Effects of a protracted induction of parturition on the incidence of retained placenta and assessment of uterine artery blood flow as a measure of placental maturation in cattle

Hartmann, D ; Honnens, A ; Piechotta, M ; Lüttgenau, J ; Niemann, H ; Rath, D ; Bollwein, H

Abstract: The objectives of the present study were to compare the effects of a protracted and a conventional induction of parturition on the incidence of retained placenta, and to evaluate the suitability of transrectal Doppler sonography of the uterine arteries as a noninvasive method for the assessment of placental maturation. Protracted induction of labor (PIP) was precipitated in 13 cows by the administration of 1.3 mg dexamethasone im twice daily between Days 268 and 273 of gestation, and 40 mg dexamethasone im on Day 274 of gestation. For conventional induction of labor (SIP), 10 cows received 40 mg dexamethasone on Day 274 of gestation. A third group was not treated and served as control (SPON; N = 11). Blood flow volume (BFV) and resistance index in the uterine arteries were measured with Doppler sonography once a day from Day 268 of gestation until labor. After each ultrasonographic examination, blood samples for determination of steroid hormones were taken. Incidence of retained placenta was lower ($P < 0.05$) in group SPON (9%) compared with groups PIP (54%) and SIP (70%). In the last 7 days before parturition uterine BFV and resistance index did not change ($P > 0.05$) and did not differ between groups SPON, PIP, and SIP ($P > 0.05$). Resistance index was higher ($P < 0.001$) in cows with retained placenta compared with cows with released placenta, and BFV did not differ ($P > 0.05$) between them. Total estrogen concentrations increased by 283% ($P < 0.001$) in group PIP and by 60% ($P < 0.05$) in group SPON between Days -7 and -1 before parturition. They stayed constant ($P > 0.05$) until Day -2 in group SIP, but increased ($P < 0.05$) after the high dosage of dexamethasone within 1 day by 140%. Total estrogen levels were higher ($P < 0.05$) in cows with released placenta than in cows with retained placenta. In conclusion, a protracted compared with a short induction of labor results in higher estrogen levels before term, but does not affect incidence of placental retention. Neither alterations in placental maturation nor changes in steroid hormones influenced uterine blood supply. Therefore, Doppler sonography of uterine arteries is unsuitable to investigate the process of placental maturation induced by glucocorticoids in cows. Nevertheless, disturbances in the placental maturation process in cows with retained fetal membranes after parturition can be detected before parturition by a higher uterine blood flow resistance in the uterine arteries.

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Short Title: Effects of a protracted induction of parturition in cattle

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Abstract

The objectives of the present study were to compare the effects of a protracted and a conventional induction of parturition on the incidence of retained placenta, and to evaluate the suitability of transrectal Doppler sonography of the uterine arteries as a non-invasive method for the assessment of placental maturation. Protracted induction of labor (PIP) was precipitated in 13 cows by the administration of 1.3 mg dexamethasone intramuscular (i.m.) twice daily between Days 268 and 273 of gestation, and 40 mg dexamethasone i.m. on Day 274 of gestation. For conventional induction of labor (SIP), 10 cows received 40 mg dexamethasone on Day 274 of gestation. A third group was not treated and served as control (SPON, $n = 11$). Blood flow volume (BFV) and resistance index (RI) in the uterine arteries were measured with Doppler sonography once a day from Day 268 of gestation until labor. After each ultrasonographic examination, blood samples for determination of steroid hormones were taken. Incidence of retained placenta was lower ($P < 0.05$) in group SPON (9 %) compared to group PIP (54 %) and SIP (70 %). In the last seven days before parturition uterine BFV and RI did not change ($P > 0.05$) and did not differ between groups SPON, PIP and SIP ($P > 0.05$). Resistance index was higher ($P < 0.001$) in cows with retained placenta (RET) compared to cows with released placenta (REL), whereas BFV did not differ ($P > 0.05$) between them. Total estrogen (E_{tot}) concentrations increased by 283 % ($P < 0.001$) in group PIP and by 60 % ($P < 0.05$) in group SPON between Days -7 and -1 before parturition. They stayed constant ($P > 0.05$) until Day -2 in group SIP, but increased ($P < 0.05$) after the high dosage of dexamethasone within one day by 140 %. Total estrogen levels were higher ($P < 0.05$) in REL than in RET cows. In conclusion, a protracted compared to a short induction of labor results in higher estrogen levels before term, but does not affect incidence of placental retention. Neither alterations in placental maturation nor changes in steroid hormones influenced uterine blood supply. Therefore, Doppler sonography of uterine arteries is unsuitable to investigate the process of placental maturation induced by glucocorticoids in cows. Nevertheless, disturbances in the placental maturation process in cows with retained fetal membranes after parturition can be detected before parturition by a higher uterine blood flow resistance in the uterine arteries.

Keywords: Placental retention; Placental maturation; Induction of parturition; Dexamethasone; Cattle

1. Introduction

On large dairy farms, there is an increasing incidence of stillbirths due to difficulties with calving management [1,2]. It has been shown that stagnation in parturition was the main reason for stillbirth in cows besides of specific dystocia, such as malposition and uterine torsion [2]. One method to reduce this problem is to terminate labor via hormonal induction of parturition for a better supervision in calving animals, especially when difficulties are expected. Conventionally used methods for the induction of parturition are single treatments with corticosteroids and prostaglandins, respectively, to mimic the naturally occurring endocrine events that trigger the onset of parturition in cattle [3,4]. Spontaneous parturition is induced by the release of cortisol from the fetus. Cortisol stimulates the enzyme 17α-hydroxylase in the fetal membranes to catalyze the conversion of progesterone (P_4) to estrogens. The increasing concentrations of estrogens lead to a higher expression of oxytocin receptors, followed by a release of prostaglandins, which in turn induce luteolysis and parturition [3,5]. It is also well documented that sexual steroid hormones play an important role in placental development [5-9]. The maturation process of the placentomes is very important for normal placental separation, and it is suggested that a disturbance of the placental maturation is caused by a multifactorial event including morphological, functional and endocrinological processes [10].

Exogenous induction of parturition by using a single treatment with either dexamethasone or prostaglandin causes a high incidence of retained fetal membranes [11-13], which relies on the failure to raise estrogens to concentrations similar to those measured during spontaneously occurring parturitions in cows [14]. This perinatal imbalance or deficiency of hormones results in an incomplete placental maturation [9,15,16].

Several attempts to modify the induction schedule achieved no effect on placental maturation [14,17-19]. The first auspicious results in reducing the incidence of retained fetal membranes in cows were observed in New Zealand [20]. It was demonstrated that a pretreatment with long-acting corticosteroids had a positive effect on placental release. By using this method, a lower incidence of retained fetal membranes was observed [4,17,19-21]. Treatment with long-acting corticosteroids gained wide acceptance in New Zealand, where dairy farming is highly seasonal and lactation needs to coincide with the maximum availability of pasture [22]. In Germany and other European countries, this procedure is not popular because, according to European law, the exogenous application of long - acting corticosteroids is not allowed in food animals. An alternative in these countries is the treatment with low doses of a corticosteroid like dexamethasone two times per day to maintain consistently elevated levels of plasma cortisol, followed by a single treatment with a high dose of this hormone [23].

However, in previous studies it was noticed that the repeated application of corticosteroids during late pregnancy decreased ovine uterine and umbilical blood flow and consequently placental and fetal growth [24,25]. Although there were some studies measuring uterine blood flow in the last period of pregnancy in cattle [26-28], until now there are no data about uterine blood flow in the last few days of pregnancy. It has been shown that the large increase in transplacental exchange during the second half of gestation depends primarily on uterine blood flow [29,30]. Placental maturation in the last term of pregnancy is characterised by the formation of new vessels via angiogenesis [31] mainly by new fetal villious trees in the centre of the placentomes [30].

Administration of corticosteroids for several days in late pregnancy of the cow might result in a better hormonal preparation for parturition and therefore in a reduced incidence of retained placenta. But uterine blood flow might be influenced through the repeated doses of corticosteroids. Therefore, the objectives of the present study were to examine the effects of a short and a protracted induction of parturition with exogenous corticosteroids during the end of pregnancy on the release of the placenta and on uterine blood supply.

2. Materials and Methods

2.1. Cattle

Thirty-four primi- and pluriparous (n = 17 each), clinically healthy Holstein Friesian (n = 24), German Black Pied (n = 8), Simmenthal (n = 1), and Red Holstein (n = 1) cows with known breeding dates were examined at a research farm in Lower Saxony, Germany, between June 2007 and December 2008. These cows were 3.2 ± 1.2 years old (range, 2 to 8), with a parity of 1.7 ± 1.0 (range, 1 to 5). Twelve days before their expected calving date, cows were brought into stables with deep-straw bedding and fed a mixed ration (corn, grass silage, ground corn, vitamins and minerals), with *ad libitum* access to water.

2.2. Study design

Cows were randomly allocated into three groups with spontaneously occurring parturition (SPON, n=11), serving as control group, or short (SIP, n=10) and protracted (PIP, n=13) inductions of parturition, respectively. Cows in group SIP received a single treatment with 40 mg dexamethasone (Dexamethason-Lösung®, cp-pharma, Burgdorf, Germany) on Day 274 post insemination (p.insem.). Because plasma cortisol starts to decrease after 2 to 4 hours after administration of dexamethasone [32], cows in PIP received 1.3 mg dexamethasone twice daily on Days 268 to 273 p.insem., followed by a single treatment with 40 mg dexamethasone on Day 274 p.insem. (all treatments were given i.m.; Fig. 1).

In group SPON, ultrasonographic examinations were performed every second day from Days 268 to 276 p.insem., and starting on Day 276, once daily until the day of parturition. Cows of groups SIP and PIP were examined once daily between Day 268 p.insem. and parturition.

All cows were controlled every 4 h for signs of an imminent parturition. If cows did not deliver the fetus within 2 h after the first visible signs of labor, the position and size of the fetus was examined by transvaginal manual exploration of the birth canal. If indicated due to the obstetric findings, assistance of parturition was provided. Calves were judged as vital if they were able to stand and drink within the first 2 h after parturition. Birth weight of 23 calves were determined (SPON, n = 5; PIP, n = 10; SIP, n = 8).

Depending on if cows expelled the fetal membranes within the first 12 h after calving or not, they were defined as cows with released (REL) and retained placenta (RET), respectively. During the first 5 d, the latter were treated once daily with 1.0 mg Ceftiofur per kg body weight subcutaneous (Excenel® RTU, Pfizer AG, Zürich, Swiss) and were examined once daily to record the time of placental separation.

2.3. Measurement of uterine blood flow

Ten minutes before each ultrasonographic examination, cows were treated epidurally with 70 mg procaine hydrochloride (Procasel 2%®; Selectavet, Weyarn-Holzolling, Germany) to reduce rectal contractions. Transrectal ultrasonographic examinations of blood flow in the main uterine arteries ipsi- and contralateral to the localization of the fetus were performed using an ultrasound device (Toshiba SSH 370A, Toshiba Co., Tokyo, Japan), equipped with a 7.5 MHz microconvex transducer. All examinations lasted approximately

30 min per cow and were performed always by the same person (DH) during the same interval of the day (between 8 and 11 a.m.).

Uterine blood flow was investigated as described earlier [33]. All blood flow velocity waveforms were obtained at an interrogation angle between Doppler ultrasonic beam and blood flow direction of 20 to 60 degrees (Fig. 2). The observations were displayed on-line and recorded on a DVD recorder (DV-RW260S; Sharp Electronics, Hamburg, Germany). To study the intraobserver reproducibility both uterine arteries were examined in the first three cows in 42 examinations for three times. The interval between each measurement in the same vessel lasted approximately 1 to 15 min. For the off-line evaluation of blood flow parameters an image analysis software (Pixelflux; Chameleon-Software, Leipzig, Germany) was used. Two uniform consecutive waveforms with a maximum ratio between diastolic and systolic frequency shift were selected for each investigation. Immediately after the Doppler measurements the vessel diameters were calculated as the mean of three diameters measured on frozen two-dimensional grey scale images. Blood flow volume (BFV) was calculated by using the time-averaged maximum velocity over the cardiac cycle (TAMV) and the vessel diameter (D), according to the following equation: $BFV\ (mL / min) = TAMV\ (cm / min) \times (D / 2\ (cm))^2 \times \pi$. The resistance index (RI) was calculated as the ratio of the difference between peak systolic frequency shift (PSF) and end-diastolic frequency shift (EDF) to PSF: $RI = (PSF – EDF) / PSF$ (Fig. 2).

2.4. Determination of steroid hormones

Blood samples were collected from the coccygeal blood vessels into tubes containing potassium-EDTA (S-Monovette®; Sarstedt, Nümbrecht, Germany). Plasma was separated by centrifugation (4000 x g, 10 min) of blood samples within 20 min after collection. Samples were stored frozen at -20 °C until analyses of endocrine parameters. All hormone analyses were performed in duplicates and a difference < 10 % between the results were considered to be acceptable and the mean value was used for further calculations.

Plasma progesterone concentrations were measured using a sequential competitive chemiluminescence enzyme immunoassay (IMMULITE® 1000; Siemens, Los Angeles, USA), with a lower detection limit of 0.6 nmol/L. The intra- and interassay coefficients of variation (CV) were 16.0%, 8.1%, and 6.3% for control sera with low (<1 ng/mL), medium (4 ng/mL), and high (8 ng/mL) progesterone levels.

Total estrogens (E_{tot}) were measured after extraction from plasma (300 µl) with ether by a direct enzyme-immunoassay (EIA) on microtiter plates using a secondary antibody coating technique and horseradish peroxidase as the enzyme label. The EIA was previously validated and described [34,35]. For analysis an antibody (antigen: estradiol-17²-hemisuccinate bovine serum albumin) reacting with estradiol-17² (100 %), estrone (100 %) and estradiol-17± (66 %), was combined with estradiol-17²-hemisuccinate horseradish peroxidase, used as steroid-enzyme conjugate. The EIA method was validated in the Endocrinology Laboratory of the Clinic for Cattle, University of Veterinary Medicine, Hannover for bovine plasma by analyzing plasma samples of cows spiked with known amounts of 20 and 40 pg/ml 17²-estradiol. The intra-assay and inter-assay CV% was calculated by repeated measurements of twenty bovine plasma samples within one test routine and within different days. The minimal detectable concentration was 8 pg/ml. The recovery was 84.6 and 96.2 %, the intra-assay CV% was 11.0 % and the inter-assay 19.6 %.

Plasma cortisol concentrations were measured using a competitive chemiluminescent enzyme immunoassay (IMMULITE® 1000 Cortisol, Siemens, Los Angeles, USA) with a lower detection limit of 5.5 nmol/L and intra- and interassay CVs of < 8.8 % and < 10 %, respectively. According to the information of the manufacturer, there is no cross - reaction between plasma cortisol and dexamethasone.

2.5. Statistical Analysis

All statistical analyses were done with the Statistical Analysis System V9.1 (SAS Institute Inc., Cary, NC, USA) and SPSS 15.0 (SPSS, Chicago, IL). Since data of BFV, RI, progesterone, estrogens and cortisol were normally distributed (Shapiro-Wilk test), they were presented as means ± SD. Blood flow volume, RI, progesterone, estrogens and cortisol as well as changes in time-interval of progesterone were compared between groups and days and between RET and REL cows using the Student´s *t*-test. Also differences in BFV and RI in uterine arteries ipsi- and contralateral to the fetus and between cows with retained and released fetal membranes, as well as differences between parity of the cows were evaluated by Student's *t*-test. Effects of breed in BFV and RI in uterine arteries could not be determined, because the number of animals of some breeds was too small. Furthermore, the relationships between BFV and RI, BFV and birth weight of the calves, as well as RI and birth weight were investigated by calculating Pearson´s correlation coefficients (r). Results with positive or negative correlation coefficients of d 0.20 were interpreted as low or no correlation, between 0.21 and 0.50 as weak correlations, between 0.51 and 0.80 as moderate correlations and e 0.81 as good correlations. The variability of plasma progesterone and cortisol concentrations between groups was expressed as the coefficient of variation (CV). One-way ANOVA for repeated measurements with a saturated model of the fixed factor group and RET and REL was used to evaluate trends overtime for Progesterone, Estrogens and Cortisol. Incidence of retained placenta was compared between groups using Chi-square distribution. Differences and relationships with $P \leq 0.05$ were considered significant. Intraobserver reproducibility of Doppler measurements results were expressed as coefficient of variation (CV) and intra-classcorrelation coefficients (ICC) as described earlier [36].

3. Results

3.1. Clinical findings

Gestation length in cows of group SPON was 282 ± 4.1 d (range 278 to 289 d, n = 11). In group SIP, eight cows calved on Day 276 and two cows on Day 277 p. insem. (30 to 70 hours after treatment with 40 mg dexamethasone). In group PIP, nine cows calved on Day 275 (24 to 36 hours after treatment with 40 mg dexamethasone), three cows between Days 272 and 274, and one cow on Day 276 p. insemination.

Parturition of all cows in group PIP that calved on Day 275 was during daytime (6 a.m. to 8 p.m.), whereas cows in groups SPON and SIP calved during daytime (n = 9) or nighttime (8 p.m. to 6 a.m.; n = 12).

Obstetrical assistance was required in two cows of groups SPON, SIP, and PIP, respectively. Four cows (SPON: 1, SIP: 2, PIP: 1) needed slight (traction force of one person) and one cow (SPON) tight assistance (traction force of two people). In one cow (PIP), the position of the calf with the head tucked back had to be corrected.

Retained fetal membranes were observed in 1 of 11 (9 %) cows of group SPON, 7 of 10 (70 %) of group SIP and 7 of 13 (54 %) of group PIP. Incidence of retained placenta was lower ($P < 0.05$) in group SPON compared with group PIP and SIP. No differences ($P > 0.05$) were found between group PIP and SIP. The abruption of placenta occurred in all cows with RET 3 to 8 d after parturition. All cows with RET showed a metritis of grade I according to SHELDON et al. [37] within two days after parturition.

In group SPON two calves died, one during the calving process because of a prolonged and difficult labor (traction force of two people for more than 30 minutes) and one was already dead, when the cow was examined to check the presentation of the calf in the birth canal. In groups SIP and PIP no calf died, but two and one, respectively, needed nursing assistance.

At birth, ten of the weighted calves were male (m) and 13 female (f). The average birth weight was 42.5 ± 3.9 kg. The male calves were heavier than females (range 36 to 52 kg; m: 44.5 ± 3.8, f: 41.1 ± 3.4, $P < 0.05$). Birth weight of calves in group SPON (39.1 ± 1.0; m = 1, f = 4) was lower ($P < 0.05$) compared with calves in groups SIP (42.7 ± 3.3; m = 6, f = 2) and PIP (44.2 ± 4.3; m = 3, f = 7), respectively. No effects ($P > 0.05$) of birth weight of the calves on the time of release of the placenta could be found. Calves from cows with RET had an average birth weight of 42.8 ± 4.3 kg (n = 12), calves from cows with REL weighed 42.3 ± 3.6 kg (n = 11).

3.2. Uterine blood flow

CV and intra-class correlation coefficient values concerning the intraobserver repeatability of BFV and RI measurements were 1% and 0.97, respectively. Values for blood flow volume and RI did not change ($P > 0.05$) during the last seven days before parturition within cows, neither the values in the ipsi- and contralateral side nor the sum values for BFV or mean values of RI of both sides (Tab. 1 and 2).

In all groups, BFV was lower ($P < 0.001$) and RI was higher ($P < 0.003$) in the contra- compared to the ipsilateral uterine arteries. There were no differences ($P > 0.05$) in BFV and RI between groups SPON, SIP, and PIP.

Resistance index was higher ($P < 0.05$) in RET compared to REL cows, whereas BFV did not differ ($P > 0.05$) between RET and REL cows (Tab. 3).

There were moderate negative correlations between BFV and RI (r e -0.54; $P < 0.001$) between Days -7 and -1. No correlation ($P > 0.05$) was observed between BFV and the birth weight of the calves (Fig. 3a), but there was a weak positive correlation (r e 0.46, $P < 0.05$) between RI and birth weight (Fig. 3b). No effects ($P > 0.05$) of parity of cows on BFV- and RI values were found.

3.3. Steroid hormones

3.3.1. Progesterone

Plasma P_4 concentrations decreased between Days -7 and -1 before parturition ($P < 0.05$) in cows of groups SPON, SIP, and PIP by 46, 20 and 59 %, respectively (Fig. 4). The relative changes between Days -7 and -1 did not differ ($P > 0.05$) between groups. The P_4 concentrations were higher ($P < 0.05$) in PIP than in SPON and SIP. Between groups the CV for concentrations ranged between Days -7 and -1 from 17 to 64 %. An effect of time ($P < 0.05$) on P_4 concentrations were found in REL- and RET cows, but no differences ($P > 0.05$) between both groups of cows (Figure 7).

3.3.2. Total estrogens

Concentrations of E_{tot} in group SPON increased ($P < 0.05$) between Days -7 and -1 by 60 %. In group SIP, E_{tot} did not change ($P > 0.05$) between Days -7 and -2, but increased ($P < 0.05$) after treatment with dexamethasone by 140 % between Days -2 and -1. In group PIP, E_{tot} increased ($P < 0.001$) continuously between Days -7 and -1 by 283 % (Figure 5). Between Days -4 and -2, E_{tot} was higher ($P < 0.05$) in SPON than in SIP cows. Concentrations of E_{tot} in PIP were higher ($P < 0.05$) than in SIP between Days -3 and -1 and higher ($P < 0.05$) than in SPON on Day -1. On Days -7 to -5 E_{tot} concentrations were higher ($P < 0.05$) in REL than in RET cows (Figure 8). Estrogen levels showed changes in

REL as well as in RET cows ($P < 0.001$). In tendency ($P = 0.069$) E_{tot} concentrations were higher in REL- than in RET cows.

3.3.3. Cortisol

There were high variabilities of cortisol concentrations between groups ranging from 7 to 83 % between Days -7 and -1. Plasma cortisol concentrations in cows of group SIP decreased ($P < 0.05$) between Days -2 and -1 (following treatment with dexamethasone) by 22 %. In cows of group PIP they decreased ($P < 0.05$) by 21 % between Days -7 and -6 and stayed on a low level during dexamethasone treatment (Figure 6). Between Days -6 and -1 cortisol concentrations were lower ($P < 0.05$) in PIP cows compared to SPON and SIP cows. On Day -1, cortisol was lower ($P < 0.05$) in SIP than in SPON cows. No time-dependent changes ($P > 0.05$) and no differences ($P > 0.05$) in plasma cortisol concentrations were found within and between REL and RET cows, respectively (Figure 9).

4. Discussion

In group PIP, 70 % of cows calved 24 to 36 h after the last treatment with dexamethasone on daytime between 6 am and 8 pm. Similarly, in a previous study, the use of a long-acting corticosteroid 4 to 6 days prior to induction of parturition with dexamethasone resulted in a more predictable calving time compared to the induction of parturition by a single treatment using one shot of a high dosage of a glucocorticoid [4]. Therefore, a protracted induction of parturition allows a better calving management and may therefore lead to a lower stillbirth rate of calves.

In the control group, the incidence of stillbirth was 5.8 %, being within the normal range in dairy cows [2]. Furthermore, the average birth weight of the calves in the present study was consistent with results in untreated cows [2], probably due to the temporal coincidence of the day of birth induction (Day 274 of parturition) and to the normal ending of the gestation period.

In the present study, 54 % of cows in group PIP had a retained placenta compared to 70 % in group SIP. Previous studies found that a conventional induction of parturition with a single application of corticosteroids or prostaglandin resulted in a high incidence of retained placenta up to 93 % [13-15,38], which has detrimental effects on postpartum fertility [4,39] and milk yield [40]. Therefore, subsequent studies aimed to reduce the high incidence of retained placenta in induced animals. It was found that in late pregnancy the application of a long acting corticosteroid formulation followed by the administration of short acting corticosteroid several days later resulted in a lower incidence of retained fetal membranes [4,17-20]. The decisive factor for placental separation seems to be the variable interval from application of a long acting corticosteroid and the subsequently administered short acting corticosteroid. Some studies have shown that a minimum pre-treatment period of six days with corticosteroids is necessary to obtain a significant effect on placental maturation and therefore on placental release [4,18,20,22]. In this period of time the placenta required exposure to exogenous corticosteroids to ensure the physiological maturation process that would allow induction of parturition without retention of the fetal membranes [17]. Therefore, a pretreatment interval of six days was chosen in the present study.

The blood flow volumes and RI values did not change in the last seven days of pregnancy. In previous studies, significant increases in blood flow of both uterine arteries throughout gestation [27,41,42], steeping mainly during the last two-thirds of pregnancy [27], as well as no differences in uterine blood flow during the last third of pregnancy [28] were observed. The large increases in uterine blood flow during the last half of gestation were explained by the continuously increasing demands of the fetus [42]. We hypothesize that the constant uterine blood flow during the last days of pregnancy in the present study was due to a maximum blood flow capacity of uterine arteries. Therefore, it seems that the considerable increase in transplacental exchange at the end of pregnancy is not maintained by an increase in uterine blood flow, but by an increase in placental function associated with a vast growth of placental vascularity [29,42]. In addition, a limitation of blood flow capacity and therefore of the fetal supply could be a possible stress factor that may be jointly responsible for the induction of birth.

Mean BFV values in the last days before parturition were consistently higher and RI values lower in the uterine artery located ipsilateral to the pregnant uterus horn compared to values measured in the uterine artery contralateral to the fetus. These results are in agreement with findings of previous studies [27,28,43]. They may be associated with the increasing demands of the fetus during the last two-thirds of pregnancy [42]. Another study attributed these observations to the fact that the pregnant horn contains more and larger placentomes than the non-pregnant horn [44].

Resistance index was significantly higher in the last days of pregnancy in RET compared to REL cows. This is probably due to the fact that the physiological ablation process of the fetomaternal adherence in cows with retained placenta failed. Physiologically, this loss of adherence occurs only after the placentome has undergone a process of maturation [10,45]. During this maturation process, vasculo-syncytial configurations are formed [10], which may result in a lower resistance index, and consequentially lead to a reduced incidence of retained fetal membranes.

In a previous study, a good correlation between BFV and birth weight of the calves was noticed in the last third of pregnancy [46]. In the present study, however, no correlation between BFV and birth weight, which is probably due to the fact that there was no increase in uterine blood flow in the last seven days of gestation. Surprisingly, a positive correlation was detected between RI and birth weight in the last days before parturition. In contrast, studies in women found a high negative relationship between resistance index in the uterine arteries and birth weight of babies during the first trimester and during 19 to 23 weeks of pregnancy, respectively [47,48]. This finding may be associated with the size of the uterus in the last stage of pregnancy; a heavier and therefore larger calf may lead to stretching of uterine vessels and thus create a higher resistance to blood flow.

In the present study, progesterone levels in group PIP declined during the glucocorticoid treatment by 59 % until parturition. The fact, that progesterone concentrations between Days -7 and -1 were higher in PIP than in SPON and SIP could not be clarified. A possible explanation might be the high inter-individual variability between animals. Nevertheless, the relative changes in this time interval did not differ between groups.

Total estrogen concentrations in group SPON increased by 60 % between Days -7 and -1. Physiologically, estrogen concentrations in the maternal peripheral circulation start to increase in the last few days before parturition and decline rapidly to zero level post partum [5]. Total estrogens in group PIP increased by 283 % between Days -7 and -1, whereas E_{tot} in group SIP increased by 140 % within one day (Day -2 to -1). It was noticed that estrogens play an important role in the maturation of placentomes [5,8,16,49]. In agreement with previous studies we found that estrogen concentrations were lower in RET compared to REL cows [8,49].

Plasma cortisol concentrations showed a high variability in group SPON and SIP, which may be due to handling stress, since cortisol is recognized as a stress hormone [5]. In group PIP plasma cortisol concentrations decreased after the first application of dexamethasone and remained on a low level during dexamethasone treatment. This phenomenon is based on the negative feedback on hypothalamo-pituitary-adrenal function [50]. Moreover, maternal exposure to exogenous glucocorticoids can lead to permanent modification of fetal hypothalamo-pituitary-adrenal function [50]. Other studies have shown that the exogenous administration of glucocorticoids has been associated with increased susceptibility to infectious diseases in cattle [51,52]. However, the immunosuppressive effect is dependent upon the dose of dexamethasone administered. Thus, the authors found little variations in haematological parameters when a dose of 0.3 mg/kg for three days was applied [53]. In the present study 0.004 mg/kg over six days followed by a single dose of 0.07 mg/g was given. Further investigations are necessary to evaluate the effects of a protracted administration of glucocorticoids on the immune system and on the hypothalamo-pituitary-adrenal function in cattle.

Conclusions

In conclusion, a protracted induction of parturition with the use of repeated treatments with low doses of corticosteroids for six days followed by one single treatment with a high dose of this hormone will result in higher estrogen levels before term, but does not affect uterine blood flow volume and the incidence of retained placentas. Nevertheless, the resistance index was higher in RET compared to REL cows, therefore disturbances in the placental maturation process in cows with retained fetal membranes after parturition can already be detected before parturition.

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Revised

List of figures

Figure 1: Treatment schedule of cows with spontaneous parturition (SPON, n = 11), as well as short (SIP, n = 10) and protracted (PIP, n = 13) induction of parturition: Cows in group SIP received a single treatment with 40 mg dexamethasone on Day 274 post insemination (p.insem.), cows in PIP received 1.3 mg dexamethasone twice daily on Days 268 to 273 p.insem., followed by a single treatment with 40 mg dexamethasone on Day 274 p.insem. Ultrasonographic examinations were performed in group SPON every second day from Days 268 to 276 p.insem., and starting on Day 276, once daily until the day of parturition. Cows of groups SIP and PIP were examined once daily between Day 268 p.insem. and parturition.

Figure 2a and b: Doppler flow mapping of blood flow in the uterine artery ipsilateral to the conceptus in a cow with protracted induction of parturition (Fig.2a) and spontaneous parturition (Fig.2b) on Day 272 of pregnancy;

left side of both pictures: spectral Mode showing pulse waves; right side of both pictures: blood flow shown in colour Mode.

Figure 3a: Relationship between birth weight of calves (n = 23) and blood flow volume (BFV in L/min) of uterine arteries in cows with spontaneous parturition (SPON, n = 5), short (SIP, n = 8) and protracted (PIP, n = 10) induction of parturition. The summation (sum) of BFV values of the uterine arteries ipsi- and contralateral to the conceptus are used.

Figure 3b: Relationship between birth weight of calves (n = 23) and resistance index (RI) of uterine blood flow in cows with spontaneous parturition (SPON, n = 5), short (SIP, n = 8) and protracted (PIP, n = 10) induction of parturition. Mean RI values of the uterine arteries ipsi- and contralateral to the conceptus are used.

Figure 4: Plasma progesterone (P_4) concentrations in cows with spontaneous parturition (SPON), as well as short (SIP) and protracted (PIP) induction of parturition. Between Days -7 and -1 before parturition, P_4 concentrations were higher ($P < 0.05$) in PIP than in SPON and SIP. There was an effect of time ($P < 0.001$) and group ($P < 0.05$).

* Difference between group PIP ($P < 0.05$) and SPON and SIP.

Figure 5: Differences in plasma concentrations of total estrogens in cows with spontaneous parturition (SPON), as well as short (SIP) and protracted (PIP) induction of parturition. There was an effect of time ($P < 0.001$) and group ($P < 0.05$).

* Differences between group PIP ($P < 0.05$) and SPON (Day -1) and SIP (Day -3 to -1) on the day indicated.

\# Differences between group SPON ($P < 0.05$) and SIP on the day indicated.

a, b Differences between days ($P < 0.05$) in group SPON and PIP (a) and SIP (b)

Figure 6: Plasma cortisol concentrations in cows with spontaneous parturition (SPON), as well as short (SIP) and protracted (PIP) induction of parturition. There was an effect of time ($P < 0.05$) and group ($P < 0.05$)..

* Differences between group PIP ($P < 0.05$) compared with SPON (Day -6 to -1) and SIP (Day -6 to -2) on the day indicated.

\# Differences between group SIP ($P < 0.05$) and SPON on the day indicated

a, b Differences between days ($P < 0.05$) in group SPON and PIP (a) and SIP (b)

Figure 7: Plasma progesterone concentrations in cows with released (REL) and retained placenta (RET). There was an effect of time (P < 0.05), but no effect of group (P > 0.05).

Figure 8: Plasma concentrations of total estrogens in cows with released (REL) and retained placenta (RET). There was an effect of time (P < 0.05) and in tendency (P = 0.069) higher total estrogen concentrations in cows of REL compared to cows of group REL.
* Differences between REL (P < 0.05) and RET (Day -7 to -5) on the day indicated.

Figure 9: Plasma cortisol concentrations in cows with released (REL) and retained placenta (RET). There was neither an effect of time (P > 0.05), nor an effect of group (P > 0.05).

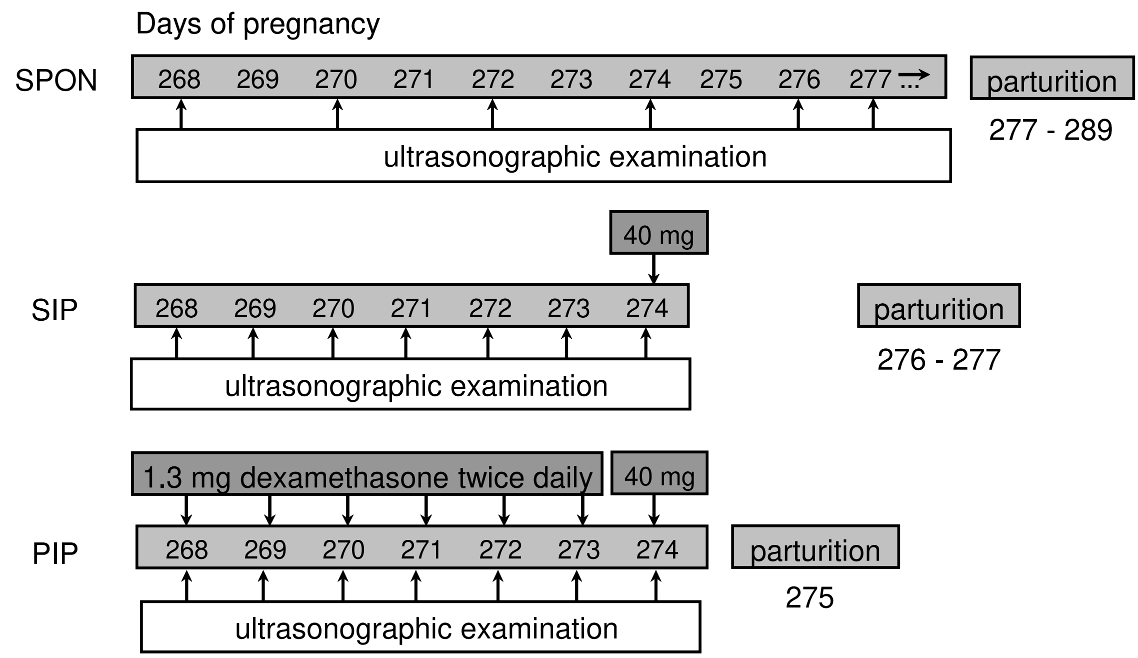
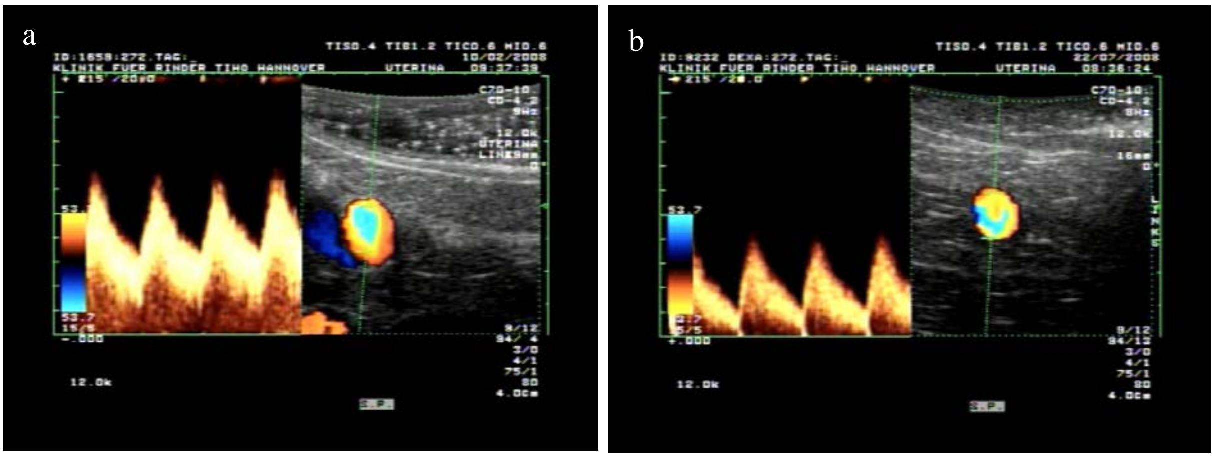


Figure 1



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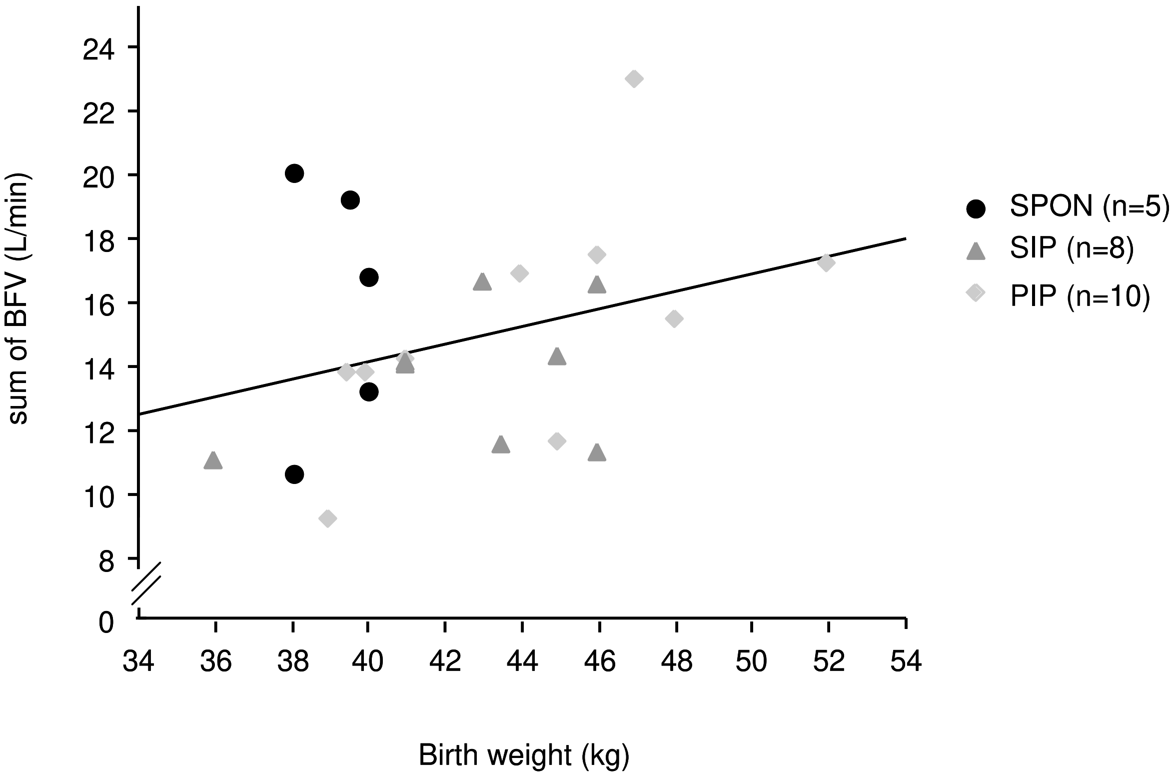
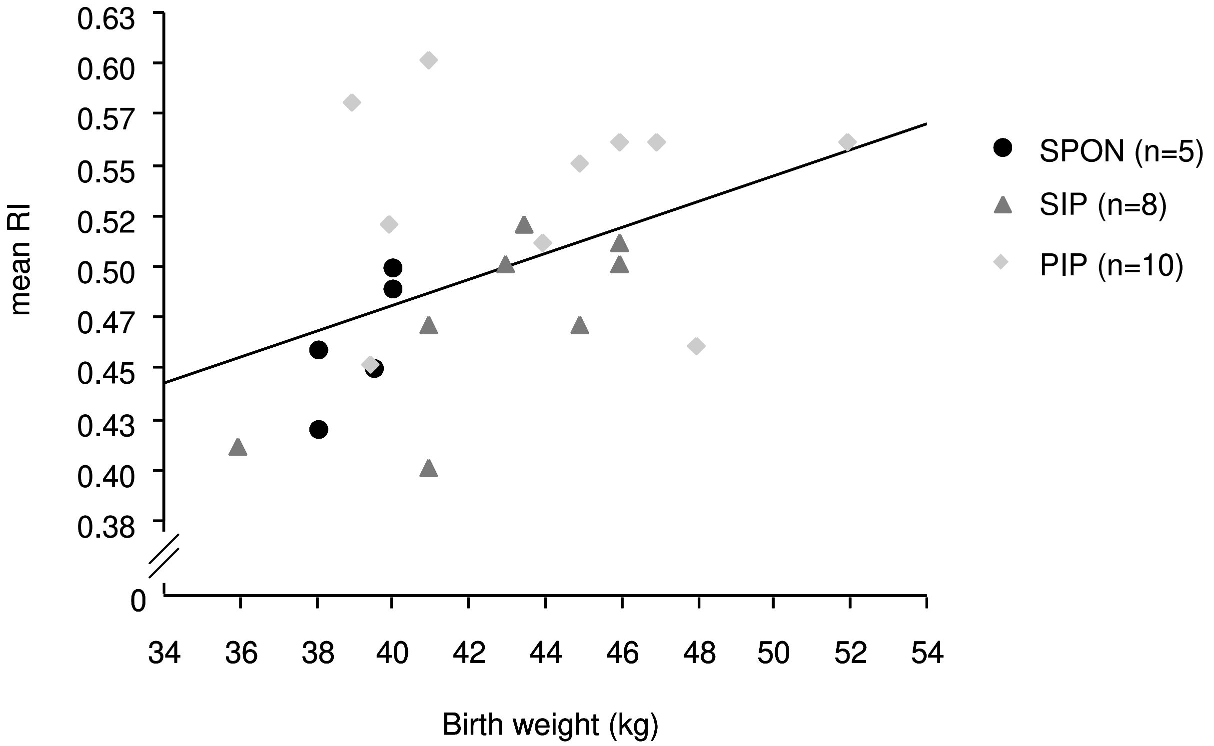


Figure 3a



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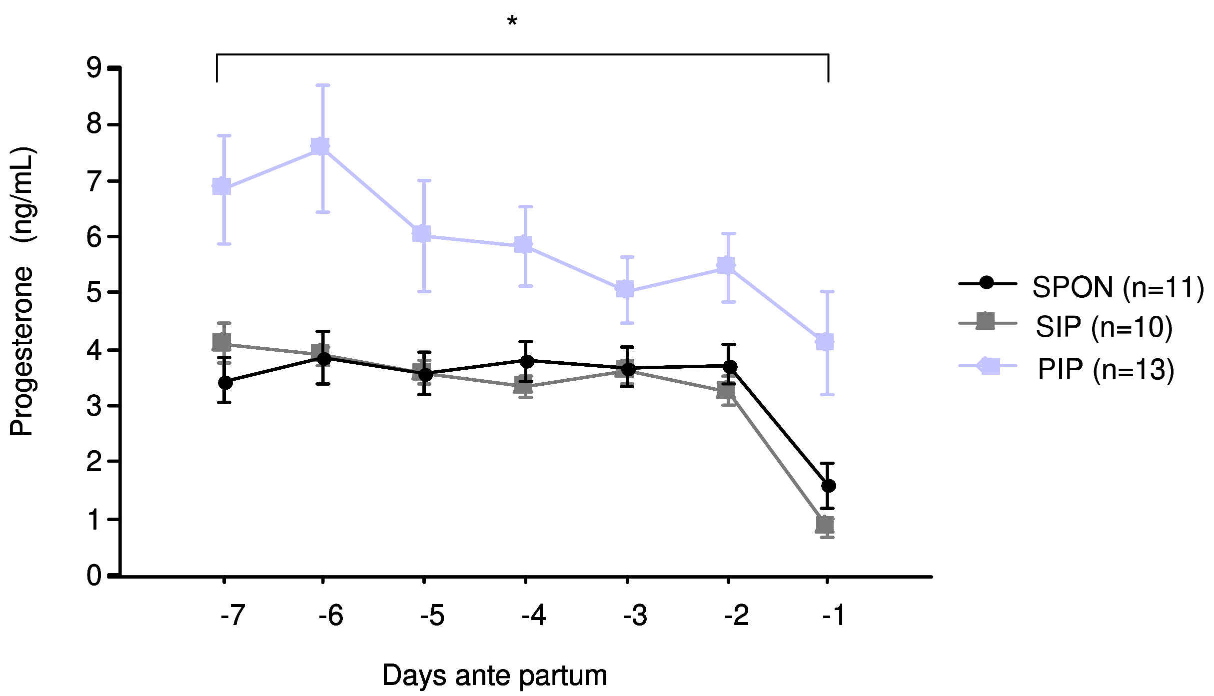


Figure 4

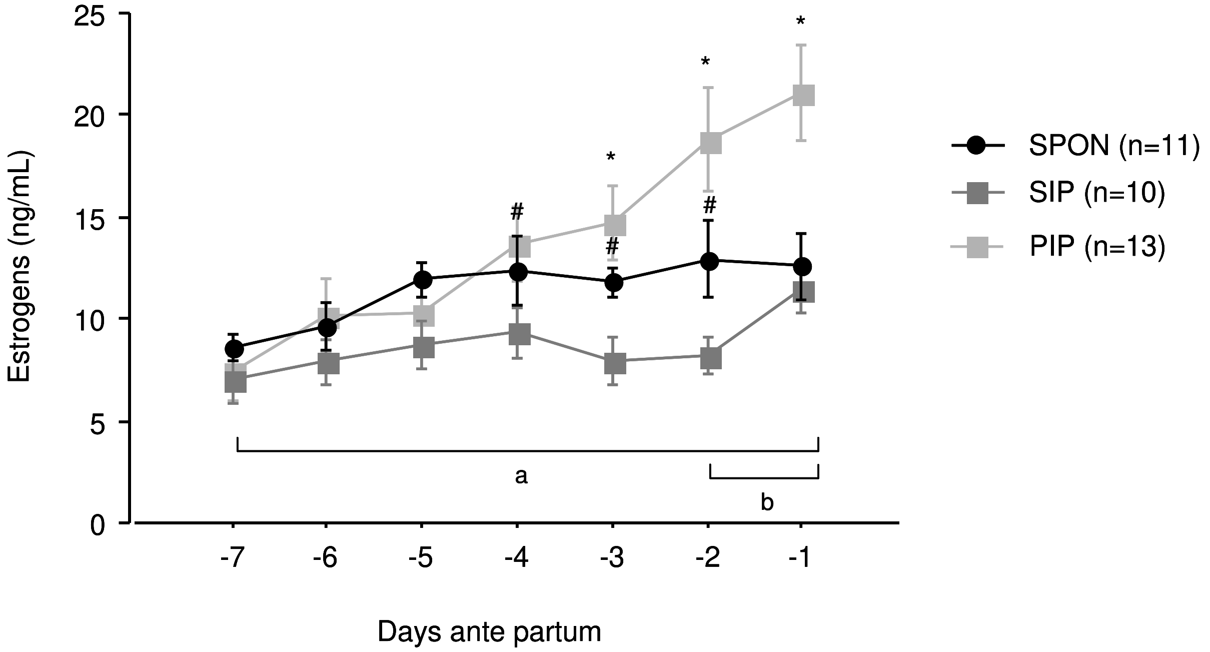


Figure 5

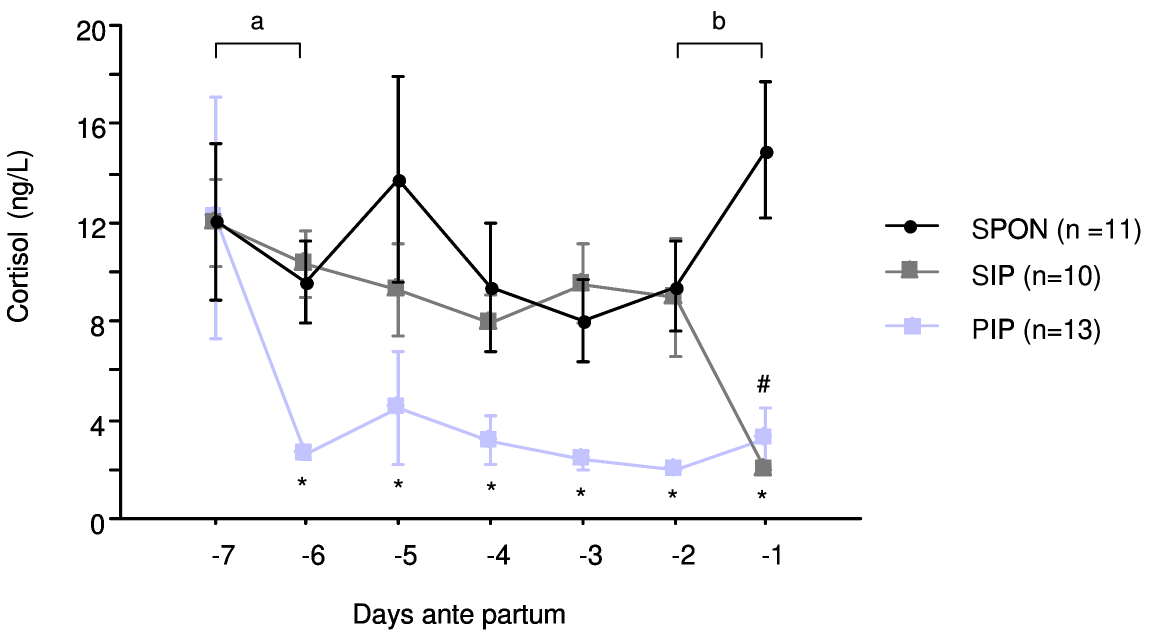


Figure 6

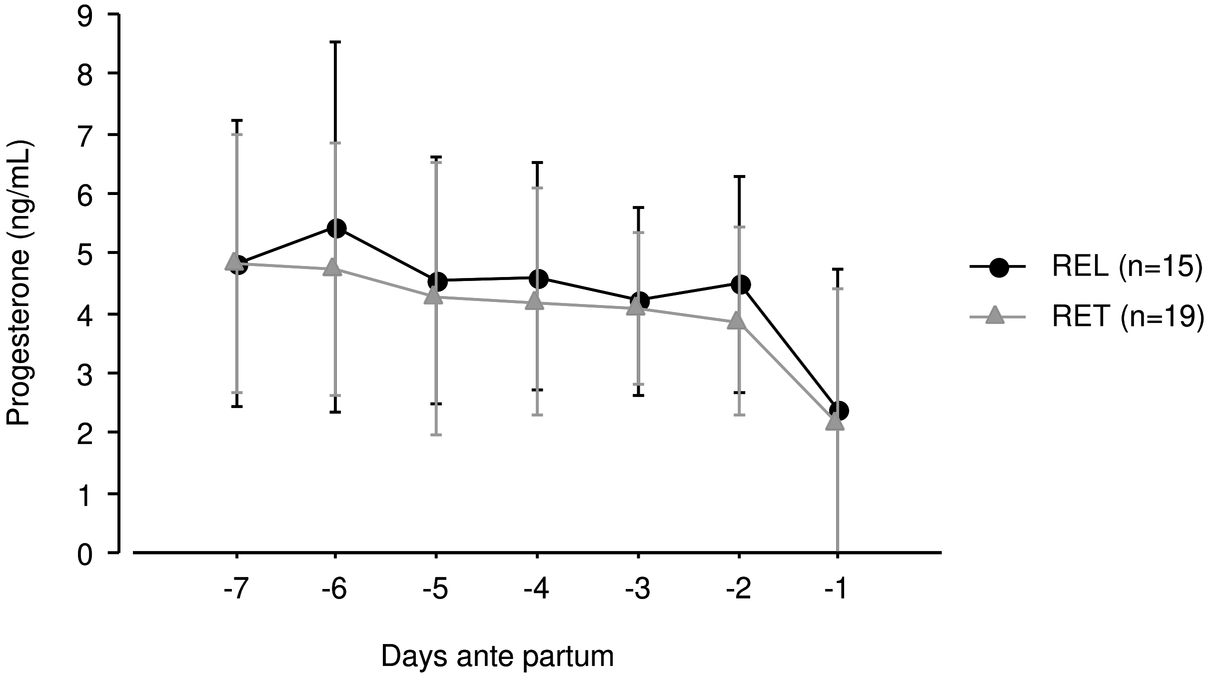


Figure 7

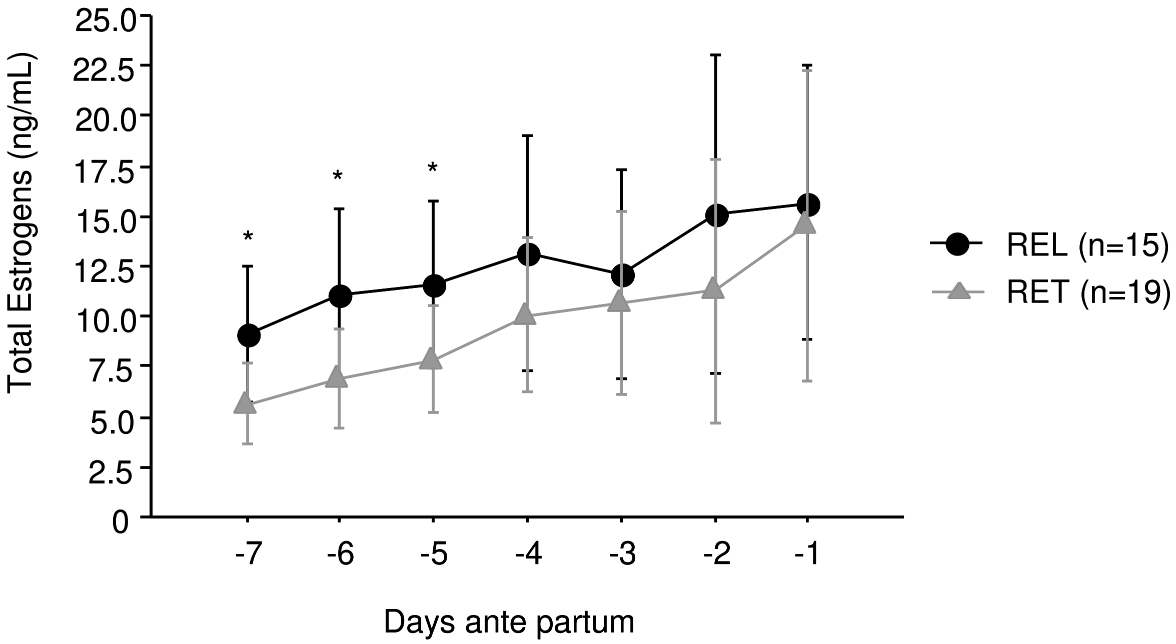
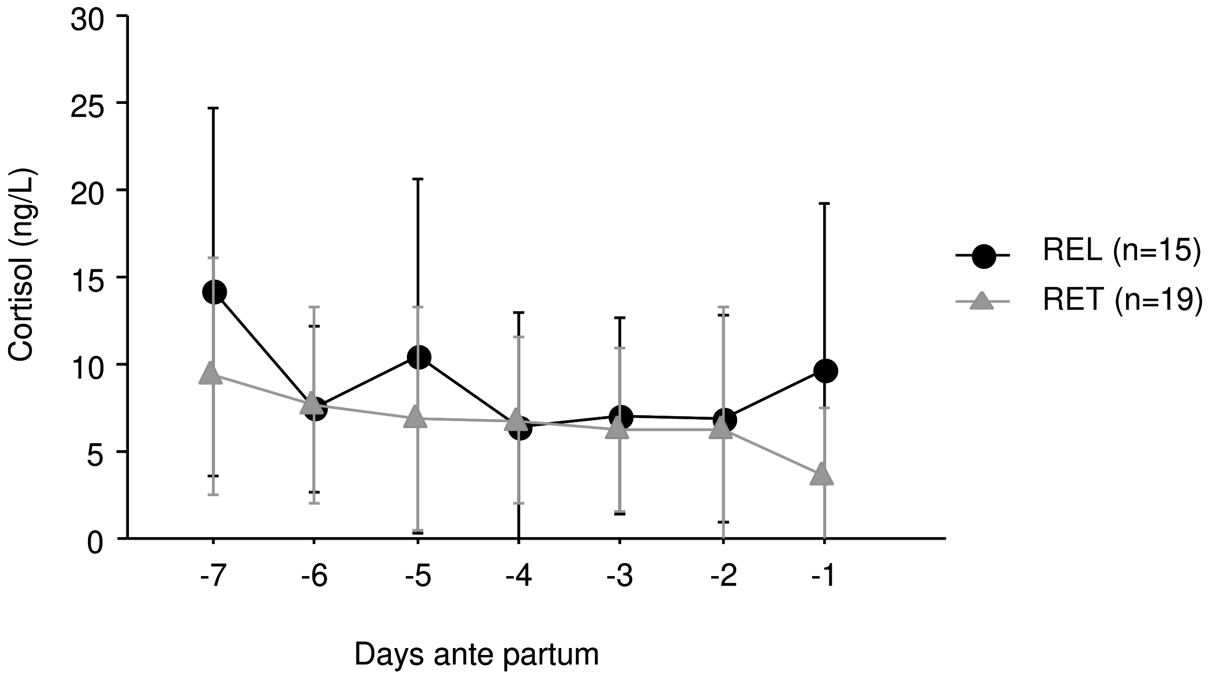


Figure 8



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