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Endocrine Profiles, Haematology and Pregnancy Outcomes of Late Pregnant Holstein Dairy Heifers Sired by Bulls Giving a High or Low Incidence of Stillbirth

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Kornmatitsuk B, Dahl E, Ropstad E, Beckers J.F, Gustafsson H, Kindahl H: Endocrine profiles, haematology and pregnancy outcomes of late pregnant Holstein dairy heifers sired by bulls giving a high or low incidence of stillbirth. Acta vet. scand. 2004, 45, 47-68. – The high incidence of stillbirth in Swedish Holstein heifers has increased continuously during the last 15 years to an average of 11% today. The pathological reasons behind the increased incidence of stillbirth are unknown. The present experiment was undertaken to investigate possible causes of stillbirth and to study possible physiological markers for predicting stillbirth. Twenty Swedish Holstein dairy heifers sired by bulls with breeding values for a high risk of stillbirth (n = 12) (experimental group) and a low risk of stillbirth (n = 8) (control group, group B) were selected based on information in the Swedish AI-data base. The experimental group consisted of 2 subgroups of heifers (groups A1 and A2) inseminated with 2 different bulls with 3.5% and 9% higher stillbirth rates than the average, and the control group consisted of heifers pregnant with 5 different bulls with 0%-6% lower stillbirth rates than the average. The bull used for group A1 had also calving difficulties due to large calves as compared to the bull in group A2 showing no calving difficulties. The heifers were supervised from 6-7 months of pregnancy up to birth, and the pregnancies and parturitions were compared between groups regarding hormonal levels, haematology, placental characteristics and calf viability. In group A1, 1 stillborn, 1 weak and 4 normal calves were recorded. In group A2, 2 stillborn and 4 normal calves were registered. All animals in the control group gave birth to a normal living calf without any assistance. The weak calf showed deviating profiles of body temperature, saturated oxygen and heart rates, compared with the normal living calves. No differences of the placentome thickness, measured *in vivo* by ultrasonography were seen between the groups. The number of leukocytes and differential cell counts in groups A1 and A2 followed the profiles found in the control group. In group A1, a slight decrease of oestrone sulphate (E1SO4) levels was found in the animal delivering a stillborn calf from the first 24-h blood sampling at 6 weeks to the second at 3 weeks prior to delivery, while the levels of E1SO4 at both periods in the animal delivering a weak calf followed the profile in animals delivering a normal living calf. During late pregnancy and at the time of parturition, the levels of E1SO4 and PAGs in animals delivering a stillborn or weak calf (from group A1) followed the normal profiles found in animals delivering a normal living calf. In group A2, low levels of E1SO4 and pregnancy associated glycoproteins (PAGs) over 24 h at both 3 and 6 weeks prior to parturition (<1.5 nmol/L) were recorded in animals delivering a stillborn calf. During late pregnancy and parturition, the levels of E1SO4 and PAGs were slightly lower dur-

ing 30-50 days prior to delivery and increased with a lower magnitude at the time of parturition. In conclusion, our results indicate that the aetiology behind stillbirth varies depending on the AI-bulls used and is associated with dystocia or low viability of the calves. Deviating profiles of oestrone sulphate (E1SO4) and pregnancy associated glycoproteins (PAGs) in animals delivering a stillborn calf not caused by dystocia were observed, suggesting placental dysfunction as a possible factor. The finding suggests that the analyses of E1SO4 and PAGs could be used for monitoring foetal well-being in animals with a high risk of stillbirth at term.

Cattle-pregnancy; parturition; endocrine profiles; haematology; placental characteristics; foetal well-being.

Introduction

According to official cattle statistics the incidence of stillbirth in the Swedish Holstein (SLB) heifers has shown an increase from 6% to 11% during the past 15 years (Swedish Dairy Association). The incidence of stillbirth in SLB heifers is twice that found in SLB cows and other cattle breeds (both heifers and cows). *Berglund* (1996) reported that about 50% of all stillborn calves were from parturitions without any calving problems, based on farmer's records reported in the milk recording system. This was also confirmed by an autopsy study, in which about half of the calves had post-mortem signs of calving difficulty (*Berglund et al.* 2003). Thus, poor neonatal calf viability was proposed to be one reason behind the high percentage of stillbirth in SLB heifers. The increased incidence of stillbirth in Sweden was also found to be related to the import and use of North American Holstein semen, with a large variability between AI-bulls to give rise to stillbirth in heifers (*Berglund & Philipsson* 1992). Many factors associated with stillbirths in cattle have been reported, such as parity of the dam, sex of the calf and gestation length (*Philipsson* 1976, *McDermott et al.* 1992). Primiparous cows showed a higher stillbirth incidence, compared with multiparous cows (*Thompson & Rege* 1984, *Berger et al.* 1992). Shorter gestation length was significantly related to increased stillbirth incidence (*Meyer et al.* 2000) and a slightly negative genetic trend in perinatal survival was found (*Meyer et al.* 2001). *Chassagne et al.* (1999) indicated that higher prepartum circulating neutrophil counts were associated with a lower risk of stillbirth. There are also some specified congenital anomalies in the Holstein breed that can cause stillbirth, e.g. complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD) (*Agerholm et al.* 2001, *Shuster et al.* 1992).

In the cow, the foetal membrane is of a "synepithelial chorial" type, in which the combined cotyledons and caruncles are formed into the so-called "placentomes". This structure plays a vital role for supporting foetal survival by transfer of nutrients from the dam and metabolic wastes from the foetus. If a placental dysfunction occurs, this might result in a restriction of foetal substrate supply. This was reported to be a major factor of altered or reduced foetal growth in sheep (*McMillen et al.* 2001). In addition, a number of hormones and growth factors including progesterone, bovine placental lactogen and pregnancy associated glycoproteins (PAGs) are produced from the foetal membranes (*Schlafer et al.* 2000). Some of these placental-derived hormones can be measured in the maternal blood circulation and typical hormonal patterns during late pregnancy and parturition have been shown in dairy cows.

It has been suggested that these hormonal parameters might be used for monitoring of the foetal well being (for a review, see *Kindahl et al.* 2002). Measurements of bovine pregnancy specific protein (bPSPB) may be useful for prediction of the foetal health status, whereas oestrone sulphate (E1SO4) may reflect placental viability (*Dobson et al.* 1993). *Zhang et al.* (1999) reported that the E1SO4 concentrations were positively correlated with neonatal viability after day 195 of pregnancy and low levels of E1SO4 might be related to some pathological conditions of foetuses and newborns (*Echternkamp* 1993). Deviating profiles of E1SO4 and PAGs were found in animals with impaired parturition (*Kornmatitsuk et al.* 2002).

The reason for, and the pathological background to, the higher incidence of stillbirth in Holstein heifers in comparison with Holstein cows and other dairy breeds are still unknown. Hence, the aim of the present study was to investigate a relationship between the occurrence of stillbirth or poor calf viability and endocrine patterns, haematology and some other related parameters such as placental characteristics, labour and the process of foetal adaptations in order to detect physiological defects as possible causes of stillbirth and which might be used as prognostic tools for identification of foetuses with low viability and high risk of stillbirth at term.

Materials and methods

Experimental design

AI-bulls for the study were selected from the official breeding records (Swedish Dairy Association). The criterion used for selection was the index of stillbirth, which had been previously analysed by the Swedish Dairy Association. The index of an individual bull was referred to the incidence of stillbirth in the cattle population, in which the bull had given in relation to the mean of all AI-bulls used.

The index of stillbirth is expressed as a relative breeding value (RBV) with a mean of 100, which results in close to 11% of stillbirth in the first calvings. Each RBV unit means 0.6% of stillbirth, e.g., a bull with a very low stillbirth RBV of 85 gave 9% stillbirth more than the average from a bull with RBV of 100, a bull with RBV of 90 gave 6% stillbirth more than the average from a bull with RBV of 100, and a bull with RBV of 110 gave 6% stillbirth less than the average from a bull with RBV of 100. In the same manner, a calving ease index below 100 indicates a higher risk of calving problems, compared with a calving ease index above 100. Thus, the bulls giving a higher incidence of stillbirth (a lower stillbirth index) were selected for the experimental group, whereas the bulls giving a lower incidence of stillbirth (a higher stillbirth index) were selected for the control group. To some extent, consideration was also taken to the bull-breeding index for calving ease, in which a bull with a low incidence of calving difficulty (a high index for calving ease) was preferred.

Heifers that had been artificially inseminated with selected bulls and declared pregnant, and that were supposed to calve at a desired point of time, were selected throughout the country in the national AI-database and brought to the experimental site (details given below) for further investigations. The bulls selected were tested and proved to be non-carriers of complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD).

Animals and animal handling

Totally, 20 late pregnant Swedish Holstein (SLB) dairy heifers (6-7 months of pregnancy) were selected and 3 groups of animals were formed (groups A1, A2 and B). The stillbirth index and the calving ease index in each individual bull at the start of experiment and at the time of writing were given as follows: Heifers

in group A1 (n = 6) were sired by the bull named "Bubba" USAHOL000002229383, SWE 90202 (stillbirth index = 85 (90 Aug-02), calving ease index = 88 (88 Aug-02)) and heifers in group A2 (n = 6) were sired by the bull named "Patron" USAHOL000002160458, SWE 99495 (stillbirth index = 94 (97 Aug-02), calving ease index = 101 (104 Aug-02)). Heifers in group B (control group; n = 8) were sired by 5 different bulls named "Marauder" USAHOL000002073968, SWE 99336 (stillbirth index = 105 (107 Aug-02), calving ease index = unknown at time of selection (100 Aug-02)), "Ilius" FRAHOL006293021462, SWE 90218 (stillbirth index = 102 (106 Aug-02), calving ease index = unknown at time of selection (111 Aug-02)), "Demand" USAHOL000002193272, SWE 90111 (stillbirth index = 101 (94 Aug-02), calving ease index = 107 (106 Aug-02)), "Häradsköp" SWE 44358 (stillbirth index = 100 (101 Aug-02), calving ease index = 110 (109 Aug-02)) and "Tegl" DNKHOL000000228028, SWE 99370, (stillbirth index = 111 (112 Aug-02), calving ease index = 108 (108 Aug-02)).

The experiment was conducted in 3 phases during 2000-2002. In the first phase, 10 heifers were used. Six of them were artificially inseminated and confirmed to be pregnant with the bull named "Bubba" (group A1) and 4 heifers were pregnant with 4 different control bulls named "Marauder", "Ilius", "Demand" and "Häradsköp" (group B). In the second phase, 6 heifers were used. Four heifers were artificially inseminated and found pregnant with the bull named "Patron" (group A2) and 2 heifers were pregnant with the bull named "Tegl" (group B). In the third phase, 4 heifers were used. Two heifers were artificially inseminated with the bull named "Patron" (group A2) and 2 heifers were pregnant with the bull named "Tegl" (group B). The animals were housed in a barn with a tied stall system at the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, Uppsala, Sweden, and fed according to Swedish standards (*Spörndly* 1993). They were serologically tested and free from Neospora caninum (*Björkman et al.* 1997) and bovine viral diarrhoea virus (BVDV) infections (*Juntti et al.* 1987). The care of the animals and the experimental design of this study were approved by the Local Animal Ethics Committee in Uppsala, Sweden.

Registration of calving performance and monitoring of calf viability

In general, the heifers were observed clinically twice daily and the body temperature was monitored once daily for health status from the 6th-7th month of pregnancy. The calving was calculated to take place 280 days after the last insemination. During the last 10 days prior to expected calving, signs of approaching parturition were carefully examined and the body temperature was measured twice daily. The intensive supervision of the calving process was undertaken by the same person, and it started when the animals showed signs of onset of parturition. In addition, the calving process was recorded by a video-recording system, and thereafter details of the calving events were evaluated retrospectively. The onset of parturition was defined as the time when the heifer started to show repeated cycles of standing and lying down or colic symptoms. Calving difficulty was classified as: 0 (unassisted), 1 (slight with light intervention, e.g., control of the calf position), 2 (moderate with mild traction, e.g., pulling out the calf by one person) and 3 (severe with heavy traction, e.g., pulling out the calf by 2 persons). The animals were allowed sufficient time to give birth naturally, and calving aids were given according to the following scheme; in time related to the rupture of allantochorion (the water bag).

– if the calving process was regarded as normal

(normal labour and the calf position was correct) but the calf was not out after 4 h, the calf was pulled out
– if in labour, but the calf was not visible within 1 h, the calf position was checked and, if necessary, corrected
– if no labour, the calf was pulled out after 1 h

No procedures for saving the life of the calves after parturition were done. Calf sex and body weight were registered, and calf viability was scored after birth as normal, weak or stillborn. The viability score was based on a willingness to lift the head, an appearance of mucous membranes and an attempt to escape from external stimuli. Body temperature was recorded, and the percentage of saturated oxygen and heart rates were measured by a Pulse Oxymeter (Hewlett Packard, Berlin, Germany) every 10 min for 1 h after birth. The site of the measurements evaluated by the Pulse Oxymeter was the tip of the tail, and the hairs were properly shaved before the measurements took place. Stillbirth was defined as when, after at least 260 days of pregnancy, a calf died prior to, during or within 24 h after birth. Time of shedding the foetal membranes was recorded, and they were kept for further investigation (see details below). Foetal membranes were defined as retained (RFM) if they were not expelled within the first 24 h after delivery and the animals were neither mechanically nor chemically treated. The stillborn calves were autopsied at the Department of Pathology, the National Veterinary Institute in Uppsala, Sweden.

Investigations of placental characteristics

Transrectal ultrasound examinations for determining changes of the placentome thickness *in vivo* were performed 3 times per week (Monday, Wednesday and Friday mornings). A real-time B-mode ultrasound scanner (485 Anser Vet, Pie Medical Equipment B.V., Maastricht, The Netherlands) with a 6.0-MHz rectal linear array transducer connected to a monitor and a video-recording system was used. For the measurements of placentome thickness, an image freezer facility and electronic callipers of the equipment were employed.

The foetal membranes were collected and macroscopically examined for evidence of infectious diseases and malformations within 1 h after they were expelled. The placental weight was registered. Then the foetal membrane was dissected into 2 parts consisting of the intercotyledonary membranes and the foetal cotyledons, which were differentiated into 3 categories, small ($\varnothing < 4$ cm), medium ($\varnothing = 4$-8 cm) and large ($\varnothing > 8$ cm). The weight of intercotyledonary membranes was registered, and in each size-category of cotyledons, the numbers and weight of the foetal cotyledons were recorded.

Blood sampling and analyses

For haematology, blood samples were collected from the heifers 3 times per week (Monday, Wednesday and Friday at 09.00) during the last 2-3 months prior to expected delivery until 10 days after parturition. Five ml of blood was taken by jugular venipuncture into EDTA evacuated tubes (Venoject, Terumo Europe N.V., Leuven, Belgium). The total leukocyte counts were performed using an automated haematology analyser (Cell-Dyn 3500, Abbott Diagnostics, Abbott Park, IL, USA).

Blood smears were made and stained with May-Grünwald/Giemsa for differential cell counts. The differential cell proportion was obtained by microscopic counting of 200 cells. The resulting percentages were multiplied by the total leukocyte count to calculate the absolute values.

Two blood sampling schemes were used for hormonal analyses; 1) twice daily from 2-3 months prior to expected delivery until 10 days after parturition, and 2) once per h over a 24-h

period on 2 occasions approx. 3 and 6 weeks prior to expected parturition. For both 24-h blood samplings, the surgical procedure was applied and previously described by *Båge et al.* (2000). In the calves, blood samples were collected at 0 and 1 h after birth for hormonal analyses. For each blood sample, 10 ml of blood was taken by jugular venipuncture into heparinized evacuated tubes (Venoject, Terumo Europe N.V., Leuven, Belgium) and centrifuged with 1000 × g for 10 min at room temperature. The plasma was separated into plastic tubes and stored at –20 °C until hormonal analyses could be conducted.

The hormone assays were applied using radioimmunoassay (RIA) technique. The levels of the $PGF_{2\alpha}$ metabolite (15-ketodihydro-$PGF_{2\alpha}$) (PG-metabolite) were determined according to *Granström & Kindahl* (1982). The procedures used for determinations of progesterone (P4), cortisol and oestrone sulphate (E1SO4) followed the manufacturers guidelines (Coat-A-Count Progesterone and Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, CA, and DSL-5400, Diagnostic Systems Laboratories, Webster, TX, USA, respectively). The levels of pregnancy associated glycoproteins (PAGs) were assayed according to *Zoli et al.* (1992). The detection limits of each hormonal assay were given in the references mentioned above. The intra-assay and the inter-assay coefficients of variation of all hormonal analyses were below 10% and 14%, respectively.

Statistical analyses

The statistical analyses were performed using the Statistical Analysis System (SAS), version 6.12 (SAS Institute Inc., Cary, NC, USA). In each parameter, the mean and a standard deviation (mean ± SD) were calculated by the MEANS procedure. The analyses of variance (ANOVA) procedure was used to test significant differences of the means (least-squares means) and the means between 2 groups were compared using the modified *t*-test (Bonferroni method). The procedure of PROC MIXED for repeated measurements was used for analyses of the effects, which influenced the hormonal levels. Probability values of less than 0.05 ($p<0.05$) were considered to be significant.

Results

Calving performance

In group A1 (n = 6), 1 stillborn calf (from animal no. 58) and 1 weak (from animal no. 716) calf were recorded. The calving process in the animal with stillbirth was scored = 2 (moderate degree of calving difficulty). After the forelegs of the calf were presented, the dam did not show any progress of the calving process. The calf was pulled out approximately 4 h after the onset of the calving process. The sex of the calf was male, and the body weight was 47 kg. In the animal delivering a weak calf (no. 716), the calving process was score = 1 (slight degree of calving difficulty). During the calving process, this animal showed signs of strong labour, but the time used for calving took over 2 h after the rupture of the allantochorion. The calf viability (male, weight: 44 kg) was scored as weak due to purple mucous membranes, unwillingness to lift the head after birth and making no attempt to escape from external stimuli. The other 4 heifers gave birth without assistance, and all calves were judged to have normal viability.

In group A2 (n =6), 2 stillborn calves (from animals no. 145 and 465) were registered. These 2 heifers gave birth without assistance, and the calf body weights were 33.5 and 34 kg, respectively. The stillborn calf from animal no. 465 had posterior presentation at birth. One heifer (animal no. 735) delivered a normal living male calf (after 283 days of pregnancy) with the body weight of 47 kg, and the calving process in this animal was scored as a slight degree of calving

Table 1. Descriptive data of calving performance in groups A1, A2 and B.

Group (n)	Gestation length (d)	First sight of calf legs (min)	Degree of calving difficulty (n)				Stillborn calves (n)	Weak calves (n)	RFM	Calf sex (n)/ (Calf weight (kg))	
			0	1	2	3				M	F
A1 (6)	285±6	78±53	4	1	1	0	1 (M)	1 (M)	0	5 (43.5±6.1)	1 (30.0)
A2 (6)	279±8	83±44	5	1	0	0	2 (M;M)	0	1	3 (38.2±7.7)	3 (35.8±4.2)
B (8)	277±6	51±29	8	0	0	0	0	0	1	2 (37.0±4.2)	6 (39.3±5.0)
Total (20)	280±7	65±43	17	2	1	0	3	1	2	10 (40.6±6.4)	10 (37.3±5.2)

Group A1 = an index of stillbirth equal to 85; Group A2 = an index of stillbirth equal to 94; Group B = an index of stillbirth ranged between 100-111; n = the number of observations; Degree of calving difficulty: 0 = unassisted, 1 = slight, 2 = moderate, 3 = severe; RFM = retained foetal membranes; M=male; F=female

difficulty. The first sight of the forelegs in this calf was observed about 2 h and 20 min before the interventions. The calf was diagnosed as large in size, thus the calf was assisted and the time used for the calving assistance was 5 min. The other heifers in group A2 gave birth without assistance to calves with viability scored as normal.

In group B (n = 8), all animals had unassisted parturitions with normal calf viability. The average body weight of the calves for males and females was 37.0 ± 4.2 kg and 39.3 ± 5.0 kg, respectively. Descriptive data of the calving performance sorted by groups is presented in Table 1. The post-mortem examination of the stillborn calves (from animals nos. 145 and 465) revealed no pathological changes or malformation, while the dead calf from animal no. 58 had post-mortem signs of trauma and was diagnosed stillborn due to calving difficulty. The average weights of vital organs from 2 stillborn calves (from animals nos. 58 and 465) (missing data from one stillborn calf) were as follows; liver = 905 ± 71 g, spleen = 63 ± 4 g, kidneys = 228 ± 32 g, heart = 368 ± 18 g, brain = 230 ± 28 g, lung = 923 ± 513 g, adrenal glands = 5 g, thymus = 123 ± 18 g.

Changes of body temperature during late pregnancy, parturition and postpartum

In all heifers of groups A1*, A2* and B (* = excluding data on animals delivering a stillborn or weak calf), the body temperature measured in the morning increased gradually within a narrow range during late pregnancy. The average body temperature in groups A1*, A2* and B were 38.6 ± 0.1 °C, 38.7 ± 0.2 °C and 38.7 ± 0.1 °C, respectively. In animal no. 58 (stillbirth), a higher increase of body temperature was recorded during the time of parturition, whereas the body temperature in animal no. 716 (weak calf) increased about 1 weeks prior to delivery and decreased markedly before the time of parturition. In animal no. 145 (stillbirth), the body temperature slightly increased during late pregnancy and decreased just before the day of parturition. The body temperature in animal no. 465 (stillbirth) also increased grad-

Table 2. Signs of calf viability during the first hour after birth in groups A1*, A2* and B.

Group (n)	Willingness to lift the head[1] (min)	Sternal recumbency[1] (min)	First attempt to stand[1] (min)	First standing[1] (min)
A1* (4)	2 ± 2 (n = 3)	5 ± 1 (n = 3)	27 ± 6 (n = 4)	63 ± 4 (n = 2), >60 (n = 2)
A2* (4)	2 ± 0 (n = 2)	6 ± 4 (n = 2)	31 ± 22 (n = 3)	49 ± 10 (n = 3)
B (8)	3 ± 1 (n = 7)	5 ± 3 (n = 5)	30 ± 18 (n = 5)	36 ± 11 (n = 3), >60 (n = 2)

Group A1 = an index of stillbirth equal to 85; Group A2 = an index of stillbirth equal to 94; Group B = an index of stillbirth ranged between 100-111; n = the number of observations; 1 = in minutes after birth

ually during late pregnancy, however, a decrease of body temperature was not clearly seen. During the postpartum period, an increase of body temperature was recorded in animal no. 489 (from group B) with RFM and in animal no. 145 delivering a stillborn calf followed by RFM. For the others, the body temperature during the postpartum period remained in the same range as the measurements during late pregnancy.

During the last 10 days before parturition, the mean values of body temperature of all animals in the evening were higher than in the morning. In group A1*, a high increase of the evening body temperature in animal no. 60 was recorded. This measurement was done 2 h before the time of parturition. For the other animals in this group, a drop in the evening body temperature prior to parturition (varied between 0.2-0.7 °C) was observed. In group A2*, all animals delivered in late evening and during the night. A decrease of the morning and evening body temperature was recorded and a drop of the evening body temperature of more than, or equal to, 0.5°C was found. In group B, a decrease in body temperature prior to the time of parturition (varied between 0.3-0.9°C) was observed in all animals. In animals with impaired parturition, a drop in the evening body temperature (about 1.0 °C) was found in animal no. 716 (weak calf). However, a slight decrease in the evening body temperature (varied 0.2-0.4 °C) in the animals carrying stillbirths (animals no. 58, 145 and 465) was observed.

Clinical signs and measurements of early postnatal calves

In all groups, the live calves with normal viability showed a willingness to lift the head within 5 min, and the sternal recumbency of the calves was recorded during 5-10 min after birth. Descriptive data of clinical signs in individual groups is shown in Table 2. In the weak calf from animal no. 716, a willingness to lift the head, time to attain the sternal recumbency, time for the first attempt to stand and time of the first standing were more than 1 h after birth.

Overall, the body temperature of all live calves decreased during the first hour after birth in all groups. Higher decrease of body temperature (about 1°C) was found in group A1*, compared with a decrease in groups A2* and B (about 0.3-0.4 °C). The body temperature in group B was more stable than in the other groups. In the weak calf born in group A1, the body temperature was lower than the average values at birth from group B, and this calf had a numerically low body temperature during the whole period studied (Fig. 1, left upper panel). The saturated oxygen in all animals of groups A1*, A2* and

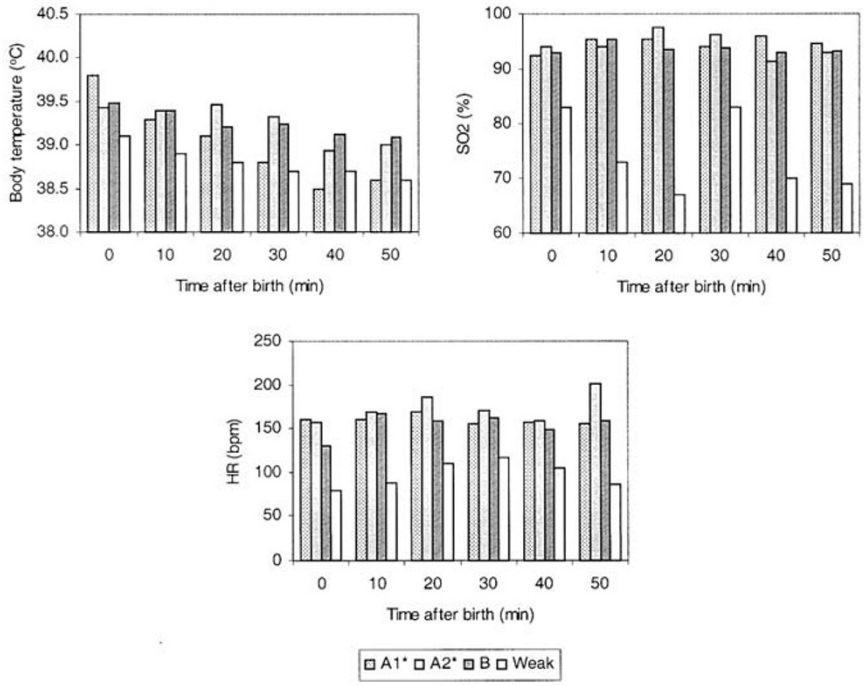

Figure 1. Body temperature, saturated oxygen (SO2) and heart rates (HR) measured in the calves during the first hour after birth.

B maintained between 92%-98%, whereas the levels of saturated oxygen in the weak calf were 67%-83% (Fig. 1, right upper panel). The heart rates in group A1* were relatively constant and maintained between 155-160 beats/min. The heart rates in groups A2* and B increased during the first hour after birth (group A2*, from 157 to 201 beats/min and group B, from 130 to 160 beats/min). In the weak calf, the heart rates varied between 80-117 beats/min (Fig. 1, lower panel). Regarding all measurements, no significant differences were found between groups A1*, A2* and B ($p>0.05$).

Ultrasonography of the placentomes

The number of placentomes observed in each session varied between 1-5 and they varied in size and shape. Changes of the placentome thickness during late pregnancy were not clearly seen individually, and the average placentome thickness was relatively constant ($p>0.05$). The average placentome thickness in groups A1*, A2* and B was 3.0 ± 0.7, 3.4 ± 0.6 and 3.2 ± 0.6 cm, respectively. In animals delivering a stillborn or weak calf, the average placentome thickness was 3.3 ± 0.8 cm. The average values of placentome thickness in animals delivering a stillborn or weak calf from Bubba and Patron were 3.2 ± 0.7 and 3.4 ± 0.9 cm, respectively.

Ultrasonography investigations without finding any placentomes were recorded in certain periods and animals. The difficulty of finding placentomes during early stages of the experimental period was recorded in 3 animals delivering a normal living calf in group A, whereas in 2

Table 3. Descriptive data of placental characteristics summarised by groups.

Group (n)	Placental wt (kg)	Cotyledons (no. / kg)				Intercotyledonary wt (kg)	Shedding time (h)
		S	M	L	Total		
A1* (4)	5.3 ± 1.8	35 ± 23 / 0.2 ± 0.1	65 ± 33 / 1.4 ± 0.5	19 ± 7 / 0.8 ± 0.2	119 ± 60 / 2.4 ± 0.7	2.8 ± 1.2	3.9 ± 1.0
A2* (4)	4.6 ± 0.9	35 ± 13 / 0.2 ± 0.1	51 ± 20 / 1.2 ± 0.4	17 ± 4 / 0.8 ± 0.3	102 ± 28 / 2.2 ± 0.4	2.5 ± 0.8	3.9 ± 0.9
B (7)	4.5 ± 1.2	38 ± 17 / 0.2 ± 0.1	55 ± 15 / 1.0 ± 0.2	20 ± 8 / 0.7 ± 0.3	113 ± 32 / 2.0 ± 0.4	2.4 ± 0.6	4.0 ± 0.9

Group A1 = an index of stillbirth equal to 85; Group A2 = an index of stillbirth equal to 94; Group B = an index of stillbirth ranged between 100-111; n = the number of observations; S = small size, M = medium size, L = large size

animals delivering a stillborn calf (animals nos. 58 and 465) the difficulty was distributed over the whole period studied.

Placental characteristics

In group A1, no RFM were observed. The data of placental characteristics in this group was derived from 4 animals (Table 3) (data from animals nos. 58 and 716 is presented separately in Table 4). The placental weight, cotyledonary weight and intercotyledonary weight in this group were numerically the largest. The total number of cotyledons and the number of cotyledons in the medium size were also higher than those in other groups. In animal no. 58 with a stillborn calf, the placental weight was large, compared with the others and the shedding time of the foetal membranes was also longest. In animal no. 716, the placental characteristics were similar to the figures recorded in group B. In group A2, animal no. 145 had RFM. The summarised data of placental characteristics in this group was from 4 animals (Table 3) (data from animal no. 465 is presented separately in Table 4). In animal no. 465, the lightest placental weight and a small number of cotyledons were recorded. In group B, one case of RFM was recorded. Thus, totally 7 foetal membranes were collected and examined. No statistical differences of the placental characteristics were found between groups of animals ($p>0.05$).

Table 4. Descriptive data of placental characteristics in animals delivering a stillborn (animals no. 58 and 465) or weak calf (animal no. 716).

Animal no.	Placental wt (kg)	Cotyledons (no. / kg)				Intercotyledonary wt (kg)	Shedding time (h)
		S	M	L	Total		
58	6.4	32 / 0.2	63 / 1.2	14 / 0.7	109 / 2.1	4.3	7.7
716	4.8	30 / 0.3	62 / 1.2	27 / 1.2	119 / 2.7	2.1	3.6
465	2.9	31 / 0.1	33 / 0.5	18 / 0.8	82 / 1.5	1.5	2.7

Group A1 = animals no. 58 and 716; Group A2 = animal no. 465; S = small size, M = medium size, L = large size

Changes of haematology during late pregnancy, parturition and postpartum

In groups A1*, A2* and B. The average number of leukocytes and neutrophils in groups A1*, A2* and B observed from the start of the study to 2 w before parturition was rather constant. Then, an increase of leukocytes was found up to the time of parturition and thereafter the number decreased during the postpartum period. The same pattern was also found for the numbers of neutrophils. The average numbers of lymphocytes and eosinophils maintained stable during late pregnancy and a slight decrease of these 2 cell types occurred during the last week of pregnancy and after parturition in all groups of animals. The number of monocytes was constant during late pregnancy until 1 w before parturition and then the number increased at about the time of parturition. After parturition, the number of monocytes decreased to the peripartal levels. No clear differences of the number of differential cell types between groups were observed.

In animals delivering a stillborn or weak calf. In animals no. 58 and 716, the number of leukocytes was relatively constant during late pregnancy and reached a high level on the day of parturition. A rapid fall of the leukocyte number was found during the postpartum period and the same pattern was observed in the number of neutrophils. The number of lymphocytes gradually decreased towards the end of pregnancy, however, the levels slightly fluctuated. An increased number of eosinophils was depicted in animal no. 58 around 40-50 d prior to parturition. Otherwise, no different patterns of eosinophils were found between these 2 animals. The number of monocytes gradually increased during late pregnancy and reached high levels a few days after parturition. Afterwards, the number of monocytes decreased to low levels at 10 d postpartum.

In animal no. 145, larger numbers of leukocytes and lymphocytes were recorded during late pregnancy than in animal no. 465, but the same patterns were observed, compared with the numbers in the control group. In animal no. 145, the number of neutrophils was elevated slightly at about day 40 prior to delivery, but the number of eosinophils was lower. The same pattern for the number of monocytes as in animals delivering normal living calves was found. The high number of monocytes was recorded during the time of parturition and during the postpartum period, the number of monocytes decreased to peripartal levels.

Endocrine changes over 24 h at 6 (24h-1) and 3 (24h-2) weeks prior to delivery

In groups A1* and B, the levels of PG-metabolite were constant during both 24-h blood samplings at 3 and 6 w before parturition. A similar pattern of the PG-metabolite was seen in animal no. 716. Higher PG-metabolite levels at the second 24-h blood sampling at 6 w before parturition were recorded in group A2* and in animals no. 58, 145 and 465. The levels of cortisol in all groups varied with a lower magnitude in both the first and the second 24-h blood samplings. In animal no. 58, a slight increase of cortisol was found in both periods, whereas the levels remained low in animal no. 716. Higher levels of cortisol were also observed in animals no. 145 and 465, in particular during the first 24-h blood sampling at 6 w prior to delivery. In groups A1*, A2* and B, the E1SO4 levels in the second 24-h blood sampling were significantly higher than the levels of E1SO4 in the first 24-h blood sampling ($P<0.05$). Higher levels of E1SO4 were recorded in groups A2* and B, compared with the levels in group A1*. In animal no. 58, the levels of E1SO4 were lower for the second 24-h blood sampling, whereas the levels of E1SO4 in animal no. 716 were higher in the second 24-h blood sampling. Low levels of E1SO4 were recorded for animals no.

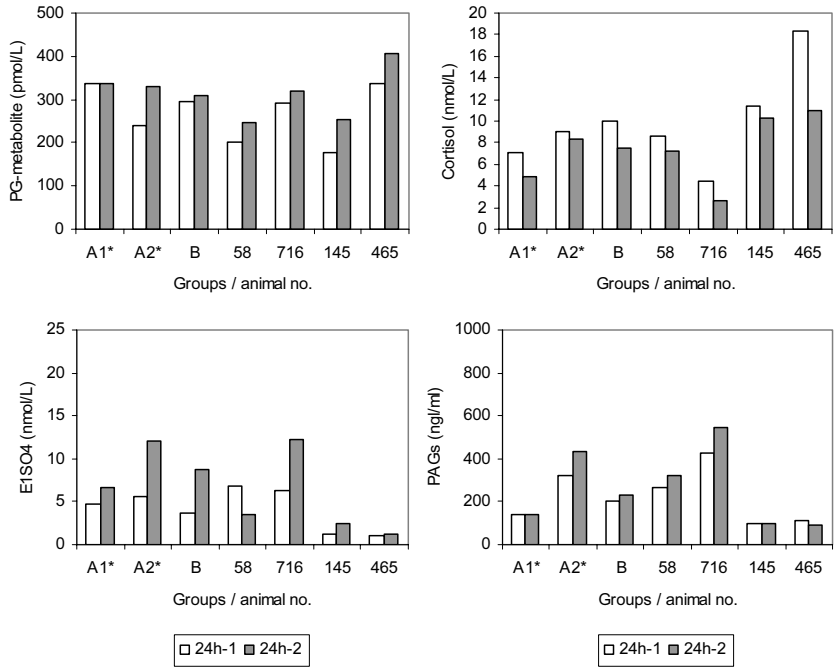

Figure 2. The average values of endocrine parameters over 24 h at 3 and 6 weeks prior to parturition in groups A1*, A2* and B and in animals delivering a stillborn or weak calf from groups A1 (animals no. 58 and 716) and A2 (animals no. 145 and 465).

145 and 465 and a slight increase was found from the first 24-h blood sampling to the second. The levels of PAGs in groups A1* and B were relatively constant and lower than the levels recorded in group A2*. An increase of PAGs for the second 24-h blood sampling was recorded in group A2* and in animals no. 58 and 716. In animals no. 145 and 465, the levels of PAGs remained low and constant.

The average values of PG-metabolite over 24 h in groups A1*, A2* and B varied between about 200-400 pmol/L. In addition, higher levels of PG-metabolite were observed in animal no. 465 (Fig. 2, left upper panel). The average cortisol levels in groups A1*, A2* and B and in animals no. 58 and 716 were lower than 10 nmol/L, whereas in animals no. 145 and 465, the cortisol levels were numerically the highest among the others (Fig. 2, right upper panel). A slight decrease of E1SO4 levels was found in animal no. 58 from the first 24-h blood sampling to the second, whereas animal no. 716 showed an increase of E1SO4 from the first 24-h blood sampling to the second. In animals no. 145 and 465, the low levels at both 3 and 6 w prior to parturition (<1.5 nmol/L) were recorded (Fig. 2, left lower panel). The levels of PAGs in all groups were relatively constant or slightly higher at the second 24-h blood sampling at 3 w prior to parturition. The levels of PAGs in animals no. 58 and 716 showed the same or higher levels as found in animals delivering a normal living calf. Low levels of PAGs were recorded in animals no. 145 and 465 carrying stillbirth (Fig. 2, right lower panel).

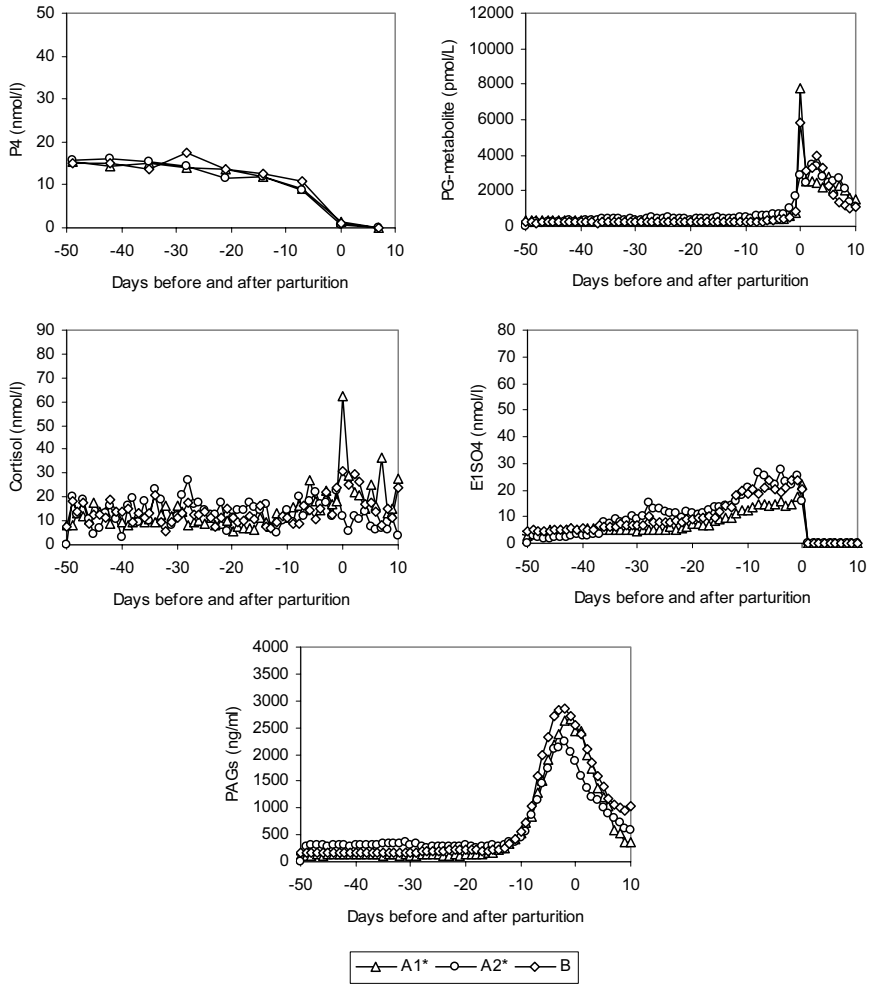

Figure 3. Endocrine profiles during late pregnancy and parturition in groups A1*, A2* and B.

Endocrine changes during late pregnancy, parturition and postpartum period

In groups A1*, A2* and B. The P4 patterns of all groups were similar to each other. Decreased levels of P4 were recorded and they gradually decreased towards the end of pregnancy. Low levels of P4 were found on the day of parturition, and after parturition the zero levels of P4 were reached. No significant differences of P4 between groups were observed (Fig. 3, left upper panel). The levels of PG-metabolite were constant during most of the period studied and the levels markedly increased on the day of parturition. Afterwards, the levels gradually declined but still the levels were higher than the peripartal levels as presented in Figure 3, right upper panel. The levels of cortisol fluctuated during late pregnancy and the

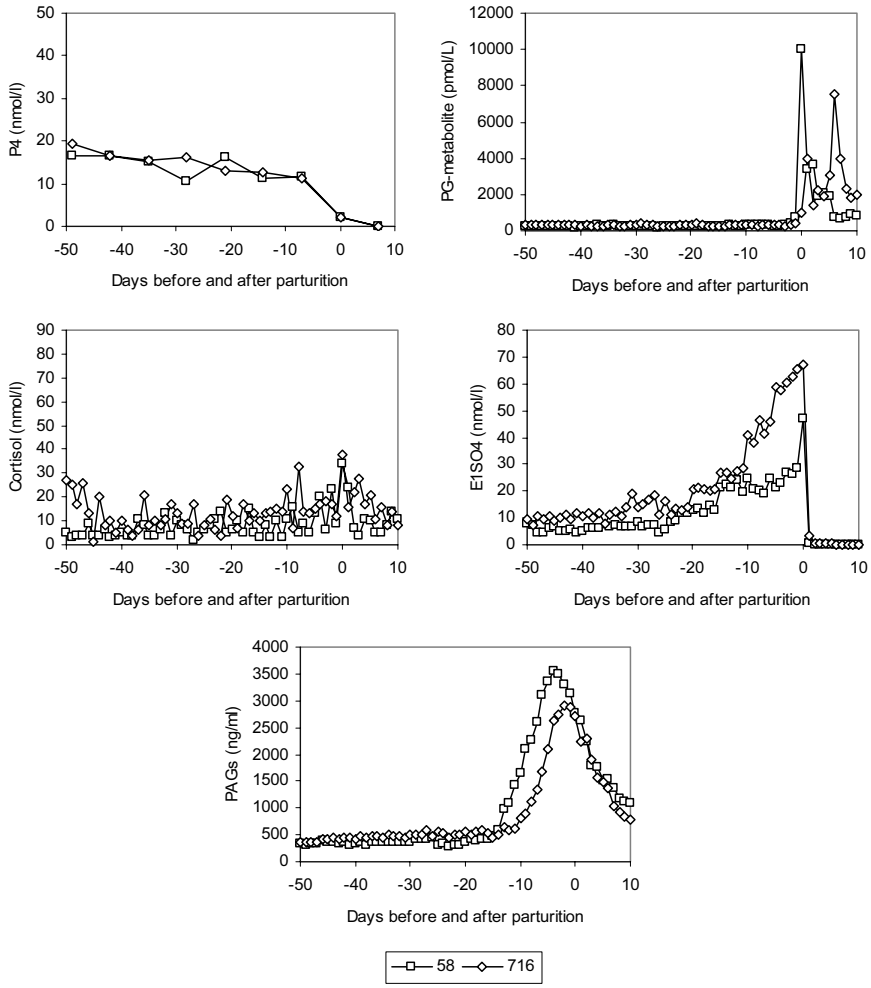

Figure 4. Endocrine profiles during late pregnancy and parturition in animals no. 58 and 716 delivering a stillborn or weak calf.

levels increased at the end of pregnancy. The average levels of cortisol on the day of parturition in group A2* were lower than the others and higher levels of cortisol were found in group A1*. After parturition, the levels of cortisol were still elevated and a higher level of cortisol was found in group A1* (Fig. 3, left middle panel). The levels of E1SO4 gradually rose from about 40 d prior to delivery in all groups of animals. The highest increases of E1SO4 were recorded on the day of parturition. Then, the levels dropped to zero levels within 1 d postpartum (Fig. 3, right middle panel). The average levels of PAGs were less than 500 ng/ml before the last 10 d of pregnancy. Then, the levels increased and reached high levels prior to the day of parturition. During the postpartum period, the levels substantially de-

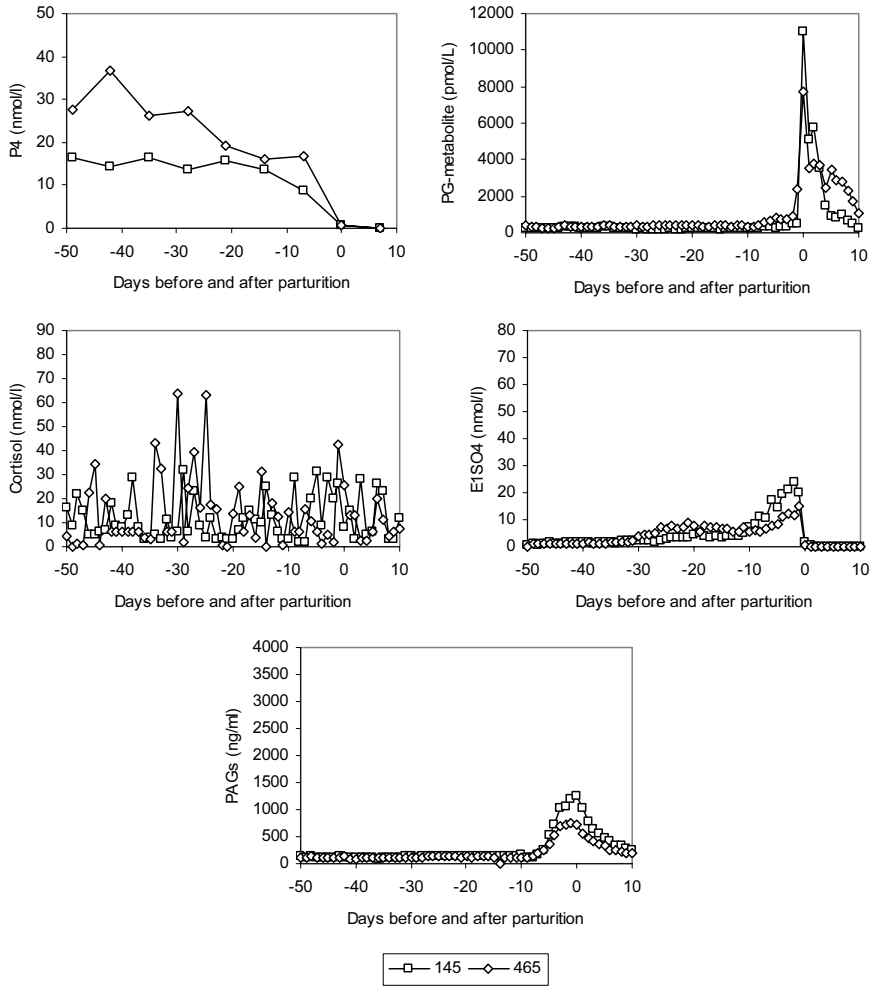

Figure 5. Endocrine profiles during late pregnancy and parturition in animals no. 145 and 465 delivering a stillborn calf.

creased to the peripartal levels (Fig. 3, lower panel). No significant differences of the endocrine parameters between groups were found ($p>0.05$).

In animals delivering a stillborn or weak calf. During late pregnancy, the levels of P4 in all animals showed the same pattern but higher levels of P4 were recorded in animal no. 465. Low levels of P4 were also found at the end of pregnancy. After parturition, the levels of P4 in animals no. 145 and 465 were very close to zero, whereas the levels were slightly higher in animals no. 58 and 716 (Figs. 4-5, left upper panels). The levels of PG-metabolite in all animals were similar during late pregnancy and parturition. The levels remained low during most of the period studied and a peak of PG-metabolite was recorded on the day of parturi-

Table 5. Endocrine concentrations in the calves at birth (0) and 1 h (1) after birth summarised by groups. Data of the weak calf from animal no. 716 is presented separately.

Group / calf no. (n)	P4 (nmol/L)		PG (pmol/L)		Cortisol (nmol/L)		E1SO4 (nmol/L)		PAGs (ng/ml)	
	0	1	0	1	0	1	0	1	0	1
A1* (4)	0.8 ± 0.8	0.6 ± 0.3	9932 ± 2581	3146 ± 642	342 ± 76	351 ± 35	224 ± 81	59 ± 23	209 ± 44	177 ± 39
A2* (4)	0.9 ± 0.6	1.3 ± 1.1	13072 ± 7399	4415 ± 3016	404 ± 140	385 ± 87	128 ± 87	46 ± 33	114 ± 42	107 ± 41
B (8)	1.0 ± 0.4	1.1 ± 1.0	9589 ± 8098	2643 ± 1160	341 ± 124	355 ± 102	104 ± 65	37 ± 11	211 ± 107	202 ± 85
716	0.5	0.3	3762	1290	302	315	90	12	236	233

Group A1 = an index of stillbirth equal to 85; Group A2 = an index of stillbirth equal to 94; Group B = an index of stillbirth ranged between 100-111; n = the number of observations

tion. In animal no. 716, a lower increase of the PG-metabolite on the day of parturition was found (< 4000 pmol/L) and the levels increased 1-2 d after parturition, whereas in other animals, the levels of PG-metabolite sharply decreased (Figs. 4-5, right upper panels). The levels of cortisol in animals no. 58 and 716 fluctuated within a magnitude of about 30 nmol/L, whereas the levels in animals no. 145 and 465 fluctuated with a higher range through the period of late pregnancy and after parturition (Figs. 4-5, left middle panels).

The levels of E1SO4 in all animals increased considerably, reaching a peak at the time of parturition and then dropping markedly to the basal levels afterwards. However, the levels of E1SO4 in animals no. 58 and 716 were high or relatively normal during late pregnancy and at the time of parturition. In animals no. 145 and 465, the levels were lower during late pregnancy and increased with a lower magnitude at the time of parturition (Figs. 4-5, right middle panels). In animals no. 58 and 716, the levels of PAGs were low and constant during late pregnancy. An increased level of PAGs was recorded during the last 10 d prior to parturition and reached high levels on the day of parturition similar to those found in animals delivering a normal living calf. In animals no. 145 and 465, the levels of PAGs were low during late pregnancy and increased with a low magnitude (<1000 ng/ml) on the day of parturition (Figs. 4-5, lower panels).

Endocrine changes in early postnatal calves

The levels of P4 and cortisol in all groups remained relatively constant over 1 h after birth but the cortisol showed very high levels (about 200-400 nmol/L) compared with the levels in the dams. The PG-metabolite and E1SO4 were very high at birth and the levels of these 2 hormones decreased significantly within 1 h ($p<0.001$). The levels of PAGs varied between 50-400 ng/ml, the average values being between 100-200 ng/ml at birth and then remaining constant afterwards. In the weak calf, lower levels of PG-metabolite and E1SO4 were found, compared with the average levels in all groups. The levels of P4, cortisol and PAGs were similar to the levels found in normal living

calves. No significant differences of endocrine parameters were recorded between groups of animals ($p>0.05$). Descriptive data of endocrine parameters summarised by groups and in the weak calf is presented in Table 5.

Discussion

The experimental animals (group A1 and A2) and the control animals (group B) were carefully selected on the criteria of having a high and a low risk, respectively, for stillbirth. In spite of the limited number of animals, the outcome of our selection was as expected, with the incidence of stillbirth in the 2 categories being 25% (3/12) and 0% (0/8), respectively. Furthermore, the viability of the live calves was slightly less in the experimental group.

However, the two bulls sired in groups A1 and A2 differed in respect to calving performance. The bull "Bubba", having a history of more calving difficulty, resulted in larger calves and a higher degree of calving difficulty. The bull "Patron", on the other hand, gave rise to smaller calves and minor calving difficulty but with low calf viability. The higher calf weight in group A1 is probably, at least in part, due to the skewed sex ratio (5 males: 1 females) in this group. Thus, there are indications that the backgrounds to the stillbirth in the 2 groups are not the same and depend on the different traits of the bulls used.

A precalving drop of body temperature of more than 0.5 °C was observed in animals with spontaneous parturition and a higher decrease of body temperature was recorded in the evening body temperature. This is a well-documented phenomenon, which has been confirmed in a recent study by *Lammoglia et al.* (1997). There is a positive correlation between a precalving decline of body temperature and P4 concentrations (*Rexha et al.* 1993, *Kornmatitsuk et al.* 2000). In the present study, only a slight precalving decrease of the evening body temperature (<0.5 °C) was recorded in animals with impaired parturition. These results may indicate that the endocrine changes preparing the dams for the parturition are not optimal regarding the P4 levels.

During the process of parturition, the average interval after the first sight of calf legs to birth in groups A1 and A2 was slightly longer, compared with the average of the control group. To some extent, this depends on a long calving process due to calving difficulties in the two dams in group A1 delivering a stillborn or weak calf. In contrast, a lower interval was observed in animals delivering a stillborn calf in group A2 and the longest intervals were recorded in 2 animals in this group giving birth to a normal living calf. This finding again indicates that there are differences between the 2 experimental groups in the process of parturition, and possibly the aetiology behind stillbirth. The shorter and the less variable calving process in the control group might indicate that the strength of labour or other factors of importance for a normal calving process might be associated with the stillbirth syndrome.

In the surviving calves, there were no clear differences between groups (A vs. B) in the levels of saturated oxygen in blood, body temperature and heart rates. Similarly, the calves showed no differences in intervals from birth to the first attempt to stand. Hence, we found no indications of lower survivability in calves born after the "high risk bulls" provided they were born alive during a normal calving process. In the weak calf, however, body temperature, saturated oxygen and heart rates were shown to be less and a longer interval to attain sternal recumbency and to the first standing were also observed. These results were in accordance with a report by *Schuijt & Taverne* (1994), who suggested that the interval between birth and sternal recumbency was an objective diagnostic tool for estimating the condition of newborn calves. The

weights of all vital organs in the 2 stillborn calves were in the same range as in a study by *Sangild et al.* (2000).

The number of cotyledons (82 cotyledons) and the placental weight in one of the animals with stillbirth (animal no. 465, group A2) was low in comparison with the other animals. These deviating placental characteristics might have affected the health status of the calf at term. Since the placentome thickness in the impaired group seemed to be thicker, one might speculate that the lower number is related to the larger size. A possible explanation is that there is a compensatory growth of the placentomes to maintain the pregnancy if the number of cotyledons is low.

During the peripartal period, the number of circulating leukocytes, especially neutrophils, increases and reaches the high levels on the day of parturition (*Saad et al.* 1989, *Cai et al.* 1994). Their functions are shown to play significant roles in early postpartum uterine defence in the cow (*Kerhli et al.* 1989). Moreover, steroids, prostaglandins and other arachidonic acid metabolites have also been proposed to influence neutrophil functions (*Kelly* 1994) and PAGs may be associated with inhibition of polymorphonuclear cell functions (*Dosogne et al.* 1999, *Heoben et al.* 2000). In our results, no clear difference was found in the number of lymphocytes but the number was larger in one of the animals with stillbirth (animal no. 145). A larger number of eosinophils was recorded during 40-50 d prior to delivery in animal no. 58, which also delivered a stillborn calf. However, these relatively small changes can not be reliably related to stillbirth and no conclusion could be drawn due to too few observations.

The pattern of P4 was similar in all animals despite the condition of the calf, indicating that the ovarian function was not influenced since most of P4 originates from the corpus luteum in the ovaries. Higher levels were seen in the two animals delivering a stillborn or weak calf after Bubba (around 2 nmol/L at parturition). Similar results have been shown in animals with calving difficulty after induced parturition (*Kornmatitsuk et al.* 2000). In animals no. 145 and 465 (sired by the bull "Patron" and both giving rise to stillbirth), the levels of PG-metabolite and cortisol were clearly higher at the second 24 h blood sampling at 3 w prior to parturition and on the day of parturition. The same relation between prostaglandin and cortisol synthesis has occurred, as shown by several reports in pigs with endotoxemia (*Magnusson et al.* 1994), food deprivation (*Tsuma et al.* 1996) and after adrenocorticotropic hormone (ACTH) administration (*Mwanza et al.* 2000). *Odensvik & Magnusson* (1996) also reported that significantly increased levels of $PGF_{2\alpha}$ metabolite and plasma cortisol were recorded after endotoxin administration in dairy heifers.

Hoffmann & Schuler (2002) mentioned that placental oestrogen is one of the important factors controlling caruncular growth, differentiation and function. *Zhang et al.* (1999) indicated that decreased oestrogen had a negative effect on neonatal viability in cattle. *Echternkamp* (1993) and *Ogata et al.* (1996) also reported that dams giving birth to weak or stillborn calves (occurring as a result of intrauterine growth retardation) had low circulating E1SO4 levels. In our experiment, the levels of E1SO4 during late pregnancy and parturition, and over 24 h at 3 and 6 w prior to parturition, were low in dams carrying stillbirth in group A2. Additionally, the levels of PAGs in these 2 animals were also low during late pregnancy and parturition, whereas the levels of E1SO4 and PAGs in animals no. 58 and 716 (delivering a stillborn or weak calf in group A1) showed a normal magnitude or higher. These results indicate that the placental function for the calves in group A2 was not the most optimal and that the calves were not in the best condition (impaired foetal

well-being). This indicates again that the two bulls ("Bubba" and "Patron") give rise to stillbirths with different pathophysiology.

Cortisol levels in the calf have been shown to be correlated to calf maturity at birth (*Nathanielsz* 1993). *Bellows & Lammoglia* (2000) indicated that severe dystocia resulted in reduced serum cortisol. However in the present study, high levels of cortisol were observed in both the normal living calves and the weak calf. The levels of PG-metabolite and E1SO4 decreased rapidly over 1 h after birth due to the rapid metabolism. The levels of PAGs were, however, maintained due to the longer half-life of this hormone (*Zoli et al.* 1992, *Beckers et al.* 1998, *Beckers et al.* 1999). The peripheral increases of PAGs prior to delivery in the dams seem to appear at the same time as marked degranulation of binucleate cells occurs (*Schlafer et al.* 2000). The process itself, and how the PAGs affect the calves at the early stage after birth, have not been clearly understood.

Conclusions

In summary, our results confirm the high incidence of stillbirth in heifers sired by certain Holstein bulls. Those stillbirths in some cases are not linked to calving difficulties but some of them are associated with calving difficulties due to large calves. This leads us to the conclusion that the nature of the stillbirth varies between different AI-bulls. We were not able to find any significant factor for causing stillbirth but the aetiology seems to be multifactorial. Our results however suggest that impaired-placental morphology and function as well as mechanisms leading to impaired labour might be parts of the problem. Deviating profiles of oestrone sulphate (E1SO4) and pregnancy associated glycoproteins (PAGs) in animals delivering stillbirth not caused by dystocia were observed also suggesting placental dysfunction as a factor. This finding suggests that the analyses of E1SO4 and PAGs could be used for monitoring the foetal well-being in animals with a high risk of stillbirth at term.

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Sammanfattning

Hormonmönster, hämatologi och kalvarnas överlevnad hos svenska holsteinkvigor i sent dräktighetsstadium inseminerade med tjurar som ger hög respektive låg frekvens av dödfödsel.

Andelen dödfödda kalvar hos kvigor av svensk holsteinras (SLB) har ökat kontinuerligt under de senaste 15 åren och ligger nu på c:a 11%. Syftet med denna undersökning var att dels leta efter orsakerna, som inte är kända, och dels att försöka hitta fysiologiska markörer för att kunna förutsäga dödfödsel. Försöks- och kontrolldjuren utvaldes baserat på relativa avelsvärden hos den tjur de inseminerats med. Tjugo mjölkkvigor av svensk holsteinras inseminerade med sperma från tjurar med hög risk för dödfödsel (n=12) utgjorde experimentgrupp och från tjurar med låg risk (n=8) utgjorde kontrollgrupp (B). Experimentgruppen delades i två undergrupper (A1 och A2) beroende på att de inseminerats med två olika tjurar med 3.5% resp. 9% högre risk för dödfödsel än medelvärdet; kontrollgruppen hade inseminerats med 5 olika tjurar som hade 0-6% lägre risk för att få dödfödda kalvar. Tjuren i grupp A1 hade också avelsvärden för kalvningssvårigheter högre än genomsnittet; medan tjuren i grupp A2 samt alla i kontrollgruppen hade avelsvärden för lägre andel kalvningssvårigheter än genomsnittet. Kvigorna köptes in dräktiga och övervakades från 6-7 månaders dräktighet fram till förlossningen. Förlossningen, samt hormonnivåer, hämatologi, olika placentamått och kalvviabilitet jämfördes mellan grupperna. Grupp A1 fick 1 dödfödd, 1 svagfödd och 4 normala kalvar; grupp A2 fick 2 dödfödda och 4 normala kalvar. I kontrollgruppen syntes inga kalvningssvårigheter och alla kalvar var normala. Födseln av den döda kalven i grupp A1 var förenad med

vissa kalvningssvårigheter på grund av en stor kalv och utdraget kalvningsförlopp. De dödfödda kalvarna i grupp A2 föddes utan problem. Den svagfödda kalven i grupp A1 avvek i kroppstemperatur, syremättnadsgrad och hjärtfrekvens jämfört med de normala kalvarna. Vid klinisk ultraljudsundersökning kunde inga avvikelser i placentatjockleken ses mellan grupperna. Likaledes avvek inte heller antalet vita blodkroppar eller differentialräkningarna mellan grupperna. Grupp A1 visade en sänkning i östronsulfatnivåerna (E1SO4) för kvigan som hade en dödfödd kalv när nivåerna jämfördes 6 veckor före beräknad kalvning mot 3 veckor före. För kvigan som fick den svagfödda kalven syntes inte några sådana skillnader och nivåerna var jämförbara med de normala djuren. Kvigorna som hade svagfödd resp. dödfödd kalv i grupp A1 visade inga avvikelser i slutet av dräktigheten eller vid förlossningen avseende E1SO4 eller PAGs ('pregnancy associated glycoproteins'). Grupp A2 visade låga nivåer av E1SO4 och PAGs för kvigorna med dödfödda kalvar både vid 6 och 3 veckor före förlossningen, samt i slutet av dräktigheten och vid förlossningen jämfört med kvigor som födde normala kalvar. Resultaten från försöket kunde inte visa en klar etiologi bakom dödfödselproblematiken, men skillnader finns mellan AI-tjurarna beroende på om de ger upphov till dödfödslar på grund av förlossningssvårigheter eller om de ger upphov till lätta kalvningar men där kalvarna föds döda. Mönstren för E1SO4 och PAGs för kvigorna som föder dödfödda kalvar utan förlossningssvårigheter (som grupp A2) tyder på att placentan är inte fullt funktionell. Dessa hormoner skulle kunna bli markörer på hur fostren mår och kunna förutsäga om dödfödsel eller ej är att vänta.

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