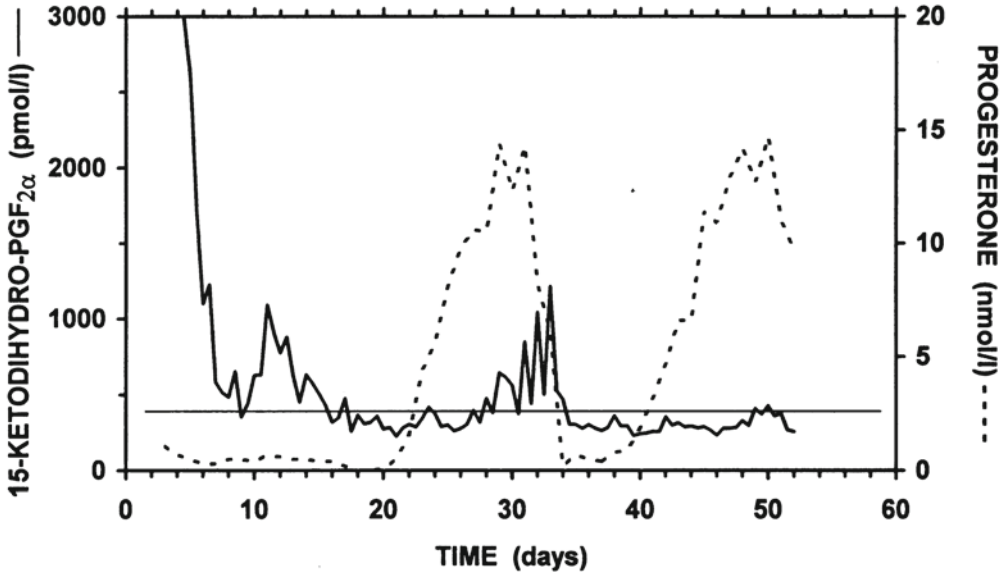

Figure 2. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 4. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; - = missing; ? = unidentified bacteria due to overgrowth of *Proteus spp.;* 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

cow no. 3 that developed pyometra wherefore the sampling was terminated 39 days pp, a few hours before the cow was slaughtered.

Hormone 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. All high levels were estimated, but for practical reasons, an upper limit was set at 3000 pmol/l in the figures. The inter-assay coefficient of variation was 14% (at 114 pmol/l), and the

E. coli	1/3	2/0	0/0	0/0	0/0	0/0	0/0	0/0
A. pyogenes	1/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0
Bact. spp.	?/?	?/2	?/1	0/0	0/0	0/0	0/0	0/0
F. necrophorum	?/?	?/2	?/1	1/1	0/0	0/0	0/0	0/0
Peptostr. sp.	?/?	?/2	?/0	0/0	0/0	0/0	0/0	0/0

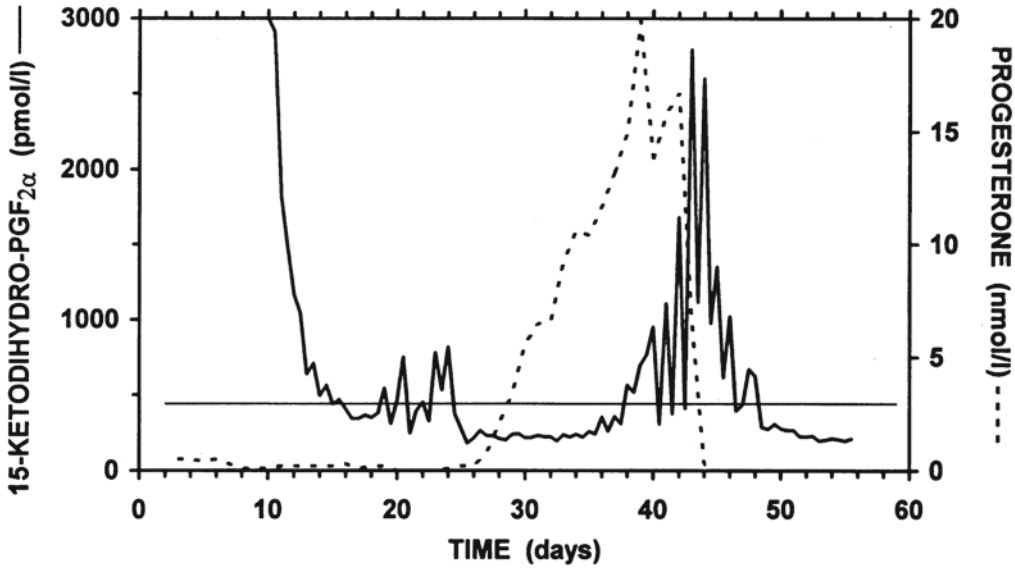

Figure 3. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 5. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; ? = unidentified bacteria due to overgrowth of *Proteus spp.;* 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

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). All prostaglandin metabolite values were used in the calculation. The higher values were removed from the data set in several cycles which were repeated until no new significant elevations were detected. For comparison of the calculations the mean basal value plus 2 and 3 SD were used and are presented in table 2. In the following discussion, the plasma levels of the $PGF_{2\alpha}$ metabolite were arbitrarily considered to be significantly elevated as long as they exceeded the mean basal value plus 2 SD.

Morning plasma samples were analyzed for the content of progesterone according to *Forsberg*

Str. sp.	-/3	2/0	0/0	0/0	0/0	0/0	0/0	0/0
E. coli	-/2	2/0	0/0	0/0	0/0	0/0	0/0	0/0
A. pyogenes	-/3	3/3	2/1	0/0	0/0	0/0	0/0	0/0
Bact. spp.	-/2	2/2	2/2	0/0	0/0	0/0	0/0	0/0
Peptostr. spp.	-/2	0/0	0/2	0/0	0/0	0/0	0/0	0/0

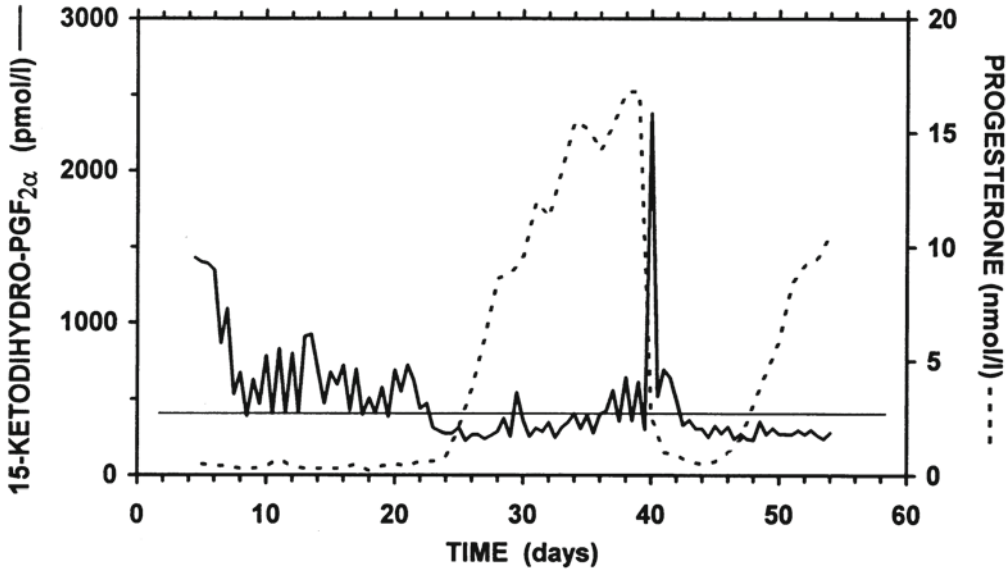

Figure 4. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 7. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; - = missing; 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

et al. (1993). The assay is an enhanced luminescence immunoassay (Amerlite®, Kodak Clinical Diagnostic Ltd, Amers- ham, England). Serial dilution of bovine plasma with high concentrations of progesterone showed displacement curves parallel to the standard curve. 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 coefficients of variation calculated from quality control samples were between 4% and 8.1%.

Statistical evaluation

Comparison and correlation test between the pp duration of the $PGF_{2\alpha}$ release in days versus the time required for completion of uterine involu-

Str. spp.	-/0	2/3	0/0	0/0	0/0	0/0	0/0	0/0
E. coli	-/3	3/3	0/0	0/0	0/0	0/0	0/0	0/0
A. pyogenes	-/3	3/3	2/2	2/1	0/0	0/0	0/0	0/0
Bact. spp.	-/3	3/3	2/2	0/0	0/0	0/0	0/0	0/0
Peptostr. spp.	-/0	0/0	2/2	2/1	0/0	0/0	0/0	0/0

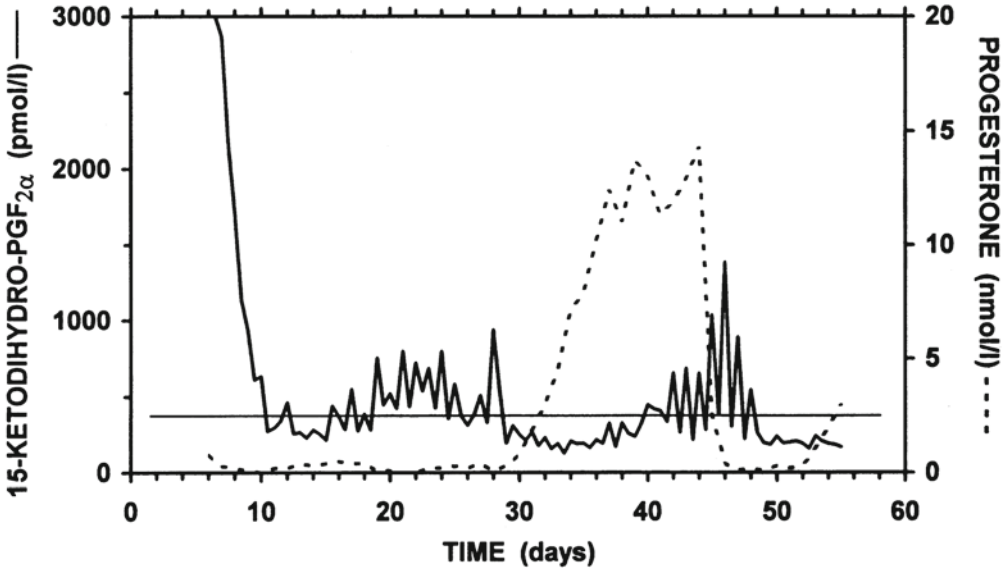

Figure 5. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 10. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; - = missing; 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

tion, placental shedding, and the last day pp of clinical signs were subjected to statistical evaluation using the Statgraphics package (STSC, Inc., 2155 East Jefferson Street, Rockville, Maryland 20852, U.S.A.) and regression analyses, respectively.

Results

A summary of the clinical pictures, including the time required for shedding of the placenta, the time required for the completion of uterine involution, and ovulation time, are presented in table 1. Seven animals; nos. 1, 2, 4-7 and 10, had their first ovulation from day 19 to 29 post partum. They all had a second ovulation between days 31 to 52, except cow no. 5 that had only one ovulation on day 27. Cow no. 6 had an additional third ovulation on day 51. In 5 of the ovulating cows (nos. 1, 4, 5, 7 and 10), ovulations occurred after (e.g. Figs. 2-5), and in

E. coli	3/3	2/1	0/0	0/0	0/0	0/0	0/0	0/0
A. pyogenes	0/0	3/1	2/0	0/0	0/0	0/0	0/0	0/0
Bact. spp.	0/2	3/3	3/0	0/0	0/0	0/0	0/0	0/0
Peptostr. sp.	0/3	3/3	3/0	0/0	0/0	0/0	0/0	0/0

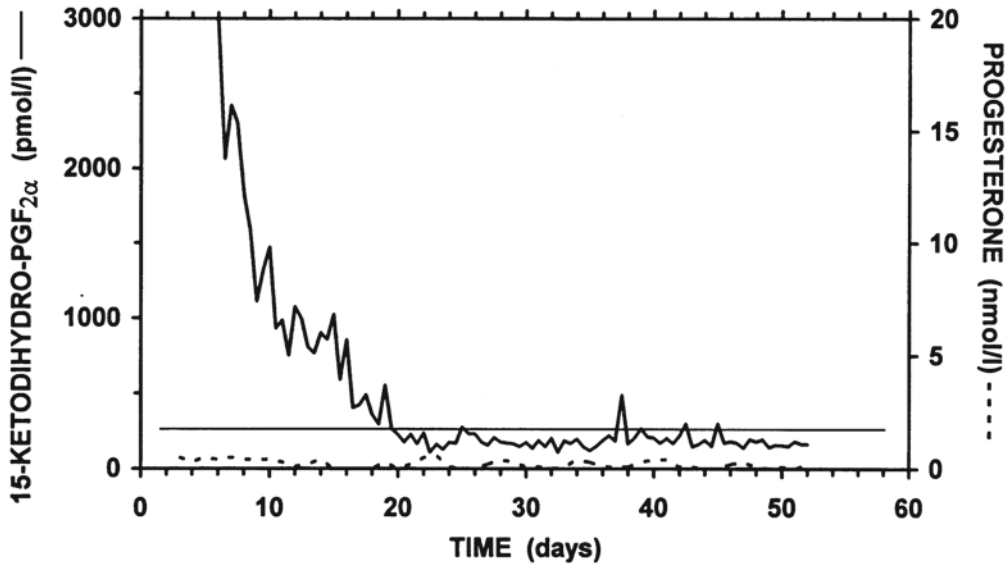

Figure 6. Peripheral blood plasma levels of 15-ketodihydro-$PGF_{2\alpha}$ (——) and progesterone (----) during the first 8-week post-partum period in cow no. 11. The horizontal line in the figure denotes the line of significance (mean basal value + 2 SD) for the prostaglandin metabolite levels. The table at the top of each graph indicates the most predominant isolated bacteria 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 2 weekly collections of endometrial biopsies during the 8-week period. The numbers in each column denote the scoring system of the bacteria; 3 = heavy growth; 2 = moderate; 1 = scanty; 0 = no growth of bacteria.

the remaining 2 cows, nos. 2 and 6, before (e.g. Fig. 1) the bacteria were cleared from the uterus. Four cows (nos. 3, 8, 9 and 11) failed to ovulate within the study period (e.g. Fig. 6). For further bacteriological data, see *Bekana et al.* (1994b).

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

The overall results of hormonal profiles for both ovulating and non-ovulating cows are illustrated in figures 1-6. Very high levels of the 15-ketodihydro-$PGF_{2\alpha}$ (>3000 pmol/l) were seen immediately after parturition. The levels were arbitrarily considered to be significantly elevated as long as they exceeded the mean basal value plus 2 SD (line of significance). For comparison reasons the mean basal values plus 2 and 3 SD, respectively, are presented in table 2. The high levels of the 15-ketodihydro-$PGF_{2\alpha}$ declined sharply soon after parturition and

Table 2. Post-partum $PGF_{2\alpha}$ metabolite levels at the line of significance (2 or 3 sd) and duration in days of the release of $PGF_{2\alpha}$ (**bold**) calculated from 2 and 3 sd, respectively, and "total" duration, including the second period of prostaglandin release

Cow no.	2 sd	3 sd	"Total"
1	479	637	
	8	**7**	**8**
2	590	762	
	10	**8**	**29**
3	392	570	
	9	**8**	**(29)**
4	382	719	
	9	**7**	**16**
5	416	1178	
	15	**13**	**24**
6*	512	865	
	-	-	(19)
7	427	966	
	8	**5**	**23**
8	406	1047	
	24	**13**	**(32)**
9	526	748	
	6	**5**	**(33)**
10	344	947	
	10	**8**	**29**
11	233	1117	
	19	**10**	**19**
Mean ± sd	428 ± 97	869 ± 199	
Days ± sd	**11.8 ± 5.7**	**8.4 ± 2.8**	**23.7 ± 7.7**

* = the duration of prostaglandin release in cow no. 6 could not be calculated. For numbers within parentheses, see text.

reached an average value of 1615 ± 1532 pmol/l (mean ± SD) by the time of placental shedding, which occurred between 4 to 10 days. Thereafter, pronounced sustained and pulsatile release occurred, but the levels continued to decline progressively below the line of significance of 233 - 590 pmo/l (mean ± SD, 428 ± 97) at 6-24 days, mean ± SD, 11.8 ± 5.7 (Table 2). No significant relationships between this duration of prostaglandin release and the time required for the completion of uterine involution, placental shedding, and the last day pp of clinical signs were observed ($p>0.10$).

In 5 of the animals (nos. 2, 4, 5, 7 and 10), increased levels above the mean basal value + 2 SD of the $PGF_{2\alpha}$ metabolite were again recorded on several days, e.g. 7, 6, 5, 14 and 14 days, respectively. If this second release is included in the pp $PGF_{2\alpha}$ release, as described above, the final decrease below the line of significance will be delayed (this has been described as the "total" duration in table 2). In cows nos. 3, 8, and 9, the levels did not show the same sustained pattern and more spurious peaks were seen. In table 2, the "total" release was estimated when these spurious peaks had disappeared and the durations of elevated prostaglandin release have been put within parentheses. Cow nos. 1 and 11 were exceptional in that the levels continued to decrease progressively below the line of significance (at 8 and 19 days after parturition) without any pronounced second elevation. Finally, although cow no. 6 showed a different release pattern of the prostaglandin metabolite with several peaks, there was no clear release of the compound up to day 28 pp. During this period, the bacteria were eliminated from the uterus, ovulation was confirmed, and a distinct peak of the $PGF_{2\alpha}$ metabolite at the time of post-oestrous bleeding was recorded. The cow also showed luteolytic prostaglandin pulses at the time of luteolysis of a corpus luteum in a short oestrous cycle. The estimated "total" release duration was 19 days if calculated up to the time of ovulation. This figure is given in table 2 within parenthesis. A correlation test between the total duration versus the number of days required for completion of uterine involution as determined by rectal palpation showed a significant positive relationship ($p<0.05$). A tendency towards a positive relationship ($p = 0.11$) was seen between the total duration and the last day of pp clinical signs. From figures 1-6 it is evident that the long duration of the elevated prostaglandin metabolite levels (recorded as "total" in table 2) is influ-

enced by the growth and/or the early elimination of bacteria. Organisms of primary pathogenic importance were *Actinomyces pyogenes* along with *Bacteroides spp.* and *Fusobacterium necrophorum* in mixed flora. It should be pointed out that the first 2 genera of bacteria were isolated in all experimental animals while the latter was not identified in 3 of the 11 cows (nos. 1, 10 and 11). It was not possible, however, to link any particular species of bacteria to the duration of the "total" $PGF_{2\alpha}$ release.

In the 7 ovulating cows, the first luteolytic pulsatile release of $PGF_{2\alpha}$ was seen prior to or in association with the luteolysis, and the levels then returned to baseline (Figs. 1-5). In cow no. 1, the release of $PGF_{2\alpha}$ was seen after both ovulations concomitant with post-oestrous bleeding.

Plasma progesterone

Low levels of peripheral plasma progesterone were seen after delivery. The levels remained <1 nmol/l for the first 19 to 30 days concomitantly with elevated levels of the $PGF_{2\alpha}$ metabolite. The first sustained rise in progesterone levels >1 nmol/l occurred after the first ovulation (Table 1), i.e. between days 21 to 32, when prostaglandin levels were at baseline (Figs. 1-5). The progesterone concentrations increased, from approximately 0.8 to its highest levels of about 17 nmol/l prior to the second ovulation in cow no. 7.

In the 4 non-ovulating animals, low levels of progesterone were maintained during the whole observation period. The levels remained <1 nmol/l in 2 cows (nos. 9 and 11) while in 1 cow (no. 8), the level varied from 1.6 to 4.1 nmol/l during the 8-week period. The remaining cow (no. 3), diagnosed with pyometra, had a short-lasting elevation in values (1.9 nmol/l) on day 21 followed by low levels of <1 nmol/l which were maintained until slaughter on day 39 after calving.

Discussion

Previous experiments have shown that elevated levels of 15-ketodihydro-$PGF_{2\alpha}$ seen at the time of parturition remains high for the first 10 to 20 days pp (*Edqvist et al.* 1978, *Lindell et al.* 1982a, *Madej et al.* 1984, *Fredriksson et al.* 1985). The levels were considered to be significantly elevated when they exceeded the mean basal value plus 3 SD of the prepartum $PGF_{2\alpha}$ levels in individual cows (*Lindell et al.* 1982a). Alternatively, they were determined on the basis of the levels found after calving using baseline levels recorded during normal cyclicity as comparison (*Madej et al.* 1984, *Fredriksson et al.* 1985). This method was considered to give a better estimation than the first method of the pp duration of the $PGF_{2\alpha}$ levels (*Fredriksson et al.* 1985). In the present study, the pp duration in days of the prostaglandin release was objectively calculated using a skewness method, and the mean basal values plus 2 and 3 SD were compared. In view of this, the levels were arbitrarily considered to be significantly elevated between 6-24 days after calving as long as they exceeded the mean basal value plus 2 SD. The use of 3 SD, excluded a number of significantly elevated prostaglandin values from the data set and was, therefore, not considered appropriate. The durations, calculated as mean basal values plus 2 SD of 15-ketodihydro-$PGF_{2\alpha}$, are comparable to those recorded previously in pp cows without RFM, possibly because the RFM was left untreated. In cows with a normal puerperium, the duration has been inversely correlated to the time required for the completion of uterine involution (*Lindell et al.* 1982a, *Madej et al.* 1984, *Fredriksson et al.* 1985, *Thompson et al.* 1987). In the present study, the dramatic fall in the concentrations of $PGF_{2\alpha}$ before placental shedding followed by a progressive decline to the baseline levels within 1-3 weeks possibly reflected an important step in the uterine repair. Thus, it seems likely that if the RFM are al-

lowed to be expelled without intervention of man, the pp duration of $PGF_{2\alpha}$ is as short as in uninfected cows. Conversely, manual removal of the RFM prolonged the pp duration of the prostaglandin release (*Fredriksson et al.* 1985, *Madej et al.* 1986).

Comparison between this duration of $PGF_{2\alpha}$ release (as presented by the mean basal value + 2 SD) and the time required for the completion of uterine involution, placental shedding, and last day of pp clinical signs showed no significant relationships. Nevertheless, the total duration of the elevated $PGF_{2\alpha}$ metabolite levels found here are positively correlated with the uterine involution judged on the basis of rectal palpation, while a tendency towards a positive correlation exists for the last day pp of clinical signs. This is in line with previous reports of a positive correlation between the duration of $PGF_{2\alpha}$ release and the time required for completion of uterine involution in cows with apparent (*Lindell et al.* 1982a) and confirmed intrauterine infections (*Fredriksson et al.* 1985).

The total duration of the $PGF_{2\alpha}$ metabolite was found to correspond to an increased frequency of intrauterine infections. The number of isolated bacteria increased from week 1 to week 2, and then declined progressively during the following weeks. It is also interesting to point out that high levels of 15-ketodihydro-$PGF_{2\alpha}$ were seen again after return to the baseline values, concomitantly with the increased frequency of intrauterine infections. This suggests that the duration of the total prostaglandin release depended on either the growth and/or elimination of the organisms from the uterus. Although it has not been possible to link any particular species of bacteria to the total duration of prostaglandin release, it should be pointed out that *A. pyogenes* along with *B. spp.* and *F. necrophorum* were found to be most common among the isolated pathogens. In fact, the first 2 genera of bacteria were isolated in all animals, while the latter was identified in 8 of the 11 cows studied. Generally, these organisms have been reported to produce a variety of potent endotoxins or other virulence determinants responsible for inflammatory processes (*Ruder et al.* 1981, *Olson et al.* 1984, *Del Vecchio et al.* 1994, *Slama al.* 1994). This presumably allowed the uterus to release $PGF_{2\alpha}$ in response to the constant tissue destruction or repair (*Kindahl et al.* 1984, 1986, *Fredriksson et al.* 1988). The high levels of the compound seem, therefore, to play an important role for mediating the inflammatory reaction in the uterus and may play a role for the early elimination of the infections.

The analysis of pp plasma progesterone in the present study revealed that the concentrations of the compound started to rise from low levels during the first 19 to 29 days in all ovulating animals. This shows that the first pp ovulations occurred after the high concentrations of the $PGF_{2\alpha}$ metabolite had returned to baseline levels. The luteolytic release of $PGF_{2\alpha}$ found here is in accordance with the termination of the luteal phase in most of the studied animals, regardless of the length of the oestrous cycles, which is in accordance with earlier findings (*Kindahl et al.* 1976). In a few cases, the high levels of prostaglandins are seen in association with post-oestrous bleeding, as has been reported previously (*Kindahl et al.* 1976). Thus, our findings confirm that cows with RFM left untreated ovulated at an early stage of the pp period. This is in line with the previous report that pp uterine infections do not appear to affect ovulations (*Peter & Bosu* 1987), while manual removal prolonged the time required from parturition to the first cyclical ovarian activity by 20 days, as compared to non-removal of the RFM (*Bolinder et al.* 1988).

In conclusion, leaving the RFM untreated does not prolong the pp duration of immediate $PGF_{2\alpha}$ release or the resumption of cyclical ovarian activities in comparison with normal

animals. However, a second release of $PGF_{2\alpha}$ was seen to correspond to an increased frequency of uterine infections indicating that the compound may play a role in the early elimination of the infections.

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Sammandrag

Hormonmönster av prostaglandin $F_{2\alpha}$-metabolit och progesteron hos postpartala mjölkkor med kvarbliven efterbörd.

Postpartal prostaglandinfrisättning och återupptagande av cyklisk äggstocksaktivitet studerades hos 11 svenska mjölkkor med kvarbliven efterbörd (RFM), där efterbörden lämnades obehandlad. Huvudmetaboliten av $PGF_{2\alpha}$, 15-ketodihydro-$PGF_{2\alpha}$, analyserades i blodplasma insamlad 2 gånger dagligen under de första 50-60 dagarna efter kalvningen. Progesteron analyserades i morgonprov under samma tidsperiod för att utvärdera återupptagandet av äggstocksaktiviteten. För att utvärdera när prostaglandinmetaboliten är förhöjd beräknades basalnivån

+ 2 standard deviationer. Värden över denna godtyckliga linje betraktades som förhöjda och detta syntes mellan 6-24 dagar hos individuella kor. En jämförelse av denna förhöjda nivå i dagar visade inget signifikant samband mot tiden för att avsluta livmoderinvolutionen, efterbördens avgång eller sista dagen för kliniska symptom. Emellertid, före en slutlig nedgång i prostaglandinnivåerna sågs markanta och pulsatila frisättningar av prostaglandin samtidigt som bakteriväxten i livmodern var ökad. Denna ytterligare ökning av prostaglandinfrisättningen kan beskrivas som en "total" frisättning adderad till den ursprungliga. Denna "totala" frisättning i dagar fanns vara positivt korrelerad till tiden som behövs för uterusinvolution ($p<0.05$) och en tendens till en positiv korrelation för sista dagen av kliniska symptom ($p=0.11$). Progesteronanalyserna visade att äggstockarna återupptog sin aktivitet och första ovulation inträffade mellan 19-29 dagar hos 70% av korna. I samband med luteolys av gulkroppen syntes återigen en pulsatil prostaglandinfrisättning. Det kan konkluderas från dessa studier att obehandlad kvarbliven efterbörd och den efterföljande intrauterina infektionen förlänger inte durationen av den omedelbara postpartala frisättningen av prostaglandin jämfört med normala djur. Däremot syns en andra frisättning som är associerad med en ökad bakterieväxt i livmodern, vilket tyder på att $PGF_{2\alpha}$ har betydelse för eliminering av infektioner i livmodern.

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