Induction of parturition with prostaglandin F$_{2\alpha}$ as a possible model to study impaired reproductive performance in the dairy cow

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Abstract

Parturitions were induced in five cows, 2 weeks before term using prostaglandin (PG) F$_{2\alpha}$. Two i.m. injections were performed with an interval of 24 h. All cows calved within 5 days (average 2.7 days) after the first injection of PGF$_{2\alpha}$. Out of five cows, four had retained fetal membranes (RFM). Each animal was sampled for bacteriological examination using uterine biopsies twice a week during 42 days postpartum (PP). Jugular vein blood samples were withdrawn for PGF$_{2\alpha}$-metabolite and progesterone analyses five times per day during the first week PP and eight times per 24 h during the 2nd and 3rd weeks PP. From the 4th week, the sampling interval was reduced back to five times per day. From the 5th week PP, the sampling was reduced to two times per day and sampling was terminated after day 46 PP. Only morning samples were used for progesterone analyses. From day 10 PP, ultrasonography (US) was performed every 3rd day until day 39 PP for detection of ovarian activity and follicular dynamics. The highest incidence of bacteriological species was found during the first 3 weeks PP. After the 5th week of collection, all animals were free from bacteria. The species of bacteria found were Arcanobacterium (Actinomyces) pyogenes, Escherichia coli, α-hemolytic streptococcae and Pasteurella multocida. Immediately after parturition, very high levels of the PG-metabolite were seen in all animals, with a sharp decrease to line of significance around days 9–12 PP. Small increases above the line of significance were detected up to day 27 PP in cows with RFM, and after that...
time the levels were considered to be at baseline. Low levels of progesterone were seen in four animals during the whole experimental time. In one animal, an increase was seen on day 43 PP, which was maintained until the end of the experimental period on day 46 PP. Based on US, follicular waves were detected in all animals during the experimental period. In three animals, three non-ovulatory follicular waves were detected and in two animals, four non-ovulatory follicular waves were detected during 39 days of ultrasound sessions. Based on progesterone levels, only one animal was considered to have ovulated around day 40 PP. Results from the present study indicate that reproductive performance of cows after PG-induced parturitions differs from those of spontaneous cases of RFM. Differences regarding the resumption of ovarian activity were also observed between previous studies of dexamethasone-induced parturitions and the present study.

1. Introduction

The postpartum (PP) period is characterised by involution of the uterus and recyclicity of the ovarian functions to prepare the animal for a new pregnancy period. The PP is strongly influenced by periparturient disturbances such as dystocia, endometritis, mastitis, etc., which to a substantial extent are caused by micro-organisms. During the PP, several hormonal changes occur, e.g. a large release of prostaglandin (PG) \( \text{F}_{2\alpha} \) concomitantly with the uterine involution (Kindahl et al., 1984, 1992; Bekana et al., 1996; Kask et al., 2000b). In the cow, the incidence of a short first oestrous cycle is very high (Kindahl et al., 1982; Bekana, 1997; Kask et al., 2000a,b). The uterus has not regained normal function at this time and after the first ovulation, an uncontrolled PG release occurs, resulting in premature luteolysis leading to the short oestrous cycle.

When making a detailed evaluation of the physiological changes during the PP, it is an advantage to have an experimental model to induce impaired reproductive PP performance. To be able to evaluate conservative vs. pharmaceutical treatments, it is necessary to have access to animals with disturbed uterine involution and with uterine infections. A possible method to get access to animals with PP disturbances is to induce parturition 2–3 weeks before expected term using dexamethasone (Kask et al., 2000a,b). The fetal membranes will be retained completely or partly in most of the cows, and pieces of the membranes are eliminated during the first week PP and uterine infections develop (Kask et al., 2000a,b). Another possibility is to use PGs for induction of parturition, resulting in a more rapid parturition process compared with dexamethasone (Arthur et al., 1996) and perhaps, also more severe PP problems.

The aim of the present experiment was to use PGF\(_2\alpha\) for inducing parturition 2 weeks before term and to evaluate the subsequent effect on the puerperium by measuring the hormone parameters and monitoring the uterine bacteriology. These results can provide information on the most suitable substances to be used, dexamethasone or PG, in an experimental model for PP studies in the cow; also, the present study could be considered as a preliminary preparing ground for future large studies on the same subject.
2. Materials and methods

2.1. Animals and induction of parturition

Five primiparous cows, two of Swedish Red and White (SRB), 2.2 and 2.3 years of age, and three Swedish Friesian Breed (SLB), two of them 2.1-years-old and one 2.5-years-old, were used in the experiment. Cows were bought from different farms and placed in the clinic at the Department of Obstetrics and Gynaecology around 1 month before expected calving. The cows were housed in individual pens and fed ad libitum according to Swedish standards (Spörndly, 1993). No treatments were given to the animals either before or after calving. In all cows, a first injection of PGF2α (Dinolytic®, Boehringer Ingelheim) was given intramuscularly 2 weeks before expected calving with a dose of 25 mg per injection, and a second injection was given 24 h thereafter. The fetal membranes were defined as retained if they were not expelled within 24 h after parturition (Bekana et al., 1994a,b; Arthur et al., 1996; Kask et al., 2000a,b).

2.2. Bacteriology and blood sampling for hormone analysis

Uterine biopsy samples for bacteriology were collected twice a week according to the method described by Fredriksson et al. (1985), Bekana et al. (1994a,b) and Kask et al. (1998), starting within 4 days after parturition and continuing until 6 weeks PP. Each biopsy was placed in a sterile tube containing thioglycolate medium for transportation to the Department of Bacteriology, National Veterinary Institute, Uppsala, where all cultivations were made according to Kask et al. (1999).

Starting on the second day after calving, cannulas were inserted in the jugular vein and 10 ml of jugular vein blood for the analysis of PGF2α-metabolite and progesterone was withdrawn via the cannulas into heparinized Venoject glass tubes (Terumo Europe N.V., Leuven, Belgium) five times per day starting at 7:00 a.m. and continuing every 3rd hour until 7:00 p.m. during the first week PP. During the 2nd and 3rd weeks PP, the sampling interval was increased to eight times per 24 h. During the 4th week, the sampling interval was reduced back to five times per day. At the same time, cannulas were removed from the veins and subsequent blood collections were performed by venipuncture. From the 5th week PP, the sampling was reduced to two times per day (7:00 a.m. and 4:00 p.m.) and sampling was terminated after day 46 PP. After immediate centrifugation, about 5 ml of plasma was removed and stored at −20°C until hormone analyses were performed.

2.3. Hormone analyses

All plasma samples were analysed for concentration of 15-ketodihydro-PGF2α according to Granström and Kindahl (1982). The relative crossreactions of the antibody raised against 15-ketodihydro-PGF2α were 16% with 15-keto-PGF2α, 4% with 13,14-dihydro-PGF2α and 1.7% with the corresponding metabolite of PGE2. The lower limit of detection of the assay was 30 pmol/l for 0.5-ml plasma. All high levels were estimated, but for a better interpretation, an upper limit was set at 3000 pmol/l in the figures. The
intra-assay coefficient of variation varied between 6.6% and 11.7% at different ranges of standard curve.

The duration of the PP PG release was calculated using a skewness method (Zarco et al., 1984). The plasma levels of the PGF$_{2\alpha}$ metabolite were considered to be significantly elevated as long as they exceeded the mean basal value plus 2 SD.

Morning plasma samples of each day were analysed for the content of progesterone according to Duchens et al. (1995). The assay used was an enhanced luminescence immunoassay (Amerlite®, Kodak Clinical, 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%.

2.4. Ovarian ultrasonography (US) and estimation of ovulation

The US equipment was a real time B-mode linear array scanner (Aloca SSD-210 DXII) with a 7.5 MHZ transducer. The instrument was supplied with a standard TV video system and the images were recorded on video tape. Prints were also obtained from a videographic printer, connected directly to the scanner. The equipment was supplied with image freezer facility and electronic calipers for taking measurements. The US examination of the ovaries started on day 10 PP and was performed every third day until day 39 PP. The largest follicle was monitored and measured by freezing the images and using calipers. Based on size measurements taken during the US session and retrospective analysis of the videotapes, graphs of the follicular dynamics were drawn. A follicular wave was defined as an emergence of a group of follicles with selection and further development of a single large follicle and regression of subordinates (Ginther et al., 1989; Knopf et al., 1989). Ovulation was judged to have occurred if the largest follicle being monitored by US, could not be detected at the next examination and also as confirmed by a subsequent increase in progesterone concentration (Kask et al., 2000a). Ovulation was postulated to occur 3 days before the first detection of sustained elevation of the plasma progesterone concentration (Duchens et al., 1995). The results regarding ovarian function from day 40 to day 46 PP were based on progesterone patterns only.

3. Results

3.1. Calving performance

All five cows calved within 5 days after the first injection of PGF$_{2\alpha}$. Normal calving performance was recorded in all animals. The average time from the first injection of PGF$_{2\alpha}$ to parturition was 2.7 days (range 1.5–5 days) and the average duration of the calving process was 5 h. Three male and two female calves were born. Retained fetal membranes (RFM) were recorded in four animals (Nos. 828, 810, 830 and 811) and one cow (No. 819) delivered the placenta within 3 h PP. Placenta was delivered on day 8 PP in animal No. 828 and on day 9 PP in animals Nos. 810, 811 and 830. Endometritis was
diagnosed in all animals based on bacteriological investigations and vaginal discharge recordings. One cow, No. 828, showed general depression and inappetance and was excluded from the study on day 35 PP. However, the cow was used in the present data presentation.

3.2. Uterine bacteriology

No bacteriologically negative animals were found during the experimental period. From five animals, altogether 55 biopsies were collected, of which 33 were found to be bacteriologically positive and the remaining 22 biopsies negative. Totally, 60 isolates were identified from the 33 positive biopsies, all of which were aerobic bacteria; no specific anaerobic infections were found. The isolates demonstrated were as follows: *Escherichia coli*, 23 cases; *Arcanobacterium (Actinomyces) pyogenes*, 17 cases; α-hemolytic streptococcae, 14 cases; and *Pasteurella multocida*, six cases.

The highest incidence of bacteriological species was found during the first 3 weeks PP in cows with RFM and during the first 2 weeks PP in the cow without RFM (No. 819). After the 5th week of collection, all animals were free from bacteria. Presence and final elimination of bacteria in individual animals can be followed in Fig. 1 and Table 1.

3.3. 15-Ketodihydro-PGF$_{2\alpha}$

Results from hormonal data are presented in Fig. 1. Immediately after parturition, very high levels of 15-ketodihydro-PGF$_{2\alpha}$ were seen. The top values of the PG-metabolite are demonstrated in Table 1. In four cows with RFM, almost identical patterns of PG-metabolite were seen, with very high values during first week PP and with a sharp decrease around 9–12 days PP (Table 1). Further elevations above the line of significance were detected, but the magnitude of these elevations were not very high and basal levels were reached around days 19–27 PP in all cows (Table 1). In one cow (No. 819) that had no RFM, the PG-metabolite release decreased after day 12 PP, but levels were slightly elevated until day 17 PP (Table 1).

3.4. Progesterone

Low levels of progesterone were seen immediately after parturition in all animals. The levels remained low (around 1 nmol/l or less) for the whole experimental period in four animals, but in one animal (No. 811), the first elevation was seen on day 43 PP. This rise continued subsequently until day 46 PP when blood sampling was terminated. This progesterone rise was considered to be the result of an ovulation.

3.5. Follicle dynamic and resumption of ovarian activity

Ovarian US was started on day 10 PP. Earlier investigations were not possible due to the large size of the involuting uterus. None of the animals were ovulating during the US period up to day 39 PP, which also was confirmed by the progesterone results. In one
Fig. 1. Peripheral blood plasma levels of 15-ketodihydro-PGF\_2\alpha (thick line) and progesterone (dotted line) during the first 48 days PP in cows with RFM (Nos. 828, 830, 811 and 810) and in the cow without RFM (No. 819). The horizontal line in the graph denotes the line of significance (mean basal value \( \pm 2\) SD) for the PG-metabolite levels. The table on the top of the graph shows the presence and elimination of bacteria in relation to hormonal profiles. The first column contains the list of isolated organisms. The subsequent columns correspond to the twice-weekly collection of endometrial biopsies. The scoring system in the table is as follows: H = heavy growth of bacteria; M = moderate growth; S = slight growth; 0 = no bacteria found; – = missing sample.

Animal (No. 811), a dominant follicle (\( \geq 1.0 \) cm) was recorded at the end of the US period on day 39, which was followed by a progesterone rise on day 43 PP.

Follicular wave patterns were detected in all animals, except No. 811, when US was started on day 10 PP. In cow No. 811, follicular waves were first detected on day 14 PP. Consecutive non-ovulatory follicular waves were recorded in all five animals. In three animals (Nos. 819, 828 and 811), three follicular waves were detected with emergence and regression of dominant follicles. The average length of these waves was 9.5 days and the average size of the dominant follicle was recorded to be 1.0 cm.
Table 1
PP PGF$_{2\alpha}$ metabolite top values, the duration of the high PG-levels (days), the duration of the low and jumping levels (days), the value at the line of significance and the time (days PP) for final elimination of bacteria

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Top values of PG-metabolite (nmol/l)</th>
<th>Duration of the high values (days)</th>
<th>Duration of the low and “jumping” values (days PP)</th>
<th>Value at the line of significance (2 SD) (pmol/l)</th>
<th>Final elimination of bacteria (days PP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>819</td>
<td>8.1 (day 4 PP)</td>
<td>12</td>
<td>12–17</td>
<td>278</td>
<td>11</td>
</tr>
<tr>
<td>828</td>
<td>16.5 (day 2 PP)</td>
<td>9</td>
<td>9–22</td>
<td>233</td>
<td>31</td>
</tr>
<tr>
<td>810</td>
<td>17.1 (day 4 PP)</td>
<td>10</td>
<td>10–19</td>
<td>236</td>
<td>34</td>
</tr>
<tr>
<td>830</td>
<td>21.1 (day 3 PP)</td>
<td>11</td>
<td>11–26</td>
<td>386</td>
<td>25</td>
</tr>
<tr>
<td>811</td>
<td>16.0 (day 3 PP)</td>
<td>9</td>
<td>9–27</td>
<td>336</td>
<td>24</td>
</tr>
</tbody>
</table>

*a Cow without RFM.

In two cows, Nos. 810 and 830, four non-ovulatory follicular waves were detected. The average length of these waves was 8.3 days and the average size of the dominant follicle was recorded to be 1.2 cm.

4. Discussion

The interval from the first injection of PGF$_{2\alpha}$ to parturition was much shorter when compared with studies made by Kask et al. (2000a,b), where dexamethasone was used for induction of parturition. In that study, the average time from injection to parturition was 5.3 days compared with 2.7 days in the present study. This is in agreement with Barth (1986) and Arthur et al. (1996) who reported calving within 3 days after PGF$_{2\alpha}$ injection, respectively. In the present study, the first injection of PGF$_{2\alpha}$ was made between days 257 and 263 of pregnancy (average 260) and no relation was observed between days of pregnancy and interval from injection to calving.

A high incidence of placental retention was reported (up to 75% of the cows) following induced parturition when PGF$_{2\alpha}$ was used around 2 weeks before expected calving time (Barth, 1986), which was also seen in the present study (four out of five cows had RFM). This meets our demands because the goal was to get a high incidence of RFM. The present study strongly supports the concept that uterine infections are a common feature in cows with RFM (Bekana et al., 1994b; Kaneko et al., 1997; Kask et al., 2000a,b). However, the most important and interesting finding was that no single case of specific anaerobic bacteria was demonstrated in these five animals. This differs from other studies performed during recent years, where the same sampling methods were used (uterine biopsies). Bekana (1996) reported that anaerobic bacteria were found to be the most common micro-organisms. In a previous study by Kask et al. (2000b) *A. pyogenes*, *F. necrophorum* and *Bacteroides* spp. were found to be dominating. In the present study only *A. pyogenes* was found among these species. This species can be present in both anaerobic and aerobic environments, thus being considered as a facultative anaerobic bacteria. There is no explanation of this difference; sampling and
cultivation methods were the same as in previous studies by Kask et al. (1999, 2000b) and the animals also were kept under the same conditions. The highest incidence of bacterial species was found during 2nd and 3rd weeks PP in all cows, followed by a successive decrease resulting in the total disappearance of bacteria after the 5th week PP. Similar results are reported from previous studies by Bekana et al. (1994b) and Kask et al. (2000b), leading to the conclusion that cows with RFM will recover from intrauterine infections quite successfully without any antibiotic treatment.

An interesting finding concerning the PGF$_{2\alpha}$ metabolite profiles in the present study was that they were almost identical. The profiles were characterised by an initial rise and followed by a sharp decrease of the PGF$_{2\alpha}$ metabolite to much lower levels after day 10–12 PP. After that, levels were found to be elevated above the line of significance until days 20–25 PP, when they reached the basal levels. This pattern is different from the studies conducted by Bekana et al. (1996), where cows with spontaneous cases of RFM were studied, and from the studies of Kask et al. (2000b) where dexamethasone-induced parturition cows were monitored. In the study from Bekana et al. (1996), where spontaneous RFM cows were used, the PG-metabolite profiles were decreasing during the first 2 weeks PP and then a second release was seen which corresponded to an increase of uterine infections. In the previous studies by Kask et al. (2000b), where dexamethasone-induced parturition cows were used, PG-metabolite levels were decreasing continuously until they reached baseline around days 15–20 in most of the cases and all later elevations were associated with luteolysis. It seems that several different patterns of PP PG-metabolite release can be seen. The first pattern is associated with spontaneous cases of RFM (Bekana et al., 1996), and a second type with PG-induced parturition as seen in this study. The induction with dexamethasone seems to be in between these two types of RFM.

One possible explanation of the PG-profiles in the present study might be that no anaerobic bacteria were found. There are studies indicating that combination or synergism of bacterias such as *A. pyogenes*, *E. coli*, *F. necrophorum* and *Bacteroides* spp. may cause more serious problems (Moberg et al., 1982; Dow and Jones, 1987; Baystone and Cohen, 1990). Since no specific anaerobic bacteria were found in the present study, this might have affected the uterine environment to a lesser extent, and thus, might explain the shorter duration of high PG-metabolite levels. Also, we should maybe consider that using PGs for induction of parturition, the parturition process will start more quickly than in the case of dexamethasone, which is considered to be in a more physiological way.

One cow, No. 819, did not retained the placenta and showed lower magnitude of the highest PG-metabolite levels, but displayed a longer duration of the high values. This pattern is more comparable with animals not having retained placenta after full-term deliveries (Lindell et al., 1982; Madej et al., 1984; Fredriksson et al., 1985). It is obvious that after induced parturition some animals will be more normal and will not have reproductive problems after parturition. The animal was positive for bacterial growth (*Arcanobacterium* and *E. coli*), but could quickly eliminate the bacteria. Thus, this cow could be considered as having normal PP performance.

None of the animals ovulated during the first 40 days PP. Only in one animal was a progesterone rise detected on day 43. This is surprising, since the prominent PG-
metabolite release had returned to relatively low levels from days 10–12 PP and to the final basal level around day 20 PP. It is documented that as long as the PG-release is dominating, ovulations do not occur and ovulations do occur in most cases shortly after the PG-metabolite levels have reached the base levels (Kindahl et al., 1984; Kindahl et al., 1992). In the present study, however, PG-levels were low approximately from day 20 PP, but no ovulations were detected until after day 40 PP. Follicular wave patterns were recorded in all animals already from day 10 PP, but none of the dominant follicles ovulated. The animals were fed properly and, based on our hormonal and bacteriological data, no severe reproductive dysfunctions were detected. Unfortunately, no data about the metabolic status of these cows are available. One cow (No. 828) was excluded from the study after day 35 due to general depression and inappetance, but until that time, she showed follicular dynamics similar to the other animals. The reason for the failure in ovulation remains unclear and should stimulate further research. It is, however, interesting to speculate that the PGF$_2$$_a$ treatment per se might influence the endocrinological events around parturition in a more unpredictable way than dexamethasone if we consider that dexamethasone initiates parturition in a more physiological manner.

5. Conclusion

Induction of parturition with PGF$_2$$_a$ is an effective method to induce RFM and endometritis during the puerperium. However, based on the present study, we have found an indication that the postpartum performance after PGF$_2$$_a$-induced parturition cows differs from those of spontaneous cases of RFM and from those of dexamethasone-induced parturition. Results from the present study can be considered as a preliminary due to the low number of animals used and further studies are underway to get a more precise picture of the studied events.

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References


