

Induced Parturition and Retained Placenta in the Cow

**Inhibition of prostaglandin F_{2α} synthesis
and antibiotic therapy**

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2001

Acta Universitatis Agriculturae Sueciae
Veterinaria 106

ISSN 1401-6257
ISBN 91-576-5933-8
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Tryck: SLU Service/Repro, Uppsala 2001

*Stolparna är mina bästa vänner.
Leif "Honken" Holmqvist*

Abstract

Königsson, K. 2001. Induced Parturition and Retained Placenta in the Cow. Inhibition of prostaglandin $F_{2\alpha}$ synthesis and antibiotic therapy Doctor's dissertation.

In order to study rapid changes in 15-ketodihydro-PGF $_{2\alpha}$ (PG-metabolite), in the period preceding parturition in cattle, pre-term parturition was induced by dexamethasone injection in four heifers on days 254 to 265 in gestation. Blood samples were collected at least every hour until 12 hours after parturition and during the second stage of labour at least 6 times per hour. The average time from injection to parturition was 7.7 days (mean). Two of the heifers had retained foetal membranes (RFM). At the start of the experiment the levels of PG-metabolite were low and increased slowly to levels between 1000 and 2000 pmol/L at one day before parturition. During the last day, however, the levels increased rapidly and the highest levels (>10000 pmol/L) were reached at the time of delivery. No pulsatile PG-metabolite elevations were seen. Immediately after foetal expulsion the PG-metabolite levels decreased rapidly in all animals. In two animals with RFM, however, this decline ceased within a few hours postpartum (pp). Instead the PG-metabolite levels started to increase and reached levels as high as during parturition.

In another experiment the effects of the prostaglandin synthesis inhibitor, flunixin (F) and oxytetracycline (T) was evaluated in cows with RFM. As a model for RFM, pre-term parturition was induced by injections of PGF $_{2\alpha}$. 22/24 cows had RFM. After parturition the cows were divided into different groups according to the treatment. Cows were treated with F, T, T+F or conservatively. There were two experiments with two different treatment periods (3–6 d pp, before placental shedding and 11–14 d pp, after placental shedding). Animals treated with T on days 11–14 had a quicker recovery from the uterine infection than other cows. T or T+F treatment on days 3–6 did not shorten the uterine infection but altered the bacterial flora. Furthermore, T or T+F treatment at this time led to improved appetite and increased energy consumption. However, T or T+F before placental shedding delayed the detachment of the retained foetal membranes compared with other cows. F did not influence clinical signs, recovery from infection or uterine involution. F suppressed PG-metabolite levels significantly during periods of treatment. However, treatment on days 3–6 suppressed PG synthesis only partially. T and T+F before placental shedding significantly altered the kinetics of the early PG-metabolite profile compared with other treatments. Late PG-metabolite elevation was significantly correlated to the duration of the uterine infection and to the cervical involution.

In a final study, we wanted to evaluate meloxicam (M) as an inhibitor of the inflammatory response elicited by endotoxin (ET). Furthermore, we wanted to evaluate a possible effect of meloxicam on Δ^{13} -reductase and 15-hydroxy prostanoate dehydrogenase, which catalyse the first steps of the metabolism of PGF $_{2\alpha}$. Four heifers in mid luteal phase were used in the experiment. ET elicited a rapid PG and cortisol release. White blood cells, iron, zinc and calcium were affected as well. The clinical effect was dramatic. Pre-treatment with M was found to abolish the PG-release totally. Furthermore, the cortisol release was reduced and the effect on general appearance and several blood parameters were suppressed. M did not prevent the pyrogenic effect of ET. M seems to have no major influence on the metabolism of PGF $_{2\alpha}$.

Key words: PGF $_{2\alpha}$, parturition, retained foetal membranes, flunixin, meloxicam, oxytetracycline, endotoxin

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Appendix

This thesis is based on the following four papers, which will be referred to by their Roman numerals.

- I. Königsson, K., Kask, K., Gustafsson, H., Kindahl, H., & Parvizi, N., 2001. 15-Ketodihydro-PGF_{2α}, progesterone and cortisol profiles in heifers after induction of parturition by injection of dexamethasone. *Acta Vet Scand* 42: 151–159.
- II. Königsson, K., Gustafsson, H., Gunnarsson, A., & Kindahl, H., 2001. Clinical and bacteriological aspects on the use of tetracycline and flunixin in primiparous cows with induced retained placenta and post partal endometritis. *Reprod Dom Anim*, in press.
- III. Königsson, K., Gustafsson, H., & Kindahl, H., 2001. 15-Ketodihydro-PGF_{2α}, progesterone and uterine involution in primiparous cows with induced retained placenta and post partal endometritis treated with oxytetracycline and flunixin. *Reprod Dom Anim*, in press.
- IV. Königsson, K., Odensvik, K., & Kindahl, H., 2001. Endocrine and clinical effects of intravenous endotoxin injection after pre-treatment with meloxicam in heifers. Manuscript.

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Introduction

Parturition is commonly divided into 3 stages. The first one comprises endocrine and mechanical preparation of uterine and cervical tissues for parturition. The second phase involves the expulsion of the foetus and the birth of an offspring. The third stage involves expulsion of the placenta (Arthur et al 1983). Thus, the normal postpartal period does not start until all these phases have finished.

The actual expulsion of a calf is the result of a chain of endocrine events starting several weeks before parturition. Perhaps, the most important of these events is removal of the pregnancy preserving action of progesterone (Janszen et al 1990).

Termination of progesterone synthesis before parturition involves at least two different phases in cattle (Edqvist et al 1978). The first occurs during the last weeks of pregnancy and is believed to be the result of a direct influence of the foetus on placental steroidogenic pathways. In cattle, cortisol from foetal adrenals induces a steroidogenic shift in the placenta (Flint et al 1979). Instead of synthesising progesterone via the Δ^4 -pathway (conversion of pregnenolone to progesterone) the steroid synthesis is switched to an alternative route, the Δ^5 -pathway (conversion of pregnenolone to 17α -hydroxy-pregnenolone). By this a placental synthesis of oestradiol- 17β is initiated and levels of this steroid increase at the end of pregnancy at the expense of progesterone synthesis (Schuler et al 1994). This can be viewed from an endocrine point of view as increasing levels of circulating oestradiol- 17β and declining levels of progesterone (Edqvist et al 1978). The effects of the steroid synthesis shift seem to prepare the uterus for parturition. As a result, the levels of prostaglandin synthesising enzymes increase in the uterus, cervix and placenta near term (Fuchs et al 1999; Wimsatt et al 1993). These enzymes permit a high synthesis rate for prostaglandins $F_{2\alpha}$ and E_2 .

Prostaglandins in general play an important role in parturition and $PGF_{2\alpha}$ in particular. $PGF_{2\alpha}$ is important for the final regression of the corpus luteum of pregnancy. This is particularly obvious in mice lacking the prostaglandin F-receptor. In such mice, $PGF_{2\alpha}$ does not terminate progesterone synthesis and as a consequence, parturition does not occur until the source of progesterone is removed by some other means (Sugimoto et al 1998). In cattle, a luteolytic dose of $PGF_{2\alpha}$ late in pregnancy induces parturition within 48 h (Kask et al 1999; Kornmatitsuk et al 2000), but if exogenous progesterone is supplied to compensate for the loss of endogenous progesterone, parturition does not take place until the exogenous progesterone is withdrawn (Janszen et al 1990).

$PGF_{2\alpha}$ has, in combination with oxytocin, an important impact on uterine contractility. In sheep, uterine prostaglandin F and E receptor levels increase at term (Ma et al 1999) and administration of $PGF_{2\alpha}$ causes myometrial contractions when administered to late pregnant (Janszen et al 1993) and postpartal cows (Thun et al 1993). Furthermore, administration of exogenous prostaglandin synthesis inhibitors can suppress uterine contraction antepartum (McKeown et al 2000). Other prostaglandins, like PGE_2 are important for the maturation of the cervical tissue (Winkler et al 1997). This maturation includes softening and dilatation of the cervix before foetal expulsion and is characterised by a

morphologic as well as a biochemical change of the connective tissue constituents (Ekman-Ordeberg & Malmström 1998).

Prostaglandins are synthesised via the arachidonic acid cascade. The first step in this pathway includes liberation of arachidonic acid from membrane bound phospholipids. Responsible for this step is the calcium-dependent, cytosolic phospholipase A₂ (cPLA₂) (Zhang et al 1996).

The second step includes the synthesis of the intermediate prostaglandins, PGG₂ and PGH₂ by two isoenzymes, cyclooxygenase-1 or -2 (COX-1 or -2) (Malkowski et al 2000). The intermediate prostaglandins are then further metabolised to PGF_{2 α} , PGE₂, PGI₂ etc.

COX-1, which was the first cyclooxygenase to be identified, is expressed in stable amounts in different tissues. It has been called the “house keeping enzyme” since prostaglandins synthesised by COX-1 mainly are involved in physiological processes. The second isoenzyme, COX-2, is inducible, short-lived and only expressed under certain conditions (Vane & Botting 1996). Inflammation (Masferrer et al 1994) and parturition (Rice et al 1995) are examples of such conditions.

Expression of COX-1 and -2 in uterine tissues during different reproductive phases has been studied in cattle and sheep. A conclusion from these studies has been that levels of COX-1 in the uterus are stable and do not vary during oestrous cycle and pregnancy. COX-2 levels on the other hand, are basal most of the time but increase during luteolysis and parturition (Johnson et al 1995; McLaren et al 1996). During late gestation and parturition, COX-2 levels increase in foetal cotyledonary epithelium, maternal caruncular epithelium and in cervix. Increased levels of COX-2 have been found in the myometrium at the time of parturition (Fuchs et al 1999; Ivell et al 2000). The increased COX-2 levels are reflected by a high synthesis rate of prostaglandins.

Prostaglandin synthesis in the uterus seems to be intimately linked to the presence of oxytocin receptors and the action of oxytocin (Ivell et al 2000). In cattle, oxytocin has been shown to induce COX-2 synthesis and release of intracellular calcium (Burns et al 1997). Both of which are important for activation of cPLA₂ and prostaglandin synthesis.

Prostaglandin F_{2 α} , like other prostaglandins, has mainly paracrine actions and its chemical properties does not allow intracellular storage. Instead, increased plasma levels are a direct result of an increased synthesis rate. Once released, however, PGF_{2 α} has a short half-life in plasma (Samuelsson et al 1975) and for this reason, measurement in peripheral blood can give a faulty picture of the “real” PGF_{2 α} synthesis. A more accurate picture of the synthesis can be achieved by measuring 15-ketodihydro-PGF_{2 α} (Kindahl et al 1976). This metabolite is more stable in plasma and measuring it gives a good estimation of the uterine PGF_{2 α} synthesis (Guilbault et al 1984a).

Studies regarding the PGF_{2α} metabolite around parturition in cattle have shown that plasma levels increase in late pregnancy and the highest levels are reached during parturition (Hunter et al 1977; Edqvist et al 1978; Aiumlamai et al 1992). After expulsion of the foetus and the foetal membranes, PGF_{2α} metabolite levels decline (Lindell et al 1982; Guilbault et al 1984b).

The physiological process of placental detachment is initiated several weeks before parturition, but is not completed until the last days before term. A maturation process leads to a successive weakening of the borderline between the cotyledonary and caruncular parts of the placentome (Grunert 1986). The process includes connective tissue remodelling (Sharpe et al 2001) and chemotactic attraction of leucocytes (Gunnink 1984; Heuwieser & Grunert 1987). The final detachment occurs along the interface between the foetal and the maternal parts of the placentome. This occurs during and after foetal expulsion. However, if the maturation process is not completed before parturition, the risk for retained foetal membranes increases (Laven & Peters 1996). In our model for retained placenta we used this matter in order to increase the frequency of retained foetal membranes.

Ideally, the postpartal period is a non-infectious event. At parturition, the contracting uterus expels the calf and the placenta. A reduction in uterine size and a unidirectional flow of uterine contents as well as a gradual closure of the cervix prevents introduction of foreign material and microbial contamination. With progression of the uterine involution the risk for infection decreases. In cows with retained foetal membranes, these membranes keep the cervical canal open and the uterine lumen distended, hence inhibiting normal uterine contraction. At the same time, the foetal membranes hang out of the vestibulum readily exhibited for faeces and other external contaminants. The membranes move in and out of the vagina when the cow moves. Hence, contaminants easily pass the mechanical protective barriers and enter the uterus. An abundance of devitalised tissue and the impaired mechanical and immunological defence are important contributing factors for a uterine infection.

Thus, an important sequel of retained foetal membranes in cows is the postpartal endometritis (Fredriksson et al 1985). The uterine infection typically has an acute and a chronic phase. In the acute phase, the dominating bacterial species are facultative anaerobic bacteria like α -haemolytic streptococci and *Escherichia coli*. They are commonly isolated from the first days after parturition until 2 to 3 weeks pp, when they gradually disappear. In the chronic phase of the infection the dominating species are instead *Arcanobacterium pyogenes* and anaerobic bacteria like *Fusobacterium necrophorum* and *Bacteroides* spp. These latter species appear during the first week pp and they usually disappear spontaneously around 5 weeks pp (Fredriksson et al 1985; Bekana et al 1994).

As mentioned earlier, PGF_{2α} has important functions in bovine reproduction and is physiologically released before, during and after parturition. However,

prostaglandins including $\text{PGF}_{2\alpha}$ are also released during inflammatory processes. From a prostaglandin point of view, retained placenta represents a condition where these two phenomena, inflammation and reproduction, meet.

Comparisons of cows with and without retained foetal membranes indicate disparities in postpartal $\text{PGF}_{2\alpha}$ metabolite profiles (Madej et al 1986; Nakao et al 1997). Postpartal $\text{PGF}_{2\alpha}$ release under physiological conditions is peaking at the time of parturition and then gradually decline to basal levels over a period of 15 to 20 days (Lindell et al 1982). The $\text{PGF}_{2\alpha}$ metabolite profile is characterised by an exponential decrease with a half-life of roughly 4 days (Kindahl et al 1982). After this initial decline, no significant release occurs until after the luteolytic episodes at the end of the first postpartal luteal phase.

In cows with retained foetal membranes the postpartal $\text{PGF}_{2\alpha}$ metabolite profile, instead of declining, increases immediately after parturition (early elevation). The levels remain high during the first week postpartum and decrease to lower, but not basal, levels at 10 to 14 days after parturition (Bolinder et al 1988; Kask et al 2000). After a short period of basal $\text{PGF}_{2\alpha}$ metabolite levels, another elevation starts. This elevation is of a smaller magnitude than the periparturient elevation, but the duration is longer. The second (late) $\text{PGF}_{2\alpha}$ metabolite elevation is correlated to the uterine infection, and is believed to be a result of the uterine inflammation (Fredriksson et al 1985; Del Vecchio et al 1992; Bekana et al 1996).

The clinical symptoms of retained foetal membranes can partly be attributed to uterine infection and inflammation. Especially during the acute phase, there is an abundance of Gram negative bacteria in the uterus. E.g. *E. coli*, *F. necrophorum* and *Bacteroides* are commonly encountered in uterine tissue during the first week pp and infections with Gram negative bacteria are known to cause depression, anorexia and fever (Langhans 2000). In cows with retained foetal membranes infection with these bacterial species coincides in time with the presence of clinical signs like depression and fever.

The high release of $\text{PGF}_{2\alpha}$ during the acute phase of the postpartal endometritis might, at least partly, be related to the presence of endotoxin from Gram negative bacteria (Bosu et al 1984; Slama et al 1994). An important property of endotoxin is its ability to activate the arachidonic acid cascade and this feature might be crucial for the pathogenesis of the clinical signs (Fredriksson 1984). However, prostaglandin synthesis as well as clinical signs of inflammation can be attenuated by administration of inhibitors of the arachidonic acid cascade (Jarløv et al 1992).

The non-steroid anti-inflammatory drugs (NSAID) form a group of substances, which has in common an ability to inhibit the prostaglandin synthesis of COX-1 and -2. Flunixin and meloxicam are two such substances registered for use in food producing animals. Both have been used for prevention and alleviation of clinical signs like pain and fever and both of them efficiently inhibit prostaglandin synthesis (Odensvik & Magnusson 1996; Salamon et al 2000). Furthermore, flunixin has been used for studies in postpartal cows. In those studies, intensive administration of flunixin already from parturition suppresses $\text{PGF}_{2\alpha}$ release, but

seems to have little influence on the uterine involution (Odensvik & Fredriksson 1993; Thun et al 1993).

Aims

The main objectives of this thesis were to:

Study the profile of 15-ketodihydro-PGF_{2α} before, during and after dexamethasone-induced parturition by frequent blood sampling in cattle with and without retained foetal membranes.

Evaluate flunixin and oxytetracycline treatments of cows with induced retained foetal membranes with regard to clinical, bacteriological and endocrine parameters.

Evaluate meloxicam as an inhibitor of endotoxin elicited disease.

Methodological considerations

Animals

A total of 32 heifers were used, 31 of the Swedish red and white breed (SRB) and 1 of the Swedish black and white breed (SLB). The ages of the animals ranged from 1 to 3 years. All heifers were clinically examined and found healthy before the start of experiments. Feeding in all experiments was according to Swedish standards.

The heifers used in papers I and IV (n=8) were bought from regular farms and brought to the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, Uppsala, Sweden. The heifers were allowed to acclimatise for at least 1 week before the start of experiments.

Heifers used in papers II and III (n=2 x 12) belonged to the Kungsängen research farm, Swedish University of Agricultural Science, Uppsala, Sweden. They were born and reared on the farm. In the winter before each experiment they were heat-synchronised and then artificially inseminated. In October, they were brought indoors and housed in individual pens. The experiments were performed in the facilities of the research farm. After calving the cows were milked twice daily.

General outline of experiments

In paper I, parturition was induced by injections of dexamethasone. The experiment lasted from first injection of dexamethasone until 12 hours after parturition. During the experiment blood samples were collected frequently (at least once per hour) and during expulsion of the foetus, at least 6 times per hour. Plasma was analysed for 15-ketodihydro-PGF_{2α}, progesterone and cortisol.

In papers II and III, 12 heifers were used in each of two experiments (altogether 24 heifers), accomplished during 2 years. As a model for retained foetal membranes, pre-term parturition was induced by injections of PGF_{2α}. After parturition the cows were divided into 4 groups according to treatment regime. The experiment lasted until 8 weeks postpartum. Blood samples were collected for analyses of PGF_{2α}-metabolite, progesterone, white blood cells and flunixin. Uterine microbiology and involution were monitored.

In paper IV, 4 non-pregnant heifers were used for investigation of meloxicam as an inhibitor of endotoxin induced inflammation. The heifers were given a low dose of endotoxin with or without meloxicam pre-treatment. In 2 additional experiments PGF_{2α} was given with or without meloxicam pre-treatment. All treatments were administered intravenously.

In the endotoxin experiments, blood samples were collected for evaluation of white blood cells, plasma levels of PGF_{2α}-metabolite, meloxicam and cortisol as well as serum levels of zinc, iron and calcium. The heifers were clinically

examined several times per day. In the PGF_{2α} experiments, plasma samples were collected for analyses of PGF_{2α}-metabolite.

Clinical examination

In papers II and III clinical examination included general health, nature and presence of vaginal discharge, body temperature, food consumption, milk production and body weight. It also included rectal palpation and transrectal ultrasonography of the inner genitals including measurement of the cervix, the uterine horns and the ovaries.

In paper IV, clinical examination was performed several times per day. It included body temperature, appetite, heart frequency and breathing frequency.

Bacteriological sampling

Papers II and III. Uterine bacteriology was investigated twice weekly by the use of a biopsy technique according to Fredriksson et al (1985). The first sample was collected in the first week postpartum. The samples were transported to the National Veterinary Institute (SVA), Department of Bacteriology. At the laboratory, the samples were used for cultivation and determination of anaerobic and aerobic bacteria, according to Kask et al (1999). Isolated bacterial strains were identified according to Bergey's Manual of Systematic Bacteriology (Holt et al 1994).

Blood sampling

Papers I and IV. On the day before the start of experiment a central venous catheter was inserted into the *V. jugularis externa*. The catheter was connected to a silicone tube, which was led to the withers. This allowed frequent blood sampling with minor disturbance of the animals. In papers II and III, blood was collected by venipuncture of *V. jugularis externa* twice daily (at 8 to 9 a.m. and at 4 to 5 p.m.).

Immediately after collection, the tubes were centrifuged and then plasma or serum was withdrawn and transferred to plastic tubes. Samples were stored at -20° C until analysis.

Treatments

Paper I. Dexamethasone (20 mg, Vorenvet vet. 1 mg/ml, BI-vet, Malmö, Sweden) was injected intramuscularly twice with a 24-hour interval on days 254–265 of pregnancy.

Papers II and III. The first PGF_{2α}-injection (Dinolitic vet. 5 mg/ml, Pharmacia-Upjohn, Stockholm, Sweden) was done between days 260 and 269 in pregnancy.

During the periods of treatment, cows were treated daily with oxytetracycline (Engemycin vet. Intervet, Göteborg, Sweden, 10 mg/kg, once daily, intramuscularly), flunixin (Finadyne vet. Schering-Plough, Stockholm, Sweden, 2.2 mg/kg twice daily, by oral route), a combination of oxytetracycline and

flunixin (dosage as above) or conservative treatment. Flunixin was suspended in a bottle of water and administered via the mouth. In the first experiment the period of treatment was days 11 to 14 (after spontaneous placental shedding) and in the second, days 3 to 6 postpartum (before placental shedding).

Paper IV. All treatments were made intravenously on the contralateral side of the intravenous catheter. Endotoxin (lipopolysaccharide from *E. coli* O55:B5 1 mg, Sigma, Stockholm, Sweden) was diluted in physiological saline to a concentration of 1 µg/ml. The dose administered was 50 ng/kg. Meloxicam (Metacam vet. 5 mg/ml, Boehringer-Ingelheim, Malmö, Sweden) was administered at a dose of 0.5 mg/kg. PGF_{2α} was diluted in physiological saline to a final concentration of 50 µg/ml. The dose administered was 0.5 µg/kg.

Analytical methods

15-ketodihydro-PGF_{2α} (papers I to IV) was analysed using a radioimmunoassay (Granström and Kindahl 1982). NaHeparin plasma was used for analysis and all samples were analysed in duplicates. Sensitivity of the method was 30 pmol/L.

In paper I, progesterone in plasma was analysed by a solid-phase radioimmunoassay technique (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). The sensitivity of the assay was 0.1 nmol/L. In papers II and III, progesterone was analysed using an enhanced luminescence immunoassay (Amerlite, Kodak Clinical Diagnostic Ltd, Amersham, England). Sensitivity of the assay was 0.2 nmol/L.

Cortisol concentration in plasma (paper IV) was measured by use of an RIA (Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, U.S.A.). Detection limit for the assay was 7 nmol/L.

Serum concentrations of calcium (paper IV) was analysed using a methylthymol blue method (Roche Unikit II, Roche Diagnostica, Basel, Switzerland).

Serum concentrations of iron (paper IV) was analysed using a colorimetric method (Roche Unimate 5, Roche Diagnostica, Basel, Switzerland).

Serum concentrations of zinc (paper IV) was analysed using a colorimetric method (Randox Zn-2341, Randox Laboratories Ltd, Antrim, UK).

An automated haematology analyser (Cell-Dyn 3500, Abbott Diagnostics) was used for evaluation of the total WBC count (papers II, III and IV). For differential polymorphonuclear (PMN) and mononuclear (MN) cell count (Paper IV), smears were made immediately after blood collection. The smears were stained with May-Grünwald/Giemsa and the proportions were obtained by microscopic counting of 200 cells.

Flunixin (papers II and III) in plasma was measured by an HPLC method described by Odensvik and Johansson (1995). The sensitivity for the assay was 47 ng/ml plasma.

Plasma levels of meloxicam (paper IV) were measured by an HPLC method provided by Boehringer-Ingelheim, Ingelheim, Germany. The sensitivity for the method was 10 ng/ml plasma.

Statistical evaluation

Statistical analyses were calculated by use of Minitab for Windows 95, release 12.2 (Minitab Inc. PA, U.S.A.). Kruskal-Wallis test was used for calculation of differences between groups (papers II and III). Pearson product moment correlation analyses were used for determination of the linear correlation between two parameters (papers II and III). Results are shown as coefficient of correlation and p-value. In order to characterise the PG-metabolite profiles, half-lives for plasma levels were used (paper III). Calculations were made by linear regression of PG-metabolite values after logarithmic transformation. For the levels of 15-ketodihydro-PGF_{2α} (paper IV), a basal level was calculated according to Zarco et al. (1984). Calcium, iron, zinc and white blood cells (paper IV) are shown with dot plots describing the differences between meloxicam and saline pre-treatments for individual heifers at each sampling occasion.

Results

PG-metabolite profiles before, during and immediately after parturition in cows with and without retained placenta (paper I)

The pre-experimental levels of PGF_{2α} metabolite, days 254–265 in pregnancy in 4 heifers ranged from 150 to 300 pmol/L. After dexamethasone injections the synthesis rate of prostaglandins increased. This could be seen as increasing levels of the PGF_{2α} metabolite. Two kinds of pattern of this increase were found. In the two heifers injected on days 264 and 265, PGF_{2α} metabolite levels continuously increased from the time of dexamethasone injection until parturition. In the heifers injected on days 254 and 258 the levels of PGF_{2α} metabolite increased initially but after a few days the levels declined to levels similar to the pre-experimental levels. After a nadir around 3 days before parturition, a final increase started and this lasted until parturition. Luteolysis occurred in all animals during the final increase of PGF_{2α}. During the luteolytic period the PGF_{2α} metabolite levels increased rapidly but showed no signs of pulsatility. The PGF_{2α} metabolite peak was sharp and clear in two heifers with a quick and easy parturition (short second stage of labour) and the highest PGF_{2α} metabolite levels were seen at the time of foetal expulsion. In two heifers with a prolonged second stage of labour, the PGF_{2α} metabolite peak was broadened. The PGF_{2α} metabolite levels at parturition were 13000–21000 pmol/L.

Immediately after foetal expulsion, the levels of PGF_{2α} metabolite declined rapidly in all heifers. However, two of the four heifers had retained foetal membranes. In these heifers the immediate postpartal PGF_{2α} metabolite decline, soon (within hours) was interrupted and changed into a new period of increasing PGF_{2α} metabolite levels. The magnitude of this postpartal PGF_{2α} metabolite elevation was in the same range as the levels recorded during parturition. In the two heifers in which the foetal membranes were shed at parturition, no postpartal increase was seen, instead PGF_{2α} metabolite levels declined throughout the experiment.

Postpartal PG-metabolite levels in 24 cows after induction of parturition with PGF_{2α} (papers II and III)

Twenty-four heifers between 260 and 269 days of gestation were injected with PGF_{2α} twice with a 24-h interval. Parturition occurred within 2 (1–7) days, median (range), of injection and was followed by a high number of retained placentas (22/24). After parturition the levels of PGF_{2α} metabolite were highly increased in retained foetal membrane cows compared with cows where the foetal membranes were shed within a few hours after parturition. Cows with retained placenta had continuously high levels of PGF_{2α} metabolite after parturition and maximum levels were measured 1.2±0.8 days after parturition. In two cows without retained foetal membranes, maximum levels were measured on the day of parturition. In non-RFM cows PGF_{2α} metabolite levels declined in an exponential fashion with a half-life of approximately 4 days. In non-treated RFM cows, the PGF_{2α} metabolite profiles during the first 10 days pp were more or less uniform.

After maximum levels around day 1 pp, PGF_{2α} metabolite levels declined. Also this decline seemed to follow an exponential decrease, but with a shorter half-life (0.7–1.6 days). Administration of flunixin on days 3 to 6 pp disturbed the decline. Cows treated in this way showed a temporary but not total inhibition of prostaglandin synthesis during the treatment period. In cows treated with flunixin on days 11 to 14 pp, prostaglandin synthesis was temporarily inhibited during the treatment period, but after the end of treatment, the levels increased. Oxytetracycline treatment on days 3 to 6 resulted in an altered PGF_{2α} metabolite profile with a prolonged half-life (2.3–3 days). In cows where oxytetracycline and flunixin were administered in combination, flunixin suppressed PG-synthesis during the treatment period but after the end of treatment PGF_{2α} metabolite levels increased again. The decline of this second elevation had a half-life of 3.5–5.4 days.

After 15–20 days the peripartal high levels of PG metabolite had declined in all cows. The next period (15–40 days), in the RFM cows was characterised by slightly elevated levels of the PGF_{2α} metabolite. This elevation was of a smaller magnitude than the peripartal PGF_{2α} metabolite elevation. The end of this elevation was significantly correlated to the end of the bacterial infection. It was also correlated to the final involution of cervix, but not to the involution of the uterine horns.

Uterine bacteriology

The main bacterial species isolated during the first weeks was *E. coli*. This species was isolated in 21/22 RFM cows. Time length of infection ranged from 9 to 20 days and more than 75 % of the cows were free of *E. coli* by day 16 pp. *E. coli* infection did not seem to be influenced by any particular treatment.

A. pyogenes was isolated in 20/21 RFM cows and *F. necrophorum* was isolated in all RFM cows. Time length of infection ranged from 12 to 45 days. Cows treated with oxytetracycline (solely or in combination with flunixin) after shedding of the placenta had a shorter period of infection with these bacteria than other RFM cows.

α-Haemolytic streptococci were isolated in 9/21 RFM cows. The last day of isolation ranged from 2 to 17 days. This bacterial species seemed less frequently found in cows treated with oxytetracycline before placental shedding compared with other cows (1/6 vs. 8/16 n.s.).

Cows treated with oxytetracycline before placental shedding had a higher proportion of *Proteus* spp. overgrowth compared with cows that were not treated with oxytetracycline before shedding, 5/6 vs. 2/16 (p<0.001). Similarly, isolation of *Pasteurella* was more common in cows treated with oxytetracycline before shedding of the foetal membranes, 4/6 vs. 3/16 (p<0.05).

White blood cells

The levels of white blood cells declined significantly during the first two days after parturition in cows with retained placenta. In the two non-retained placenta cows such postpartal decline was not seen. In untreated RFM cows the postpartal

drop was followed by a period of low white blood cells levels during the first 2 to 3 weeks postpartum. The levels then gradually increased to steady levels at about 4 weeks postpartum. Oxytetracycline treatment alone or in combination with flunixin days 3–6 tended to counteract the drop in the period immediately post partum and instead of remaining on a low level, the total white blood cell count increased after oxytetracycline treatment. In cows treated with oxytetracycline days 11–14 as well as in cows solely treated with flunixin, the profiles were similar to the profiles of the control group.

Clinical findings

In all RFM cows, the foetal membranes were shed 6 to 15 days postpartum. In cows treated with antibiotics before shedding, the placentas were shed approximately 2 days later (10 vs. 12 days pp) than in cows where no antibiotics had been given.

The two non-RFM cows had better appetite and higher energy intake than RFM cows during the first 20 days. Accordingly, cows with RFM had reduced appetite for various times after parturition. In addition, all RFM cows had increased body temperature starting 2 (0–3), median (range), and ending 11 (3–18) days pp.

Flunixin or oxytetracycline treatment days 11–14 had no effect on appetite or fever. Furthermore, flunixin treatment solely on days 3–6 had no effect on appetite or fever. Cows treated with oxytetracycline either solely or in combination with flunixin had better appetite (seen as a higher mean energy intake days 6 to 10 and a shorter period of reduced appetite after parturition) than other RFM cows.

Milk production and mean energy intake over the entire experimental period did not differ between different treatments and energy intake and milk production in the non-RFM cows was in the same range as in the RFM cows.

All RFM cows had various types and amounts of vaginal discharge during the postpartal period. The different types of discharge could not be correlated to any particular bacterial strain, but the last day for observation of mucopurulent discharge was highly correlated to the end of the bacterial infection.

Flunixin and PGF_{2α} metabolite (papers II and III)

Plasma levels of flunixin during the periods of treatments were 0.17 ± 0.01 (mean \pm SD) $\mu\text{g/ml}$ for cows treated days 11–14 and 0.38 ± 0.29 $\mu\text{g/ml}$ (one cow had significantly higher levels than the other cows) for cows treated on days 3–6. Simultaneous oxytetracycline and flunixin treatments did not influence the plasma levels of flunixin. Flunixin treatment both during days 3 to 6 pp and days 11 to 14 pp suppressed PGF_{2α} synthesis. This was seen as higher mean PGF_{2α} metabolite levels during the treatment period (3–6 or 11–14 days pp) for non-flunixin treated than for flunixin treated cows.

Clinical and endocrine effects of meloxicam on endotoxin elicited disease (Paper IV)

Four heifers were given an intravenous bolus injection of endotoxin (*E. coli* O55:B5). Ninety minutes before the injection the heifers were pre-treated with either physiological saline or meloxicam.

Endotoxin with saline pre-treatment resulted in an immediate release of prostaglandins seen as a sharp peak of the $\text{PGF}_{2\alpha}$ metabolite. In addition, all heifers were severely depressed with fever, increased breathing and heart rate as well as total anorexia during 5–9 hours. Cortisol levels were increased during 20–50 hours after injection and the levels of calcium, iron and zinc were affected. The levels of polymorphonuclear white blood cells declined immediately after endotoxin injection. After a transient leucopenia of ca 10 hours, levels higher than the pre-experimental levels were seen. This leucocytosis lasted until the end of the experiment.

Pre-treatment with meloxicam resulted in a total inhibition of endotoxin induced $\text{PGF}_{2\alpha}$ release. In addition, clinical parameters like anorexia, breathing and heart rates were less affected. Furthermore, cortisol release was reduced and serum profiles of iron and zinc. The profiles of the polymorphonuclear cells were less severely affected when meloxicam was injected before endotoxin and no period of leucocytosis was seen. Meloxicam had no effect on the endotoxin elicited fever.

General discussion

Induction of parturition by dexamethasone injection might mimic the physiological mechanisms by which the foetus induces parturition in cattle, but normally parturition usually occurs around day 280 in pregnancy and not as early as in our study. Similarly, induction of parturition by injections of PGF_{2α} days 260 to 269 in pregnancy is a non-physiological way of inducing parturition. In a way, though, it is a suitable model for the studies of retained foetal membranes in the cow since infectious and inflammatory diseases at the end of pregnancy are known to cause abortion and, as a common sequel, retained foetal membranes. PGF_{2α} induced retained foetal membranes as a model for postpartal disturbances in cattle has been evaluated by (Kask et al 1999) and according to that study, it might serve as a suitable model for this disease.

Furthermore, intravenous injections of endotoxin is an extreme model for inflammatory research. No natural infections start as a bolus dose of endotoxin. More commonly, infectious agents enter the body via some other routes, inducing local inflammatory reactions, which in a later stage might spread to the rest of the body. However, for evaluations of anti-inflammatory properties of drugs, it is one of many models.

The results in paper I indicated that PGF_{2α} is not released in a pulsatile fashion at the end of pregnancy. Thus, the release pattern of PGF_{2α} at term differs from the typical “on-off” pattern described in cyclic cattle (Basu & Kindahl 1987). The same non-pulsatile PGF_{2α} metabolite profile has been shown earlier in spontaneously calving cows (Aiumlamai et al 1992) and in goats (Ford et al 1999). Instead, of luteolytic pulses, the levels of the PGF_{2α} metabolite were increasing smoothly with an increasing rate close to parturition.

The capacity of uterine tissue to synthesise PGF_{2α} increases at the end of pregnancy. Studies in sheep have shown that levels of cPLA₂ and COX-2 increase in uterine tissue after glucocorticoid induced parturition (Zhang et al 1996). Furthermore, COX-2 levels in uterine tissue in cows reach their highest levels during labour (Wimsatt et al 1993; Fuchs et al 1999). Progesterone has an important role in this since this hormone regulates both the function (Grazzini et al 1998) and the levels of the oxytocin-receptors as well as the expression of COX-2 in uterine tissue (Ivell et al 2000). Binding of oxytocin to its receptor induces prostaglandin synthesis and is an important stimulator of COX-synthesis (Burns et al 1997; Asselin et al 2001).

In paper I the levels of the PGF_{2α} metabolite were the highest at the time of expulsion in cows with a normal shedding of the foetal membranes, then the PGF_{2α} metabolite levels declined throughout the experiment. Cows with retained foetal membranes similarly showed high levels at expulsion and declining levels immediately afterwards, but this decline was soon changed into a new period of increasing levels. The change occurred within a few hours after parturition and the magnitude of the postpartal PGF_{2α} metabolite elevation was as high as during foetal expulsion.

The postpartal PGF_{2α} metabolite profiles of the cows used in paper I have been described in another paper (Kask et al 2000). In that study and others (Bosu et al 1984; Madej et al 1986; Bolinder et al 1988) the PGF_{2α} metabolite profiles of the retained foetal membrane cows were highly increased for several days after parturition. A similar pattern of the PGF_{2α} metabolite profile was seen in paper III.

The shedding of the foetal membranes at the time of parturition seems to have major consequences for the plasma levels of the maternal PGF_{2α} metabolite in the peripartal period. This might in part be linked to a loss of the foetal part of the placenta.

During the period preceding parturition, the levels of COX-2 increase in foetal cotyledons and the COX-2 expression reaches its highest level during labour (Fuchs et al 1999). Cotyledons have been suggested to synthesise large amounts of prostaglandin at term (Wimsatt et al 1993). Thus, the shedding of the placenta might remove an important source of prostaglandins from the maternal circulation. In cows without retention of the foetal membranes this is seen as rapidly falling PGF_{2α} metabolite levels after the foetal expulsion.

In cows with retained foetal membranes, on the other hand, the foetal part of the placenta remains in contact with the maternal blood circulation. Thus, cotyledonary tissue might contribute to the high PGF_{2α} metabolite levels found after foetal expulsion in cows with retained foetal membranes. This possibility is supported by another study where cotyledonary tissue in cows with retained placenta maintained its prostaglandin synthesising capacity at least 6 hours after foetal expulsion (Slama et al 1994). Interestingly, in paper I, the profiles of the PGF_{2α} metabolite after foetal expulsion in heifers A and D seem to be a continuance of the prepartal PGF_{2α} metabolite profile. A pure speculation could be that from an endocrine perspective, parturition is not over until the placenta is shed.

An increased prostaglandin synthesis linked to the parturition might be responsible for the high levels immediately post partum. However, prostaglandins are mediators of both physiological and pathological events. Bearing in mind the uterine infection in cows with retained placenta, the PGF_{2α} metabolite elevation during the first days postpartum in retained foetal membrane cows might illustrate a transition from a physiological prostaglandin release to an inflammatory release.

Dominating bacterial species isolated during the acute phase of the postpartal endometritis are Gram negative bacteria like *E. coli* and *F. necrophorum*. Infections with these species are known to cause general depression, fever as well as an activation of the arachidonic acid cascade.

From this perspective, anti-inflammatory treatment with the prostaglandin synthesis inhibitor flunixin might have been a palliative alternative to antibiotic therapy for cows with retained foetal membranes. Unfortunately, our studies gave no such indications. Reasons for this might have been the route of administration, or the dose.

Oral administration of flunixin is an alternative to other routes of administration in heifers (Odensvik 1995) and orally administered flunixin was found to prevent endotoxin-elicited prostaglandin release and disease in cattle (Odensvik & Magnusson 1996). Therefore, as an alternative to parenteral administration, this route was used in our study. Results from the effect on the $\text{PGF}_{2\alpha}$ metabolite levels showed that the treatment via this route suppressed prostaglandin synthesis. Flunixin administration days 11 to 14, was more efficient from this perspective than treatment during days 3 to 6.

However, flunixin treatment in the first days postpartum did not suppress prostaglandin synthesis entirely. Similar results were found in another experiment in cattle (Odensvik & Fredriksson 1993). In that study, flunixin had to be administered 4 times per day for complete suppression of the prostaglandin synthesis in cows with a normal puerperium. In RFM cows, where the $\text{PGF}_{2\alpha}$ metabolite levels are higher than in non-RFM cows, a more intensive treatment might have been needed for an efficient anti-inflammatory effect.

For future research we wanted to evaluate meloxicam as an inhibitor of endotoxin elicited prostaglandin release in heifers. In those studies meloxicam completely inhibited prostaglandin release and efficiently reduced the clinical signs of disease. In other species meloxicam has been well tolerated, with few negative side effects even after long time treatment (Turck et al 1996). A further advantage of meloxicam versus flunixin is the longer half-life in plasma, 20 to 30 h for meloxicam (paper IV) and less than 10 h for flunixin (Odensvik & Johansson 1995). This would allow administration once daily and might be a better alternative for treatment of retained foetal membranes.

Oxytetracycline treatment had more influence on the clinical symptoms compared with flunixin. From this point of view, the time of treatment in relation to the shedding of the foetal membranes seemed important. Oxytetracycline treatment on days 3 to 6 had a positive effect on the appetite. It also resulted in a positive response for the white blood cells. From an infectious point of view, though, oxytetracycline treatment before placental shedding did not shorten the period of infection, but it altered the uterine micro-flora.

An interesting effect of this treatment regime was its influence on the placental shedding. According to the ultrasonographic examination in these studies, the detachment of the retained foetal membranes is completely different from the physiological shedding of the placenta.

Physiological shedding of the placenta occurs along the interface between the foetal and the maternal parts of the placentomes. According to the ultrasonographic examinations of the delayed shedding, the caruncle and the remnants of the cotyledons are shed as one unit. This has been reported by Roberts (1986). Interestingly, on the caruncular pedicle a hypoechoic zone became visible around one week postpartum. During physiologic uterine involution a demarcation zone has been described on the caruncular pedicle and shedding of the caruncle occurs along this zone (Rasbech 1950).

It is tempting to conclude that a bacterial component is involved in the pathological shedding of the foetal membranes, since oxytetracycline treatment on days 3 to 6 delayed the detachment process. Thus, shedding of the caruncle and the placentome might be favoured by bacterial growth and delayed by antibiotic therapy. A delayed shedding after antibiotic treatment has been described (Roberts 1986), but other effects of oxytetracycline and tetracycline derivatives than purely anti-microbial are reported. For instance, oxytetracycline has an inhibiting effect on collagenases and metalloproteinases (Fecteau & Eiler 1996) which might contribute to the delayed shedding after oxytetracycline treatment.

Oxytetracycline treatment on days 3 to 6 not only delayed placental shedding. It seemed to influence the postpartal $\text{PGF}_{2\alpha}$ metabolite profiles. The half-life of the $\text{PGF}_{2\alpha}$ metabolite in bovine plasma after infusion of $\text{PGF}_{2\alpha}$ has been estimated to 8 minutes (Kindahl et al 1976). The $\text{PGF}_{2\alpha}$ metabolite profiles in the postpartal cows decline with a "half-life" of several days (Kindahl et al 1982). The discrepancy in half-lives probably describes two different events. The short half-life is a metabolic half-life while the long half-life probably reflects a decreasing synthesis rate. Interestingly, this decline seems to follow a pattern, which can be influenced by oxytetracycline or flunixin treatment.

Since flunixin inhibits prostaglandin synthesis this is an obvious cause for rapidly falling levels during anti-inflammatory treatment. Oxytetracycline, on the other hand, seemed to prolong the half-life of the $\text{PGF}_{2\alpha}$ metabolite profile. Tetracycline has been shown to induce COX-2 synthesis in bovine chondrocytes (Attur et al 1999). Thus, oxytetracycline might stimulate prostaglandin synthesis by inducing COX-2. However, no effect on $\text{PGF}_{2\alpha}$ metabolite levels was seen when oxytetracycline treatment was done days 11 to 14.

Oxytetracycline treatment on days 11 to 14, after shedding of the foetal membranes, led to quicker recovery from the uterine infection. This was in contrast to oxytetracycline treatment before shedding of the foetal membranes, where no effect on the length of the infection was seen. In earlier studies of the endometritis in cows with retained foetal membranes, increased levels of $\text{PGF}_{2\alpha}$ metabolite were found during the period of uterine infection. The end of this elevation has been correlated to the end of the uterine infection and hence, associated with an inflammatory response of the uterus (Fredriksson et al 1985; Del Vecchio et al 1992). Also during our experiments a significant correlation between the end of uterine infection and $\text{PGF}_{2\alpha}$ metabolite elevations was found. Furthermore, the end of the $\text{PGF}_{2\alpha}$ metabolite elevations was correlated to the end of cervical involution, leading to the suggestion that these two were linked to one another. In another study (Oltenacu et al 1983), postpartal cows with abnormal vaginal discharge and postpartal endometritis had larger cervixes and the cervical involution was delayed compared with postpartal cows without discharge. Since inflammatory prostaglandin release includes several other prostaglandins in addition to $\text{PGF}_{2\alpha}$, we suggested that also PGE_2 could be released during the endometritis. A well-known effect of PGE_2 is its capability to soften cervical tissue (Winkler et al 1997). In a study by Duchens et al. (1993) intra-cervical

application of PGE₂ in heifers not only widened the cervix, it also could be measured as increased plasma levels of the PGF_{2α} metabolite. Our final suggestion was that inflammatory mediators might induce a softening of the cervix. This inhibits the final closure of the cervix, which remains patent until all infectious agents in the uterus are removed.

General conclusions

Frequent blood sampling before, during and after parturition in cattle indicated that prepartal 15-ketodihydro-PGF_{2α} profiles increases in a continuous way during the prepartal luteolysis and that PGF_{2α} is not released in a pulsatile fashion.

The highest levels of the PGF_{2α} metabolite were found during the foetal expulsion. In cows without retained foetal membranes, levels decline after parturition. In cows with retained foetal membranes however, this decline is changed into increasing levels within a few hours after expulsion of the foetus.

Oral administration of the prostaglandin synthesis inhibitor flunixin at the dose 2.2 mg/kg twice daily, on days 3 to 6 or 11 to 14 suppressed the synthesis of prostaglandins in cows with retained foetal membranes. However, no clinical effects of the treatments were found.

Intramuscular administration of oxytetracycline (10 mg/kg) on days 11 to 14 (after shedding of the foetal membranes) led to a shorter period of uterine infection compared with other retained foetal membrane cows.

Oxytetracycline treatments on days 3 to 6 (before shedding of the foetal membranes) had no effect on the length of the uterine infection, but seemed clinically beneficial for the cows. However, treatment at this time led to a delayed placental shedding and an alteration of the postpartal PGF_{2α} metabolite profile.

Meloxicam administered as a single dose 90 minutes before an intravenous bolus injection of endotoxin efficiently inhibited prostaglandin synthesis and suppressed clinical symptoms of endotoxemia. Furthermore meloxicam had no influence on the primary PGF_{2α} metabolism. Our results indicate that meloxicam could be a valuable contribution to other treatments of septicemic and inflammatory conditions in cattle.

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Acknowledgements

This study was carried out at the Swedish University of Agricultural Sciences, Department of Obstetrics and Gynaecology. The study was financially supported by the Swedish Council for Forestry and Agricultural Research and the Swedish Farmers Foundation for Agricultural Research. Thanks also to Schering-Plough, Stockholm, Sweden and Boehringer-Ingelheim, Ingelheim, Germany for generous gifts of pharmaceutical products.

First of all, the years I have spent at the Department of Obstetrics and Gynaecology have really been among the most interesting and joyful in my life. Not only because of an intellectual atmosphere and interesting research, but far more because of all friends and colleagues. I would like to thank all of you for being what you are!

Especially, I would like to thank Hans Kindahl, my main supervisor for his endless patience, his enthusiasm over small things, his encouraging attitude and for bringing me to this department. Thank you!

I would at the same time like to send my thanks to Kristina Odensvik Kindahl, and Hans Gustafsson, my associate supervisors for their expertise and prompt help whenever I asked for it!

I would also like to thank Stig Einarsson, Head of the Department of Obstetrics and Gynaecology. Your support has been important for me!

Kalle Kask, Anders Gunnarsson and Nahid Parvizi, my co-authors for fruitful discussions and constructive criticism.

All colleagues: Swedish or foreign, and especially. Renée, Susanne, Bodil, Bengt, Ann-Marie, Malin, Pia, Fredrik, Uffe, Bunlue, Eva, Annelie, Helle, Thomas, Håkan, Charina, Ingrid, Ulrika, Inge, Ann-Sofi, Cristina, Padet, Wichai, Alfred, A, Lennart, Kampon, Inger, Ana, Ingemar, Eva, Birgitta, Alessandra, Kulla... and many more.

A thank to all of you who have helped me with analyses and blood sampling and other other matters, Heléne, Birgitta, Kjell-Ove, Ove, Ulrika, Åsa, Karin, Annika, Carola, Marie, Lotta, Tom, Bosse, Kjell-Åke. The staff at the Department of Clinical Chemistry, especially Marianne, Åsa and Karin. The staff at Kungsängen and Nigel.

Familjerna Backlund, Hoppe och Sköldberg. Mamma och Pappa, Kalle, Malin, Petrus, Lotta, Ulla, Harald, Sture och Gunnel. Ni har alla funnits där när jag behövt er.

Tack också till Tetta, min bästa vän samt Gustaf, Oscar och Carl Johan.

