Master's thesis

Progesterone concentrations before parturition in cows

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Preface

Before you lies the master's thesis 'Progesterone concentrations before parturition in cows'. This thesis is written in as part of my graduation from the master Farm Animal Health/Veterinary Public Health at the faculty of Veterinary Medicine in Utrecht. Research was conducted at the clinic of the Department of Farm Animal Health, from April 2016 to December 2016.

The analysis and report were drafted with the help of my research supervisor, Joyce Parlevliet. Her I want to thank for the guidance and support during this project and for answering my questions. I also want to thank Hans Vernooij answering all of my questions regarding the statistical analyses and the interpretation and application of the results.

Finally, I want to thank my friends for their advice and support during the writing process. I also want to give special thanks to my parents and boyfriend, their wisdom and motivational words have helped me to finish this thesis.

I hope you enjoy reading this study.

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Samenvatting

Het doel van deze studie was om te evalueren was het nut is van het gebruik van progesteronconcentraties als diagnostisch middel om de dag van afkalven te voorspellen in koeien meer dan 265 dagen drachtig. Ook werden de incidentie en oorzaken van het aan de nageboorte blijven staan geëvalueerd. Alle data werd gehaald uit de patiëntverslagen, die opgeslagen zijn in het online administratieprogramma van de kliniek landbouwhuisdieren van de faculteit Diergeneeskunde in Utrecht. Alle progesteronconcentraties zijn gebruikt, evenals de inseminatiedatum, inductiedatum, de dag van de partus of sectio, de inductiemedicatie die is gebruikt, de klinische signalen wijzend op een naderende partus en gegevens of de koe aan de nageboorte stond. De progesteronconcentraties werden elke maandag, woensdag en vrijdag gemeten vanaf 265 dagen dracht tot aan de partus. Inductie werd gedaan door middel van de toediening van dexamethason, en in 50% van de gevallen werd prostaglandine toegediend, 12 uur na de toediening van dexamethason. De drie factoren van invloed op het niet afkomen van de nageboorte waren of de koe een sectio had gehad ($P < 0{,}01$), de drachtlengte ($P < 0{,}05$) en of de koe was geïnduceerd ($P < 0{,}01$). Het onafhankelijke effect van een sectio was de belangrijkste factor in het niet afkomen van de nageboorte, maar ook inductie had veel effect. De progesteronconcentraties van niet geïnduceerde koeien waren op dag 1 voorafgaand aan de partus significant lager (1,14 ng/ml; $P < 0{,}01$) dan die van geïnduceerde koeien. Op dag 0 daarentegen, was er geen significant verschil. De progesteronconcentratie van beide groepen eindigde ongeveer op het zelfde gemiddelde (1,0 ng/ml ± 0,17 [gemiddelde ± SEM]). Om de invloed van verschillende variabelen op de normale progesteronconcentraties te bepalen, zijn alleen koeien gebruikt die aan de tijd waren. Een significant verband is aangetoond tussen progesteronconcentraties en de laatste 7 dagen voorafgaand aan de partus ($P < 0{,}05$). Vanaf dag 8 en verder was er geen significante relatie met progesteron. De progesteronconcentraties daalden sterk vanaf 2 dagen voor de partus (Est. –4,58; $P < 0{,}01$). Voor elke koe en elk interval in dagen voorafgaand aan de partus was de gemiddelde stijging en daling berekend in progesteronconcentraties. Dit is weergegeven in absolute getallen (in ng/ml) en in percentages, en voor alles is het 95% betrouwbaarheidsinterval en het 95% referentie interval bepaald. De spreiding in progesteronconcentraties en de spreiding in daling en stijging was erg hoog, met veel overlap voor elk interval. In de gemiddelde progesteronconcentraties en de gemiddelde stijging en daling (met het 95% referentie interval), wordt er zo'n grote spreiding gezien in concentraties dat het meten van progesteron niet als een nuttig middel wordt gezien om de dag van afkalven van de koe van tevoren te bepalen.

Summary

The objectives of this study were to evaluate the use of progesterone concentrations in predicting calving time in cows over 265 days of gestation. The incidence of retained fetal membranes after induction to parturition is evaluated as well. Data were collected from the online electronic patient files at the clinic of the Department of Farm Animal Health of the Faculty of Veterinary Medicine in Utrecht. Information was collected about progesterone concentrations, date of insemination, induction and C-section or spontaneous parturition, induction medications used, clinical signs prior to parturition and retained fetal membranes. P4 levels were checked every Monday, Wednesday and Friday from ± 265 days of pregnancy until parturition. The cows were induced to parturition using dexamethasone, and in approximately 50% of the cases prostaglandin was given as well, administered 12 hours after dexamethasone. The incidence of retained placenta was affected by three factors: C-section ($P < 0,01$), gestation length ($P < 0,05$) and induction treatment ($P < 0,01$). The independent effect of a C-section had the greatest influence, but induction was highly associated as well. Progesterone concentrations of non-induced cows were on Day 1 before parturition significantly lower (1,14 ng/mL; $P < 0,01$) than those of induced cows. On Day 0, on the other hand, there is no significant difference. Progesterone concentrations of both groups end around the same average concentration (1,0 ng/mL ± 0,17 [Mean ± SEM]). To analyze the influence of several variables on normal progesterone concentrations, only cows at full-term gestation were used. A significant correlation was calculated between progesterone concentrations and Days 0–7 before parturition ($P < 0,05$). From 8 days before parturition and further, no significant relationship is seen. Progesterone concentrations decline markedly within 2 days of parturition (Est. –4,58; $P < 0,01$). For each cow and each interval in days before parturition, the average decrease or increase in progesterone concentrations was calculated, in absolute numbers (ng/mL) and in percentages, as well as the 95% confidence interval and 95% reference interval. The dispersion in progesterone concentrations and decrease/increase was very high, with a lot of overlap for each interval. Based on the mean progesterone concentrations and the average decrease and increase in progesterone concentrations (all with 95% reference interval), progesterone concentrations were found to not be a useful means to predict calving time in cattle, because of the high dispersion in concentrations.

Index

1. Introduction

1.1 Study introduction

Accurate prediction of calving time is essential in livestock farming. It is of vital importance because precise prediction can be crucial for the health of both calves and dams. Difficult parturition is a major cause of stillbirths (up to 47%) and is reported to be an important cause of periparturient diseases such as retained fetal membranes (Villarroel & Lane, 2010). Human supervision and calving assistance, when necessary, can significantly decrease calf mortality and retention of fetal membranes, thus helping prevent injuries to the newborn calf and dam. In accurate calving time prediction, the time-consuming process of monitoring is not necessary, and the effects of dystocia (delayed or difficult parturition) are minimized (Saint-Dizier & Chastant-Maillard, 2015; Streyl et al., 2011).

Performed at the clinic of the Department of Farm Animal Health of the Faculty of Veterinary Medicine in Utrecht, this study focuses on predicting time of parturition through the use of hormone concentrations. Each year, approximately 50 pregnant cows are bought by the clinic for educational purposes. After 275 or more days of gestation, an elective C-section is done by two students. This way, students can learn how to perform one under professional guidance and in nearly perfect circumstances. This elective procedure must be tightly scheduled. Because of its educational purpose, it has to be done within working hours when animal caretakers, the veterinarian and the students are all available. Consequently, cows are usually induced to parturition, and a C-section is planned within 24 to 36 hours afterwards. It is not always clear, however, if and when to induce for parturition.

In general it is established that cows have an average gestation length of 280 days (270–290 days); therefore, the dams are closely monitored from 265 days of gestation onward. The exact moment for the C-section is established based on several factors. First, clinical signs are taken into account. Those used to predict time of calving are relaxation of the pelvic ligaments, udder distention and filling of the teats. In addition, blood samples are taken every Monday, Wednesday and Friday and plasma progesterone is measured in ng/mL. A decrease in blood serum progesterone (P4) concentrations less than 1,2 ng/mL has been shown to be an accurate way to predict calving time within 12 to 24 hours (Matsas, Nebel & Pelzer, 1992). If clinical signs indicate imminent parturition and/or if P4 concentrations are less than 1,2 ng/mL, the cow is induced or a C-section is planned without prior induction. Following this course of action, chances are high that the cow will calve naturally during the evening or night. However, clinical signs and P4 concentrations are not always taken into consideration. To avoid spontaneous parturition, the cow is induced around 275 days of gestation in many cases, and a C-section is done within 36 hours. The risk of the latter course of action is that the dam is not at full-term gestation. The consequences of early induction are a higher rate of calf mortality (Mansell, Cameron, Taylor & Malmo, 2006) and the occurrence of retained fetal membranes. If parturition is induced within 30 days of parturition, the incidence of retained placenta (RP) can be up to 85% (McNaughton & Murray, 2009); however, induction closer to the expected date of parturition results in a decreased incidence (Peters & Poole, 1992; Villarroel & Lane, 2010). Savc, Kenny and Beltman (2016) even reported that cows induced within two days of due date had no difference in RP incidence compared with non-induced cows. The preferred course of action is to plan the C-section during working hours, as close to the actual parturition date as possible, without the cow giving birth naturally. Ideally, the induction treatment starts 3 to 5 days before the actual parturition date, and as a result, a C-section can be done within 4 to 2 days before the actual parturition.

As mentioned, when the P4 concentrations drop below 1,2 ng/mL, parturition will take place within 12 to 24 hours (Matsas et al., 1992), but measurements of P4 concentrations within 24 hours of parturition are not useful in determining the optimal moment of induction. The purpose of this study is to evaluate whether a decrease in progesterone levels in blood serum within 10 days of natural parturition can be indicative of the moment of parturition.

1.2 Progesterone

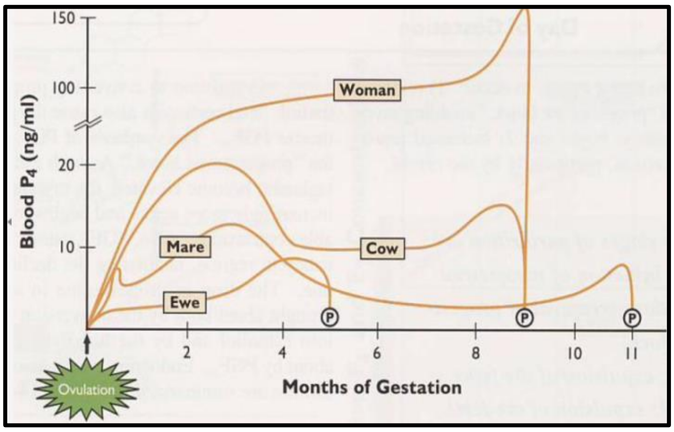


Fig. 1.1 Progesterone profiles in various pregnant females. Reprinted from *Pathways to Pregnancy and Parturition* (p. 317) by P. L. Senger, 2005, Pullman, WA: Current Conceptions, Inc. Copyright 2005 by Current Conceptions, Inc.

During early gestation, progesterone is produced by the corpus luteum, but from 150 to 200 days, the placenta takes over most of the production. Nonetheless, the corpus luteum continues to produce small amounts of progesterone until the end of gestation. These levels fluctuate during pregnancy, but they stay between a certain high range (Figure 1, Senger, 2005, p. 317). In this manner, myometrial contractions are blocked by progesterone. This feedback system is called the 'progesterone block'. In the last days of pregnancy, the fetus grows, and the volume within the uterus decreases. As a result, the fetus becomes stressed. This stress is believed to be one of the main reasons for an increased production of adrenocorticotropic hormone (ACTH), produced by the fetal pituitary gland. ACTH causes fetal adrenal cortisol concentrations to rise in the last 20 days of gestation, with a peak in the last 2 to 3 days before parturition (Senger, 2005) Elevated cortisol concentrations are the first step of many changes in the cow. First, high levels of cortisol result in the conversion of progesterone (P4) to oestrogen by promoting the synthesis of three enzymes involved in different intermediate steps in this production process (Senger, 2005; Taverne & Noakes, 2001).

The increasing concentrations of estradiol lead to a higher expression of oxytocin receptors, which leads to the removal of the blockage of myometrial contractions (Hartmann et al., 2013; Senger, 2005; Taverne & Noakes, 2001; Weems, Weems & Randel, 2006). A second change connected to high cortisol levels is that through activation of the enzyme phospholipase A_2, oestrogen causes the placenta to synthesize and release PGF2a. PGF2a also helps to remove the progesterone block (Taverne & Noakes, 2001), as seen in Figure 3 (Senger, 2005, p. 321). When estradiol and PGF2a concentrations are both elevated, the myometrium becomes more active and starts to push the calf into the right position for expulsion. PGF2a leads luteolysis as well, causing P4 concentrations to decrease even more, because the corpus luteum is still producing a small amount of it (Senger, 2005; Taverne & Noakes, 2001). The decrease in P4 concentrations begins about 10 days before parturition (Taverne, as cited in Saint-Dizier & Chastant-Maillard, 2015); therefore, the P4 concentrations of this period will be analyzed.

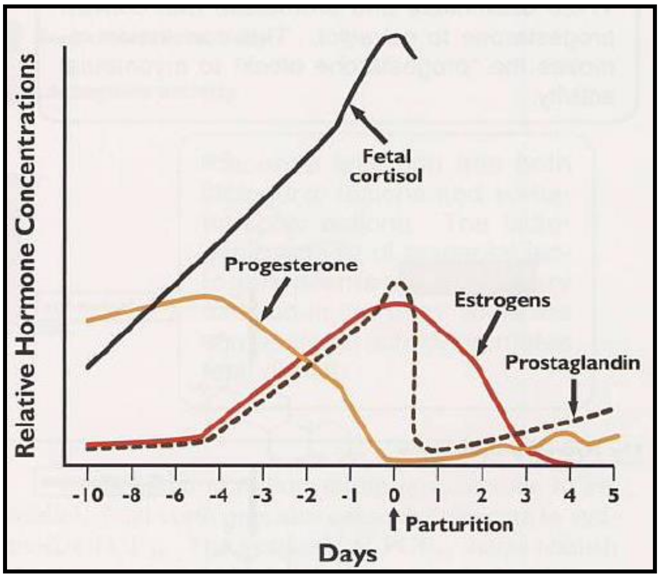


Fig. 1.2 Relative hormone profiles in the cow during the periparturient period. Reprinted from *Pathways to Pregnancy and Parturition* (p. 321) by P. L. Senger, 2005, Pullman, WA: Current Conceptions, Inc. Copyright 2005 by Current Conceptions, Inc.

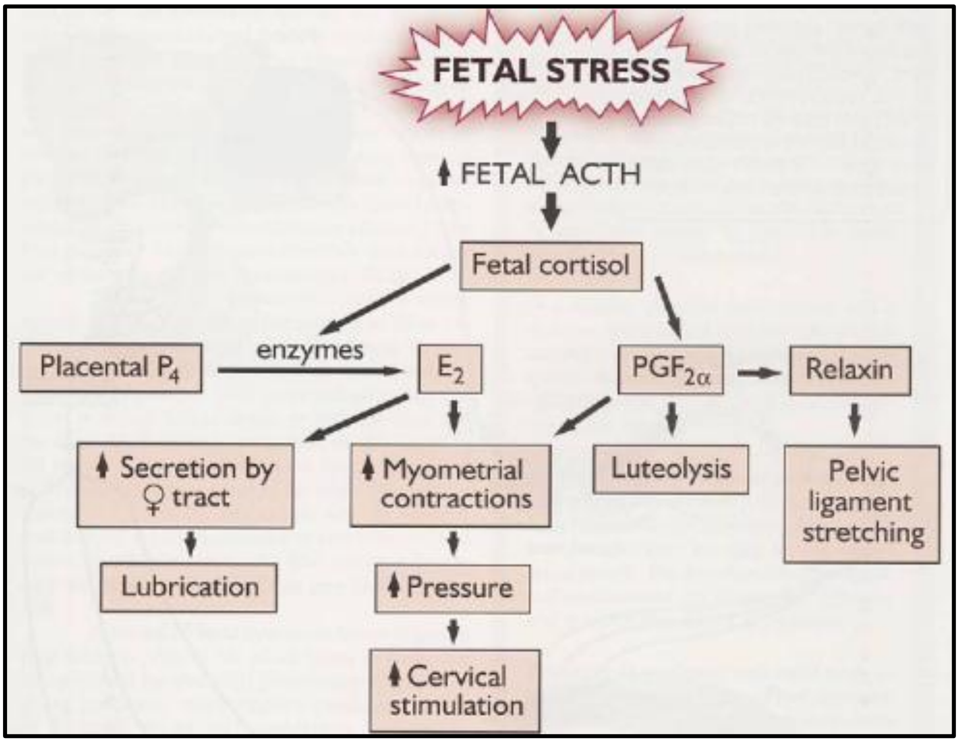


Fig. 1.3 Cascades of events prompted by fetal cortisol. Reprinted from: *Pathways to Pregnancy and Parturition* (p. 321) by P. L. Senger, 2005, Pullman, WA: Current Conceptions, Inc. Copyright 2005 by Current Conceptions, Inc.

1.3 Objective

The objective of this study is to answer the following question:

To what extent are progesterone levels of predictive value for the moment of parturition, and can they be used to decide whether to induce parturition or to wait?

H0: There is no significant decrease in P4 levels in approximately the last 10 days of pregnancy, before the actual parturition date (not the induced date).

H1: There is a significant decrease in P4 levels in approximately the last 10 days of pregnancy, before the actual parturition date (not the induced date).

If P4 concentrations do show a significant decrease a few days before natural calving, this information can be used to predict actual parturition date. Either induction can be done at the right moment or a C-section can be done without prior induction. Either can lead to a significant decrease in placental retention and postpartum uterine infections, together with an increase in cow welfare and a reduction in the use of medication. Because of the latter, the influence of (early) induction on the occurrence of RP and calf mortality is also analyzed in this study.

2. Materials and Methods

2.1 Experimental design

The datasets were obtained from electronic patient files accessible through the clinic's online administrative program Orbis VetWare (AGFA HealthCare, Vienna, Austria).

Information was used regarding each pregnant cow in the clinic of the Department of Farm Animal Health of the Faculty of Veterinary Medicine in Utrecht during the period from 01/04/2010 to 30/04/2016. Cows had a parity of 1,58 ± 1,04 (Mean ± SD). The following information was collected:

- Progesterone concentrations
- Date of insemination
- Induction date and medications used
- Date of C-section
- Clinical signs prior to parturition: udder distention or edema, relaxation of pelvic ligaments, dilated cervix, lactating
- Retained fetal membranes
- Information about the calf: male or female / born dead or alive or died within a few hours after being born

Induction for parturition was allowed by the Ethical Committee from 273 days of pregnancy.

The cows were housed in a tie-stall barn. Food and water were given ad libitum according to the feeding standards of dry cows. P4 levels were checked every Monday, Wednesday and Friday from ± 265 days of pregnancy until parturition. All cows were monitored every day for signs of imminent parturition.

The animals were induced to parturition using 36–42 mg dexamethasone (Rapidexon, as dexamethasonnatriumfosfaat 2mg/mL, Eurovet Animal Health BV, Bladel, NL) or 12–14 mg dexamethasone (Voreen, as dexamethasonisonicotinaat 1mg/mL, Boehringer Ingelheim BV, Alkmaar, NL). In approximately 50% of the cases, 25 mg prostaglandin (Enzaprost®, as dinoprost trotetamol 5 mg/mL, CEVA Sante Animale B.V., Naaldwijk, NL) was given as well, administered 12 hours after dexamethasone.

2.2 Data collection

Data were collected on 274 parturitions/C-sections. Six datasets were excluded beforehand: four because the cow aborted the fetus during pregnancy and two because the estimated length of pregnancy was less than 7 months when a C-section based on medical criteria was performed.

Datasets available were 167 from cows with a C-section and 101 from cows with spontaneous parturition. One dataset contains all of the information available on one pregnancy and C-section/natural parturition. Multiple datasets on one cow can be available, depending on the number of pregnancies. P4 concentrations were measured in all cows over 265 days of gestation, including the ones not used for educational purposes.

2.3 Blood sampling and analysis

Blood samples were collected from the jugular vein using a vacutainer system and heparin-coated tubes. The tubes were transported to the laboratory within 15 minutes. The serum was analyzed using radioimmunoassay (RIA; Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) until 8-12-2014. The test was then changed, and RIA commercial kits (IM1188; Beckman Coulter, Roissy CDG, France) were used. The sensitivity of this competitive binding immunoenzymatic assay is 0,04 ng/mL, and it can measure all P4 levels within a range of 0,08–49,0 ng/mL. All P4 results measured with the new kit were 1,33 times higher than the same results measured with the old test. All concentrations measured with the new kit are corrected for this difference.

2.4 Data Analysis

Data were analyzed using Microsoft Excel and SPSS 10 (IBM SPSS 10, IBM, Armonk, NY, USA).

2.4.1 Retained Placenta

A binary logistic regression analysis was conducted to evaluate the influence of several individual independent variables on the occurrence of RP. An explanation of all the variables used in the statistical analyses is shown in Table 2.1. A chi-square test was conducted to determine the significance of the test. The variables 'Induction', 'Induction_group' and 'Induction_med' were not used at the same time in the binary logistic regression, because all three groups represent the same parameters.

The following variables were used for the analysis of RP in all datasets:

- Full-term
- C-section
- Induction
- Induction_group
- Induction_med
- Parity
- Gestation length
- Day of P4 measurements

The variable 'Extraction' was included in the natural parturition group only. For the analyses three groups were made:

1. All datasets (268 datasets)
2. C-section only (167 datasets)
3. Spontaneous parturition only (101 datasets)

For each group, a binary logistic regression analysis was conducted. The best-fitting model was selected as that with the minimum P value for the chi-square test. Relationships with a P value less than 0,05 were considered significant.

2.4.2 Progesterone

Linear mixed models were used to examine the relationship between P4 concentrations and several individual explanatory variables. To judge the model fit, a Q&Q plot was made of each model. To study constant variance, a scatter diagram was made of each model as well. To see whether parameters influenced the dependent variable, the parameters were divided into different explanatory variables (an explanation of the variables used in the statistical analyses is shown in Table 2.1).

For the mixed-model analysis, the following variables were used:

- Induction
- Parity
- Gestation length
- Day of P4 measurements

Two groups were made for the analyses. Each dataset contains one or more P4 measurements.

1. All datasets (261 datasets)
2. Cows at full-term gestation (158 datasets)

For each group, a linear mixed model analysis was conducted. The best-fitting model was selected as that with the lowest Akaike's Information Criterion (AIC) value. Relationships with P < 0,05 were considered significant.

Each dataset contains several P4 concentrations, measured on the days before parturition. The difference in P4 concentrations between two measurements was calculated and converted to a percentage and analyzed using Excel. The 95% confidence interval was calculated using the standard error of means, and the reference interval was calculated using the standard deviation.

2.5 Explanation of classification of variables

Variables are divided into several categories, as seen in Table 2.1. Full-term gestation is explained further below.

Table 2.1 Variables and their categories used in SPSS

Variable	Variable (full)	Category
Full-term	Full-term	(0) No (1) Yes
C-section	C-section	(0) No (1) Yes
Induction	Induction	(0) No (1) Yes
Induction_group	Induction groups based on parturition or C-section	(1) C-section induced (2) C-section non-induced (3) Spontaneous parturition induced (4) Spontaneous parturition non-induced (reference category)
Induction_med	Induction groups based on medication	(1) Dexamethasone (2) PGF2a (3) Dexamethasone + PGF2a (4) Non-induced (reference category)
RP	Retained placenta	(0) No (1) Yes
Parity	Parity	Divided by parity (first calf = 1 and so on)
Parity_binary	Primiparous and multiparous	(0) Parity 1 (1) Parity ≥ 2
Calf	Calf dead?	(0) No (1) Yes
Extraction	Extraction	(1) Heavy extraction (and *extraction forcée*) (2) Normal extraction (3) Spontaneous parturition without help (reference category)
Gestation	Gestation length	In days
P4_day	Day of P4 measurements	P4 concentrations divided by the number of days before parturition on which it was measured

Full-term gestation
Without hormonal induction of parturition, spontaneous calving will take place within the next 24 hours. Obviously all cows calving without induction are at full-term gestation.

2.5.1 Non-induced C-section

Full-term based on 'in labour' written in the obstetric reports.

Full-term based on administration of clenbuterol (Planipart, as clenbuterol hydrochloride 0,03 mg/mL, Boehringer Ingelheim B.V., Alkmaar, NL) or isoxsuprine (Duphaspasmin, as isoxsuprine lactate 11,58 mg/mL, Fort Dodge Animal Health, Benelux B.V., Naarden, NL).

Full-term based on progesterone concentrations (P4 ≤ 1,2). As mentioned, a decrease in P4 concentrations ≤1,2 ng/mL indicates the onset of parturition within the next 24 hours.

Full-term based on external clinical signs. Several external clinical signs can be used to predict the time of calving. Relaxation of the pelvic ligaments was said to be the best individual predictive clinical parameter, for most cows will show this sign in contrast to others (e.g., filling of the teats). Using more than two clinical parameters does not increase the exactness of predicting calving time within 12 hours (Streyl et al., 2011). The period of relaxation of the pelvic ligaments and maximum filling of the teats can be divided into several steps or points. Streyl et al. (2011) used a scale of 0–3 for filling of the teats and a scale of 0–6 for relaxation of the pelvic ligaments (points weight double). Based on these criteria, when given 5 points and a P4 level ≤1,2 ng/mL 54,4% of the cows gave birth in 12 hours. Shah, Nakao and Kubota (2006) mentioned that the probability of calving within the next 24 hours was 94% if the depth of the ligaments increased ≥5mm in one day. Matsas et al. (1992) established that a P4 concentration lower than 1,2 ng/mL is an accurate way to predict parturition time within 24 hours. Using this information, the probability of a cow with a non-induced C-section being at full-term gestation is based on the following clinical signs:

a. Complete relaxation of the pelvic ligaments
b. Complete relaxation of the pelvic ligaments + slightly to completely filled teats
c. Mildly softened pelvic ligaments + complete filling of the teats
d. Dilation of the cervix and entry of the fetus into the cervical canal
e. Dilation of the cervix + slightly to completely filled teats

2.5.2 Induced C-sections and induced parturitions

After induction, P4 levels decrease as well, and external clinical signs change rapidly (Boos, Kohtes, Stelljes, Zerbe & Thole, 2000; Kornmatitsuk et al., 2000). However, decreasing P4 concentrations and a quick change in external clinical signs are a consequence of the medication given and do not indicate that the dam is at full-term gestation. Therefore, these P4 concentrations of induced cows are not useful in establishing normal P4 concentrations prior to parturition. Some of the animals induced for parturition are still useful for this study, but a selection was made based on the onset of parturition within a specified number of hours after induction. It takes some time from the start of the treatment to the onset of parturition, and some parturitions started early (and therefore spontaneously), so these are not a consequence of the given medication. Therefore, based on other studies, a time limit was set to establish the difference between cows calving spontaneously despite induction versus cows calving as a consequence of induction. Cows calving before this time limit are at full term. Calving of cows after this time limit was the consequence of termination of pregnancy via hormonal induction of parturition. As for the dosage, in the clinic 36–42 or 12–14 mg dexamethasone is given. In about 50% of the cases, prostaglandin is also administered 12 hours later. Both compounds are short-acting corticosteroids, and because their dosage is prescribed by the manufacturer they are considered equally potent.
Calving is defined as follows: 'in labour' written in the obstetric reports and/or clenbuterol or isoxsuprine being administered. The cut-off point for these thresholds is based on the following previous studies: Savc et al. (2016); Lewing, Proulx and Mapletoft (1985); and Hartmann et al. (2013).

2.5.2.1 Induction with dexamethasone < 24 hours

Table 2.2 Induction of parturition using dexamethasone

Range (h)	Mean (h)	P <	Gestation period up to the first injection (d)	Animals (*n*)	Dosage (mg)	Reference
30–46	39,7 ± 11,6 Mean ± ?	0,0001	285	27	40	Savc et al. (2016)
29–65 63% < 48	43,3 ± 2,4 Mean ± SEM	0,05	280	19	25	Lewing et al. (1985)
30–70	46.2 ± 6.9 Mean ± SD		274	10	40	Hartmann et al. (2013)

As seen in Table 2.2, the cows calved within 29–65 hours in all 3 studies. Hartmann et al. (2013) induced a few days earlier in comparison with the average in our study, and only 10 cows were used in their study. Savc et al. (2016) induced 2 days before the average gestation length of 287 days and Lewing et al. (1985) induced on 280 days (no herd average), but with a lower dosage of dexamethasone. All cows calved 29 hours after induction of parturition. To be sure that all cows included in our study truly calved spontaneously (all cows below a certain threshold), the time limit (or threshold) is set at 24 hours. So when the onset of calving was within 24 hours after the administration of dexamethasone only, it was a spontaneous parturition and the data of these cows are therefore included in this study.

2.5.2.2 Induction with prostaglandin < 28 hours

Table 2.3 Induction of parturition using prostaglandin (C: µg Cloprostenol)

Range (h)	Mean (h)	P < …	Gestation period up to the first injection	Animals (*n*)	Dosage	Reference
48% < 48	39,1 ± 1 Mean ± SEM	0,05	276 ± 1	26 < 72 5 > 72	C: 500	Lewing et al. (1985)
33–57 44% < 48	44,9 ± 2,1 Mean ± SEM	0,05	280	16	C: 500	Lewing et al. (1985)

In the clinic, dinoprost is used to induce parturition, whereas cloprostenol is the preferred treatment in the literature.

Substance (brand name)	**Concentration**	**Dosage (mg)**	**Type**
Dinoprost (Enzaprost, Dinolytic)	5 ml/mL	25	Natural
Cloprostenol (Alfaglandin C)	0,25 mg/mL	0,5	Synthetic

The doses of the different forms of prostaglandin are set by the manufacturer, so the brands are considered equally potent when administered in the proper dosage. The proper dosage of each medicine was verified using the Veterinary Medicines Information Bank of the Medicine Evaluation Board. In all studies, the cows calved within 33 to 66 hours (not all studies mentioned a time range). All cows calved 33 hours after induction of parturition. To be sure that all cows included in our study indeed calved spontaneously (all cows below a certain threshold), the time limit (or threshold) was set at 28 hours. So when the onset of calving was within 28 hours after the administration of prostaglandin only, a spontaneous parturition occurred, and the data of these cows are therefore included in this study.

2.5.2.3 Induction with prostaglandin and dexamethasone < 20 hours

Table 2.4 Induction of parturition using dexamethasone and prostaglandin (D: Dexamethasone; C: Cloprostenol)

Range (h)	**Mean (h)**	**P <**	**Gestation period up to the first injection**	**Animals (*n*)**	**Dosage**	**Reference**
	32,6 ± 12,1 Mean ± ?	0,0001	285	25	D 40 mg C 500 µg	Savc te al. (2016)
< 48	29,4 ± 2,3 Mean ± SEM		290		D 25 mg C 500 µg	Lewing et al. (1985)
25–42	34,6 ± 1,4 Mean ± SEM	0,05	280	19	D 25 mg C 500 µg	Lewing et al. (1985)
< 72 97% < 48	51,9 ± 3,4 Mean ± SEM	0,05	276 ± 1	30	D 25 mg C 500 µg	Lewing et al. (1985)

In both studies, cloprostenol and dexamethasone are administered at the same time. All cows calved within 25 to 72 hours after the first injection (not all studies mentioned a time frame). Most cows calved before 48 hours. This table shows that induction closer to the expected parturition date results in a quicker response to the administered induction medication. To be sure that all cows included in our study indeed calved spontaneously (all cows below a certain threshold), the time limit (or threshold) was set at 20 hours. The induction regimes used in the studies are different from those used in the clinic. It is likely that cows in the clinic will calve several hours later as a result of induction with dexamethasone and prostaglandin, because prostaglandins are administered 12 hours after the dexamethasone injection. Therefore, when the onset of calving was within 20 hours after the administration of dexamethasone (followed by an injection of prostaglandin), a spontaneous parturition occurred, and the data of these cows are therefore included in this study.

3. Results

To avoid confusion, the animals are divided into different groups and named as shown in Table 3.1.

Table 3.1 Naming of different groups into which data was divided

Group	Sub	Delivery	Induction
CI	CDex	C-section	Dexamethasone
	CPg	C-section	PGF2a
	CDex+Pg	C-section	Dexamethasone + PGF2a
CN		C-section	No prior induction
PI		Spontaneous parturition	Dexamethasone (with and without PGF2a)
PN		Spontaneous parturition	No prior induction

A total of 268 datasets were analyzed, containing 167 C-sections (173 calves) and 101 parturitions (107 calves). The calves born through C-section numbered 62% and those born through natural parturition numbered 38%.

Only known data were used to make proper calculations (e.g., if out of 167 C-sections the occurrence of RP was known for only 143, the incidence of RP was based on a total amount of 143 C-sections).

3.1 Dead calves

Twenty calves were born dead or died shortly after birth. Seventeen of them died within 2 hours after the C-section and three died during parturition. Of the calves that died during or shortly after birth, 85% were born through C-section. The calf mortality rate is 9,8% following C-section (9,8% of the calves born through C-section died) compared to 2,8% for natural parturitions.

Of the calves born through induced C-section, 7,8% died, compared to 13,8% of the calves born through non-induced C-section.

A cause of death is not always mentioned in the operation report. Most of the calves stopped breathing or trying to take a breath within a few minutes after birth. Three calves were already dead when taken out. All three calves born through natural parturition died during parturition, when still alive at the onset of parturition.

Table 3.2 Dead calves by type of parturition

Type of parturition	No. of dead calves	%	Explanation
All	20	7,1%	Of all calves (280)
C-section	17	9,8%	Of all calves born through C-section (173)
Induced	9	7,8%	Of all calves born through induced C-section (115)
Non-induced	8	13,8%	Of all calves born through non-induced C-section (58)
Natural parturition	3	2,8%	Of all calves born through natural parturition (107)

3.2 Retained Placenta

Cows were grouped based on induction medication. (See Table 3.2 for denotation of groups.)

Table 3.3 Incidence of RP in induced vs. non-induced groups

Group	**RP**		**Total (*n*)**
	%	*N*	
CI	67,0	65	97
CN	39,1	18	46
CI + CN	58,0	83	143
PI	12,5	1	8
PN	25,4	15	59
PI + PN	23,8	16	67

The overall incidence of RP among the 143 C-sections was 58% (83 out of 143). Among the 68 natural parturitions, the overall incidence of RP is 23,8% or 16 out of 68 (Table 3.3). RP was affected by three factors: C-section (P < 0,01), gestation length (P < 0,05) and induction treatment (P < 0,01). The CI group had the greatest chance of suffering from RP (Exp(B) = 8,691; P < 0,01), in comparison to the PN group (Table 3.4). The standard regression coefficients indicated that the independent effect of C-section had the greatest influence (Exp(B) = 3,255; P < 0,01), but induction had a strong influence as well (Exp(B) = 3,018; P < 0,01; Table 3.5).

The influence of several induction treatments on the occurrence of RP is compared to the reference group (non-induced). Induction with dexamethasone gives a higher chance of RP (Exp(B) = 2,78; P < 0,05). There is no significant relationship between induction with PGF2a and RP. Induction with dexamethasone and PGF2a gives a higher chance of RP (Exp(B) = 3,204; P < 0,05; Table 3.5).

Gestation length is related to RP, and an increase in gestation length decreases the chances of its occurrence (Exp(B) = 0,917; P < 0,05; Table 3.4). For every day the gestation length increases, the odds of RP decrease. There was no significant influence of the parity on the occurrence of RP.

Table 3.4 Summary of logistic regression analysis

Chi^2 sig 0,000

Explanatory variables	**Exp(B)**	**95% CI for Exp(B)**	
		Lower	Upper
Constant	22,337		
Parity	1,293	0,957	1,747
Gestation*	0,917	0,852	0,988
Induction_group**			
Induced C-section**	8,691	3,761	20,083
Non-induced C-section	2,115	0,863	5,182
Induced parturition	0,536	0,058	4,994
Non-induced parturition	Reference		

Significance * 0,01 < P < 0,05 ** P < 0,01

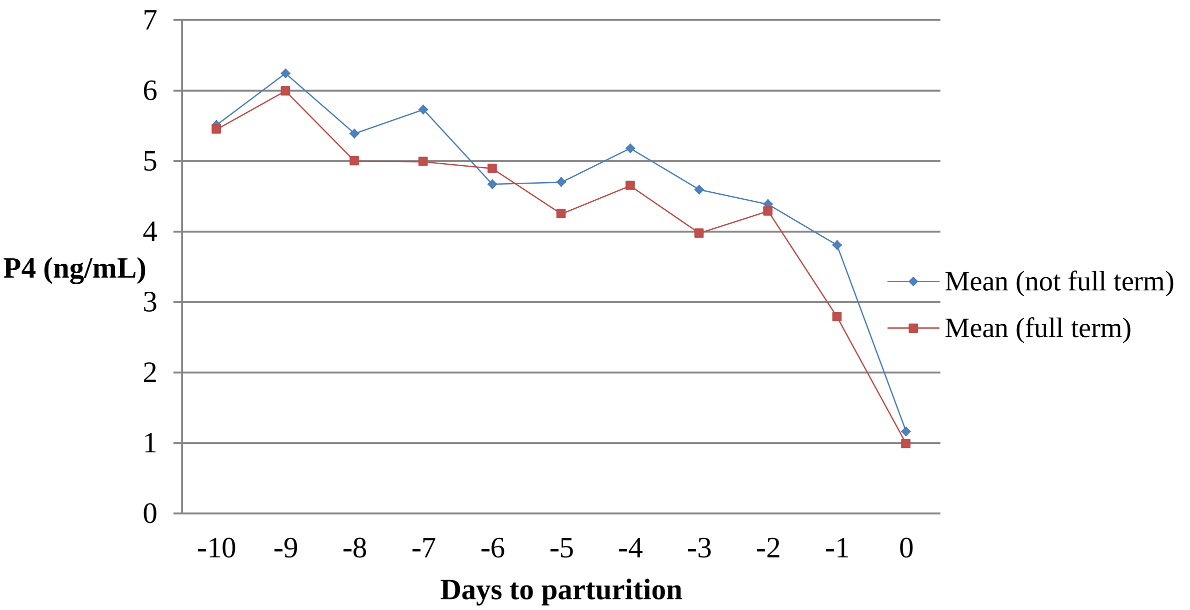
Table 3.5 Summary of logistic regression analysis						
	Model A			Model B		
	Chi² sig 0,000			Chi² sig 0,000		
Explanatory variables	**Exp(B)**	**95% CI for Exp(B)**		**Exp(B)**	**95% CI for Exp(B)**	
		Lower	Upper		Lower	Upper
Constant	1,062E+11			1,554E+1		
C-section**	3,255	1,474	7,188	1	1,477	7,218
Gestation*	0,906	0,840	0,977	3,265	0,838	0,977
Induction**	3,018	1,490	6,112	0,905		
Dexamethasone*				2,780	1,196	6,461
PGF2a				4,001	0,316	50,72
Dexamethasone+PGF2a**				3,204	1,417	1
Non-induced						7,243
Parity	1,313	0,977	1,766	1,313	0,977	
						1,766
Significance * 0,01 < P < 0,05 ** P < 0,01						

3.3 Progesterone

3.3.1 Progesterone analysis (all data)

All datasets were used to evaluate the effect of several explanatory variables on the dependent variable P4. The explanatory variables used are parity, gestation length, number of days before parturition on which P4 was measured (Day_P4) and if the cow was induced or not. Variables are used on their own and in interaction with each other. In the best model (with the lowest AIC), the day_P4 and the interaction between induction and day_P4 were of significant influence (Table 3.6). On Day 1 there is a significant difference between P4 concentrations (P < 0,01), where the mean P4 concentrations of non-induced cows is 1,14 ng/mL lower than those of induced cows. On Day 0, on the other hand, there is no significant difference. On Days 3, 5, 7, 8 and 11 there is a significant difference as well (P < 0,05).

Fig. 3.1 P4 concentration at the end of gestation, divided by cows at full-term pregnancy and induced cows not at full-term pregnancy



For all of these days, the non-induced cows have lower P4 concentrations compared to induced cows, as can also be seen in Figure 3.1. The points in this figure represent the mean P4 concentration on the number of days before parturition. Figure 3.1 shows a less steep decrease in P4 from Days 2 to 1 for the induced cows (not at full-term gestation); however, they have a much higher decrease from Day 1 to Day 0, ending at almost the same P4 concentrations as did the cows at full-term gestation.

*Table 3.6 Summary of mixed model analysis. (The data of the explanatory variable day_P4*Induction (0) are the results for the P4 concentrations in non-induced cows compared to P4 concentrations on the same day in induced cows.)*

Explanatory variables	**Sig.**	**Estimate**	**95% CI**[A]	
			Lower	Upper
Intercept	0,000	6,862	6,291	7,433
Day_P4	0,000			
Day_P4*Induction (0)	0,002			
Day 0	0,715	0,121	–0,529	0,771
Day 1	0,001	–1,139	–1,794	–0,484
Day 2	0,980	–0,007	–0,559	0,545
Day 3	0,023	–0,710	–1,320	–0,100
Day 4	0,302	–0,324	–0,940	0,292
Day 5	0,017	–0,655	–1,192	–0,118
Day 6	0,681	–0,137	–0,790	0,516
Day 7	0,042	–0,557	–1,094	–0,019
Day 8	0,029	–0,773	–1,466	-0,080
Day 9	0,616	–0,156	–0,768	0,456
Day 10	0,627	–0,173	–0,870	0,524
Day 11	0,002	–1,417	–2,314	–0,520
Day_P4*Induction (1)[B]	0	0	—	—

[A] *95% CI (Mean ± 1.96*SE)*
[B] *Reference category*

3.3.2 Progesterone and intervals to parturition: full-term

To analyze the influence of several variables on normal P4 concentrations, only cows at full-term gestation were used. The variables parity or parity_binary and day_P4 were used. There was no significant relationship between parity and P4 concentrations ($P > 0,05$). The model with only day_P4 was the best fit. As seen in Table 3.7, there is a significant correlation between P4 concentrations and Days 0–7 before parturition ($P < 0,05$). From 8 days before parturition and further, no significant relationship is seen. Table 3.8 and Figure 3.2 show the mean P4 concentrations ± 95% confidence interval, divided by days before parturition. Figure 3.3 shows the mean P4 concentrations ± 95% reference interval. The standard deviation is high, and therefore the 95% R.I. is wide. P4 concentrations decline markedly within 2 days of parturition (Est. –4,58; $P < 0,01$).

Table 3.7 Summary of mixed model analysis for P4 concentrations in cows with full-term gestation. (The data of Days 0 to 10 are compared to Day 11 before parturition, the reference category.)				
Explanatory variables	**Sig.**	**Estimate**	**95% CI[A]**	
			Lower bound	Upper bound
Intercept	0,000	5,636	4,987	6,285
Day_P4	0,000			
Day 0	0,000	–4,584	–5,321	–3,847
Day 1	0,000	–2,767	–3,460	–2,075
Day 2	0,000	–1,321	–2,011	–0,632
Day 3	0,000	–1,658	–2,355	–0,960
Day 4	0,000	–0,946	–1,627	–0,265
Day 5	0,007	–1,298	–1,992	–0,604
Day 6	0,000	–0,869	–1,545	–0,193
Day 7	0,012	–0,530	–1,233	0,173
Day 8	0,139	–0,641	–1,343	0,060
Day 9	0,073	0,447	–0,267	1,162
Day 10	0,219	–0,124	–0,863	0,615
Day 11[B]	—	0	—	—

[A] *95% CI (Mean ± 1.96*SE)*
[B] *Reference category*

Table 3.8 Summary of descriptive statistics for P4 concentrations on 0 to 11 days before parturition										
No. of days before parturition	**Total (*n*)**	**Min.**	**Max.**	**Mean (ng/mL)**		**SD**	**95% CI[A]**		**95% RI[B]**	
				Statistic	**SE**		Down	Up	Down	Up
0	44	0,1	6,1	1,0	0,17	1,11	0,6	1,3	–1,3	3,2
1	59	0,4	8,1	2,9	0,20	1,55	2,5	3,3	–0,2	6,0
2	61	0,8	13,1	4,3	0,26	2,01	3,8	4,8	0,3	8,3
3	65	1,3	6,5	4,1	0,14	1,17	3,8	4,3	1,7	6,4
4	54	2,3	13,2	4,7	0,23	1,70	4,2	5,1	1,3	8,1
5	69	1,4	16,3	4,5	0,25	2,06	4,0	5,0	0,4	8,6
6	59	2,2	10,0	4,8	0,20	1,50	4,4	5,2	1,8	7,8
7	62	0,7	19,4	5,0	0,31	2,43	4,4	5,6	0,2	9,9
8	48	1,8	12,3	5,0	0,25	1,74	4,6	5,5	1,6	8,5
9	46	1,7	20,1	6,1	0,40	2,70	5,3	6,9	0,7	11,5
10	41	2,2	8,1	5,6	0,23	1,45	5,1	6,0	2,7	8,5
11	24	2,7	11,1	5,9	0,38	1,88	5,1	6,6	2,1	9,6

[A] *95% CI (Mean ± 1.96*SE)*
[B] *95 RI (Mean ± 2*SD)*

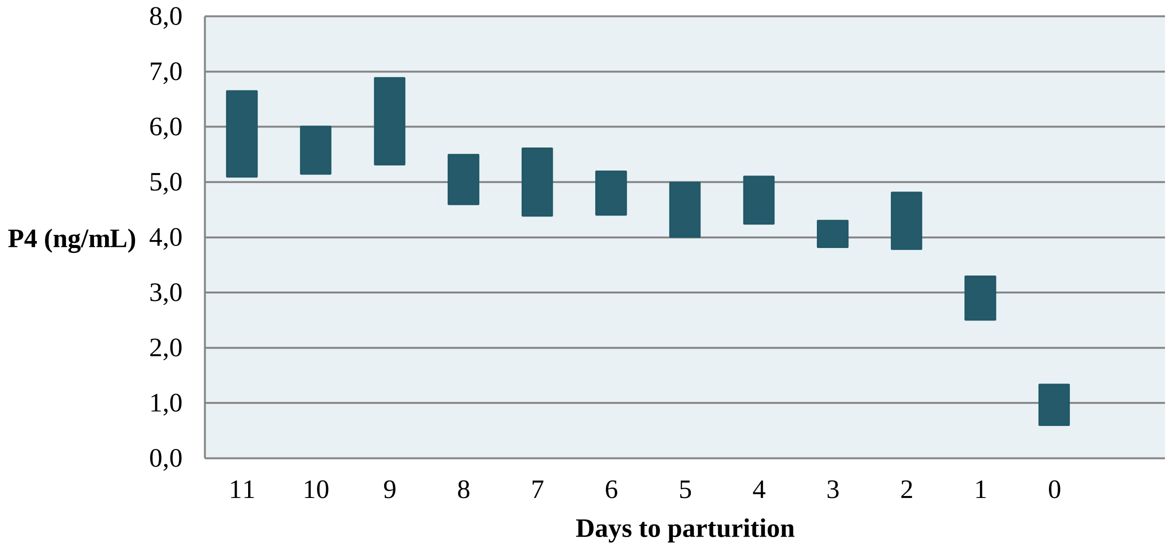


Fig. 3.2 Full-term datasets: P4 concentrations (with 95% confidence interval)

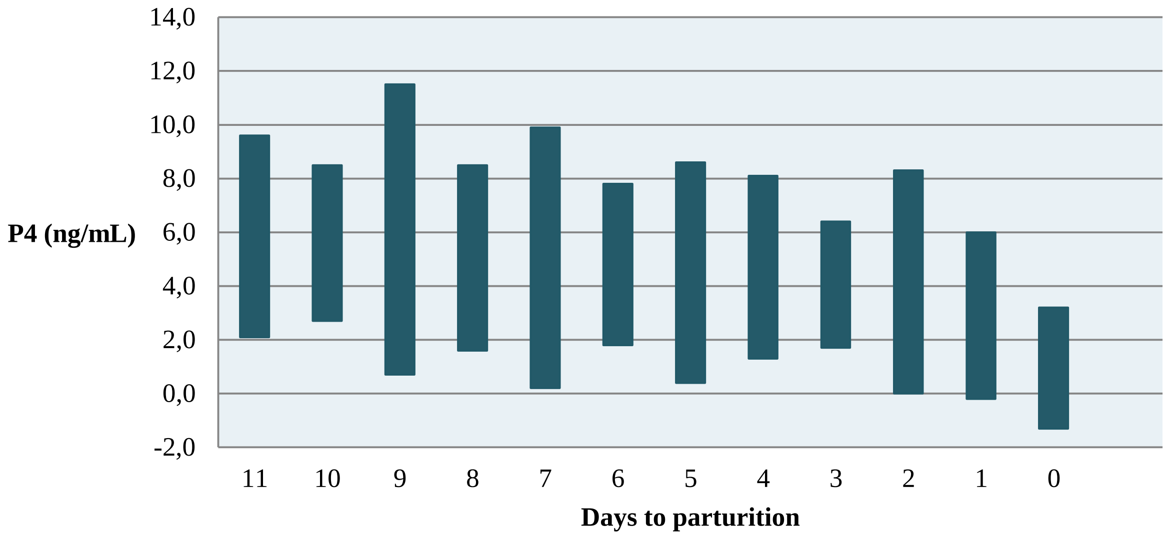


Fig. 3.3 Full-term datasets: P4 concentrations (with 95% reference interval)

Not only do the P4 concentrations play a role in predicting the day of parturition, but the average decrease between an interval of days can be useful as well. The intervals are different from each other, because P4 was not measured for every cow on the same days before parturition. For example, for cow A, P4 was measured on Days 0 and 2 before parturition and for cow B it was measured on Days 2 and 5 before parturition. For each cow and each interval the average decrease or increase in P4 concentrations was calculated, in absolute numbers (ng/mL) and in percentages, as well as the SEM, 95% CI and 95% RI. These results can be seen in Tables 3.9 (P4 in ng/mL) and 3.10 (P4 in percentages). They are also visualized in Figures 3.4 to 3.7 (with 95% CI). A negative number means an increase in P4 concentrations. Figures 3.5 and 3.7 give us an idea of the decrease in P4 concentrations in 95% of the population. The dispersion is very high. The decrease in P4 concentrations between Days 2 and 0 is calculated as well; however, this figure not very useful because many cows will have begun calving on Day 0.

Table 3.9 Descriptive statistics for the fluctuations in P4 concentrations. (Each interval consists of the two days of measurement + the mean decrease or increase in P4 concentrations in this interval. A negative number represents a mean increase in P4, a positive number represents a mean decrease.)

Intervals between no. of days before parturition	**No. of cows (*n*)**	**Mean (ng/mL)**		**SD**	**95% CI[A]**		**95% RI[B]**	
		Statistic	**SE**		Down	Up	Down	Up
Day 0–2	26	3,3	0,39	2,01	2,52	4,06	–0,7	7,3
Day 1–3	35	1,1	0,25	1,49	0,59	1,57	–1,9	4,1
Day 1–4	20	1,7	0,41	1,85	0,93	2,55	–2,0	5,4
Day 2–4	29	0,6	0,30	1,61	0,00	1,17	–2,6	3,8
Day 2–5	27	0,3	0,23	1,17	–0,18	0,70	–2,1	2,6
Day 3–5	39	0,3	0,24	1,49	–0,17	0,76	–2,7	3,3
Day 3–6	20	1,0	0,30	1,36	0,37	1,56	–1,7	3,7
Day 4–6	36	0,2	0,19	1,15	–0,22	0,53	–2,1	2,5
Day 4–7	14	–0,1	0,45	1,69	–0,93	0,83	–3,4	3,3
Day 5–7	47	0,6	0,17	1,17	0,24	0,91	–1,8	2,9

[A] *95% CI (mean ± 1.96*SE)*
[B] *95% RI (mean ± 2*SD)*

Fig 3.4 Variations in the decrease or increase in P4 concentrations (mean ± 95% RI)

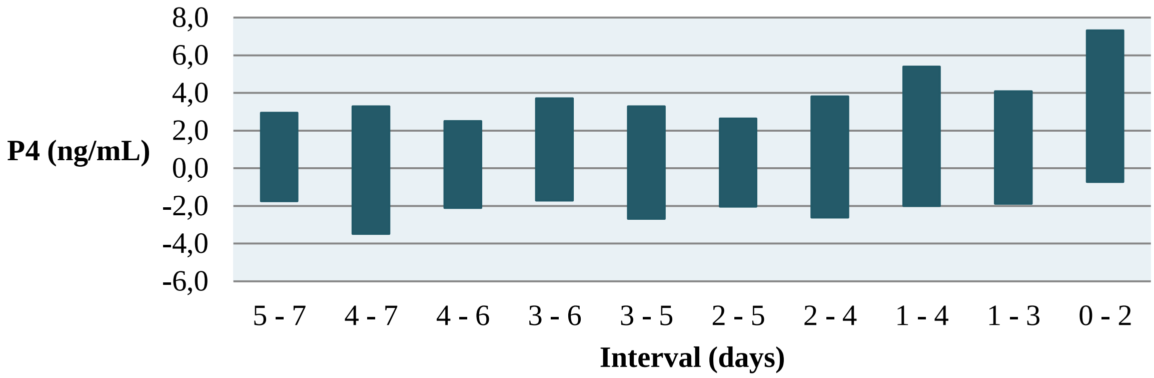


Fig 3.5 Variations in the decrease or increase in P4 concentrations (mean ± 95% RI)

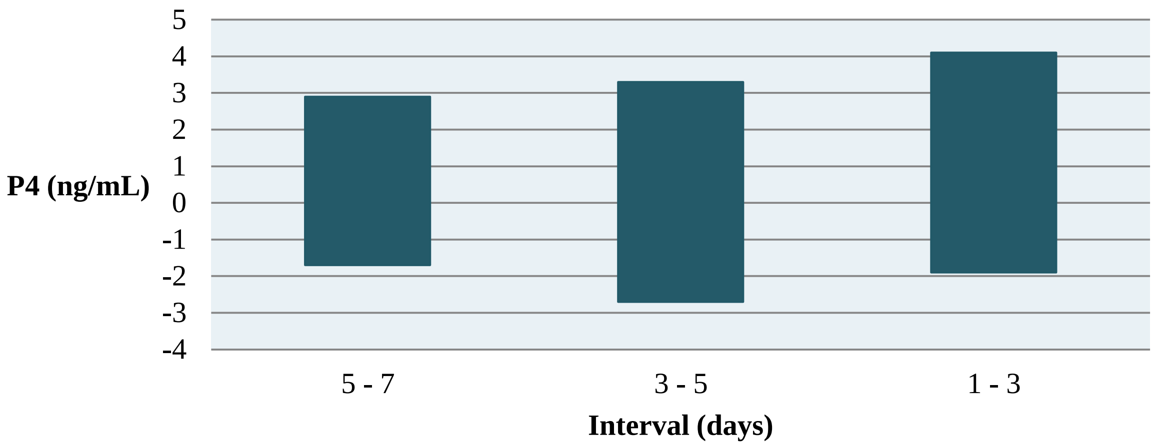


Table 3.10 Descriptive statistics for the fluctuations in P4 concentrations, converted to percentages (Each interval consist of the two days of measurement + the mean decrease or increase in P4 concentrations in this interval. A negative number represents a mean increase in P4, a positive number represents a mean decrease.)

No. days before parturition	Total (*n*)	Mean (%)		SD	95% CI[A]		95% RI[B]	
		Statistic	SE		Down	Up	Down	Up
0–2	26	74,8	4,4	22,5	66	83	30	120
1–3	35	24,4	5,8	34,1	13	36	–44	93
1–4	20	33,4	7,1	31,7	20	47	–30	97
2–4	29	12,5	5,5	29,8	2	23	–47	72
2–5	27	0,8	4,0	21,0	–7	9	–41	43
3–5	39	–0,8	7,3	45,5	–15	13	–92	90
3–6	20	15,8	6,4	28,4	3	28	–41	73
4–6	36	2,2	3,7	22,4	–5	9	–43	47
4–7	14	–6,2	8,8	32,9	11	-23	–72	60
5–7	47	2,9	8,4	57,4	–14	19	–112	118

[A] *95% CI (Mean ± 1.96*SE)*
[B] *95% RI (Mean ± 2*SD)*

Fig 3.6 Variations in the decrease or increase in P4 concentrations (in percentages; mean ± 95% RI)

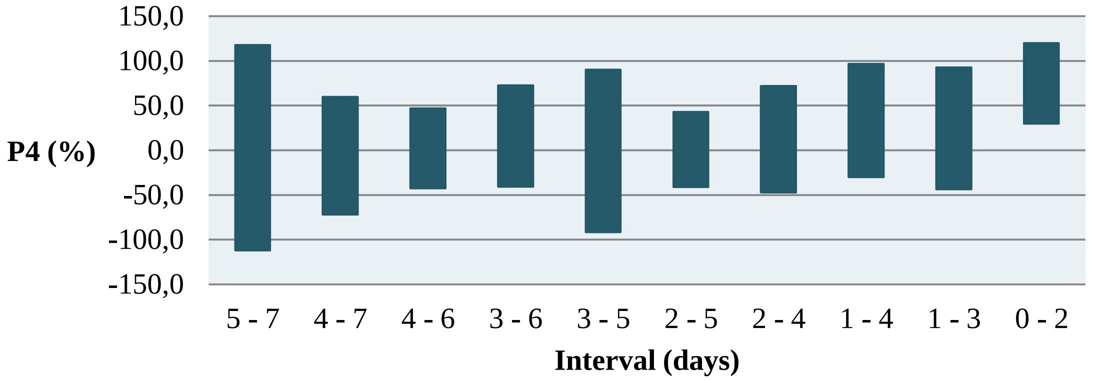
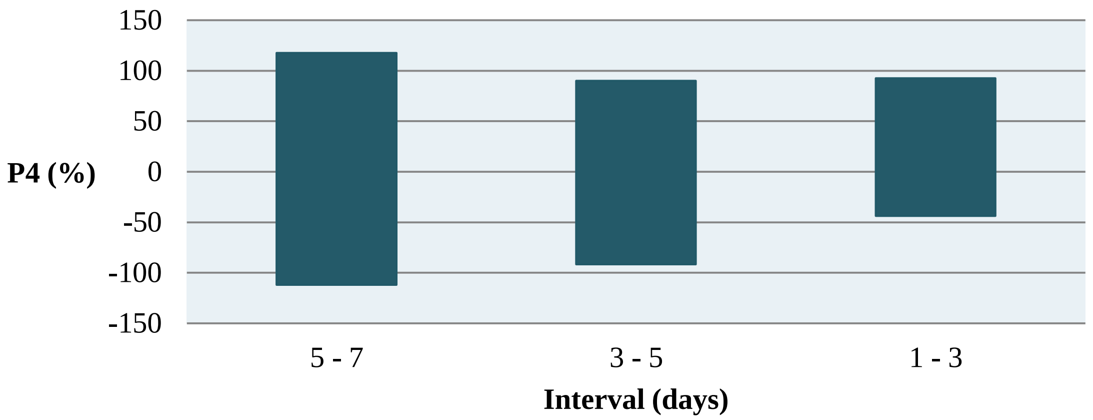


Fig 3.7 Variations in the decrease or increase in P4 concentrations (in percentages; mean ± 95% RI)



4. Conclusions and discussion

The goal of this study was to investigate whether or not a significant decrease in progesterone concentrations occurs in the days leading up to parturition, on the basis of which the day of parturition can be predicted. In the clinic, progesterone concentrations would then be used to induce cows for parturition at the most optimal moment and with the least adverse effects. With induction too early, parturition may result in higher calf mortality and retained fetal membranes. To see whether that is the case, calf mortality and the occurrence of RP are briefly evaluated as well.

This study is based entirely on patient files accessible in the clinic's online administration program. Many different people (students as well as employees) have access to these files and fill them in. A great deal of information was missing, especially regarding how many cows suffered from retained fetal membranes. It was not always clear at what time induction was initiated or what medication was used. Although all unclear or inconclusive information was omitted from this study, it is possible that some incorrect data were inadvertently used in the analyses because of their reliance on the information in the files. Also, not all students have the same definition of criteria (e.g., different interpretations of dilation of the cervix were used, as well as entry of the fetus into the cervical canal).

4.1 Calf mortality

The calf mortality rate in this study was 9,8% following C-section, compared with 2,8% for natural parturitions. In the literature, a calf mortality rate of 12% following cesarean operations is reported, compared with 5% for natural parturitions (Barkema, Schukken, Guard, Brand & Weyden, 1992). The calf mortality within the CI group was 7,8%; therefore, it is within normal limits according to the literature. In conclusion, calf mortality does not seem to be affected by the induction regime used in the clinic.

4.2 Retained placenta

Around parturition, the fetal membranes and the uterus begin to detach, resulting in the elimination of the former. One calving-associated reproductive problem is failure to expel the fetal membranes, resulting in an RP (Benedictus, Jorritsma, Knijn, Vos & Koets, 2011). The definition of RP varies, but most studies define it as failure to expel the membranes within 12 hours after parturition. The majority of cows, however, will pass their fetal membranes within 6 hours after parturition. RP results in economic loss because of a longer time to first service, decreased pregnancy rates, increased services per conception, a decrease in milk production, and an increased risk for metritis, ketosis and mastitis (Attupuram, Kumaresan, Narayanan & Kumar, 2016; Beagley, Whitman, Baptiste & Scherzer, 2010).

A number of risk factors are associated with RP, such as induced parturition, short gestation, twinning, dystocia, need for C-section and many infectious diseases such as bovine viral diarrhea (Beagley et al., 2010; Han & Kim, 2005; Joosten, Eldik, Elving & Mey, 1987). The mechanisms behind these risk factors are not always completely understood. Normal placental delivery involves maturation of the placentomes (Hartmann et al., 2013; Parkinson 2001) and separation of the cotyledonary villi from the caruncular crypts (Attupuram et al., 2016). Multiple hormonal and biochemical changes lead to normal placental delivery, and interruption or disturbance of one or more steps in these pathways can lead to RP (Attupuram et al., 2016; Beagley et al., 2010; Benedictus et al., 2011; Parkinson, 2001).

In cases of easy or normal calvings, about 4 to 18 percent of cows suffer from RP (Han & Kim, 2005). Joosten et al. (1987) found an overall incidence of 4% of RP in easy or normal calving and an increased risk of RP in higher parities. The incidence of RP after a C-section was 10,9 percent for heifers and 20,7 percent for multiparous cows (parity ≥ 3). However, an increasing chance of RP in advanced parities is not consistent with the report of Han and Kim (2005), who found that an increased risk of RP is not related to cow parity. In this study, no significant relationship between parity and RP ($P > 0,10$) was found.

Traumatic events, such as a C-section, can lead to a higher incidence of RP. A possible explanation for this is that trauma causes reaction and edema of the chorionic villi, which results in the inability of the villi to separate from the crypts (Beagley et al., 2010). Also, dystocia and trauma associated with atony of the uterus could inhibit expulsion and lead to RP (Parkinson, 2001). In this study, a higher chance of the occurrence of RP after a C-section was found as well (Exp(B) = 3,255; $P < 0,05$).

This study found that a C-section had the greatest influence on the occurrence of RP (Exp(B) = 8,691; $P < 0,01$), but induction had a strong influence as well (Exp(B) = 3,018; $P < 0,01$). Induction of parturition is one of the established risk factors for RP in cows (Beagley et al., 2010; Lewing et al., 1985). The incidence of RP increases when induction occurs more than one week before expected parturition (McNaughton & Murray, 2009; Kornmatitsuk et al., 2000) and it decreases when induction of parturition is closer to the expected moment of parturition (Peters & Poole, 1992; Savc et al., 2016; Villarroel & Lane, 2010). Lewing et al. (1985) also found that after induction on Day 280 of gestation, a significantly lower incidence of RP occurs after induction with dexamethasone (19%), in contrast to induction with dexamethasone with PGF2a (53%) or with PGF2a only (50%). In this study, the influence of several induction treatments on the occurrence of RP was compared to that in the reference group (non-induced). Induction with dexamethasone and PGF2a gives the highest chance of RP (Exp(B) = 3,204; $P < 0,05$), but induction with dexamethasone is a risk factor for its occurrence as well (Exp(B) = 2,78; $P < 0,05$). There was no significant relationship between induction with PGF2a and RP. This is probably due to limited sample size, which means that statistical significance was not achieved. Short-acting corticosteroids, as used in the clinic, have a time interval (time between treatment and effect) of 1 to 6 days (Taverne & Noakes, 2001). Induction of parturition with short-acting corticosteroids provides sufficient time to induce a shift in the progesterone concentrations, but maturation of the placenta seems to be a more time-consuming process than induction of labour. Lack of time can lead to placental retention (Boos, Janssen & Mülling, 2003; Benedictus et al., 2011). The use of corticosteroids as an induction treatment can also lead to immunosuppression, which puts the cow at increased risk of the development of RP as well (LeBlanc, 2008).

Several studies show that shorter gestation lengths are associated with a higher incidence of RP as well (Han & Kim, 2005; Joosten et al., 1987; Parkinson, 2001). In this study, gestation length is related to RP, and an increase in gestation length decreases the chances of RP occurring (Exp(B) = 0,917; $P < 0,05$). For every day the gestation length increases, the odds of RP decrease. In conclusion, induction close to the expected moment of parturition is most important. This can result in a more mature placenta, a more successful detachment of the fetal membranes and a lower incidence of RP (Benedictus et al., 2011; Peters & Poole, 1992). C-section is another important factor in the occurrence of RP, but this unavoidable. All other important factors must therefore be optimized, to keep the incidence of RP as low as possible.

4.3 Progesterone

The objective of this study was to answer following question:

To what extent are the progesterone levels of predictive value for the moment of parturition, and can they be used to decide on whether to induce parturition or to wait?

H0: There is no significant decrease in P4 levels in approximately the last 10 days of pregnancy, before the actual parturition date (not the induced date).

H1: There is a significant decrease in P4 levels in approximately the last 10 days of pregnancy, before the actual parturition date (not the induced date).

The normal course of P4 concentrations within 11 days before parturition were analyzed using the P4 measurements of cows at full-term gestation. Taverne, as cited in Saint-Dizier and Chastant-Maillard, (2015) showed that fetal cortisol increases approximately 10 days before parturition, which leads to a decrease in P4 levels in maternal blood. This study shows a significant correlation between P4 levels and the last 7 days before parturition ($P < 0,05$). From 8 days before parturition and beyond, no significant relationship was seen between P4 levels and the number of days before parturition. Based on this finding, the expectation is that the average P4 levels of cows not at full-term gestation would be (significantly) higher the last 7 days before induced parturition than the average P4 levels of non-induced cows. Also, the P4 concentrations measured on Day 8 before parturition or later cannot be used to determine the date of parturition.

P4 levels of full-term cows and those not at full term show a significant difference on Days 1, 3, 5, 7, 8 and 11 before parturition ($P < 0,05$), but not on the other days between 0 and 11 days. For all of these mentioned days, cows at full-term gestation have lower P4 concentrations compared to cows not at that stage. However, on the days on which no significant difference was seen between the two groups, P4 levels were also (slightly) lower for the cows at full-term gestation.

Also, as seen in Figure 3.1, P4 levels start to decrease markedly around Day 2 before parturition in full-term cows and on Day 1 in cows not at full-term gestation. On Day 1 the mean P4 concentrations of cows at full-term gestation is 1,14 ng/mL lower than those of induced cows. On Day 0, on the other hand, there is no significant difference. P4 concentrations of both groups end around the same average concentration (1,0 ng/mL ± 0,17 [Mean ± SEM]). These results are consistent with research by Kornmatitsuk et al. (2000) and Boos et al. (2003), who found a rapid decrease in P4 concentrations after the start of induction treatment.

The data show that normal P4 levels start to decrease slowly around Day 7 before parturition and decrease greatly within 2 days beforehand ($P < 0,01$). To see whether the number of days before parturition can be predicted based on the P4 concentration, the 95% confidence interval and 95% reference interval were calculated. The SEM and the standard deviation were both high; therefore the 95% reference interval is very large, as seen in Figure 3.3. Because of the large dispersion of individual P4 concentrations, P4 levels on Days 0 to 7 before parturition also cannot be used to determine the day of parturition.

The average decrease in P4 concentrations between an interval of days was calculated as well to predict the day of parturition. For each cow and each interval the average decrease or increase in P4 concentrations was calculated, in absolute numbers (ng/mL) and in percentages, as well as the SEM, 95% confidence interval and 95% reference interval. Figures 3.5 and 3.7 give an idea of the decrease in P4 concentrations of 95% of the population. The dispersion is high, with significant overlap for each interval. For example, if the P4 of a cow is measured on Days 275 and 277 of gestation, and the decrease is 1,5 ng/mL, she can be within 0 to 7 days before parturition, because each interval of 2 days contains 1,5 ng/mL (Fig. 4.1). If the decrease is 95%, the cow can still be within 0 to 7 days before parturition (Fig. 4.2). The decrease in P4 concentrations between Day 2 and Day 0 is calculated as well; however, this figure not very useful because many cows will have already begun calving on Day 0.

Fig 4.1 Variations in the decrease or increase in P4 concentrations (mean ± 95% R.I.)

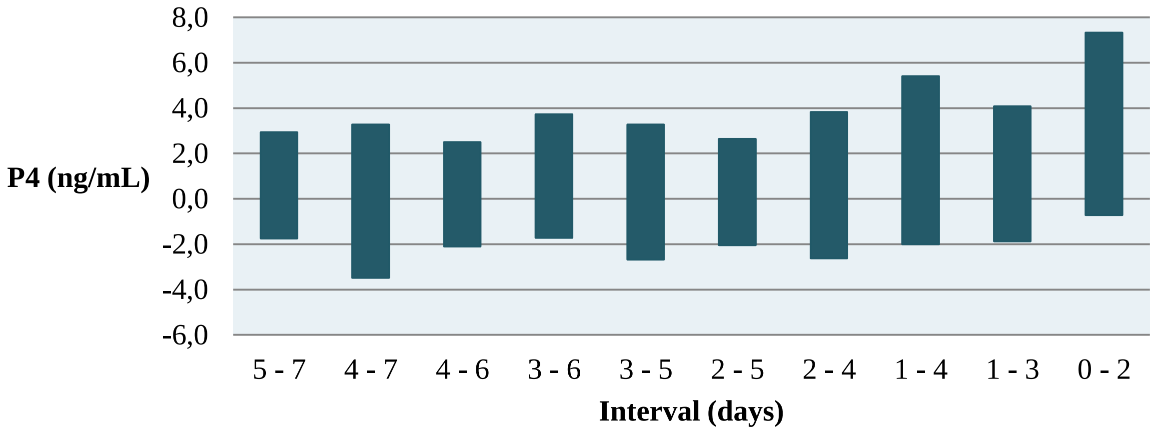
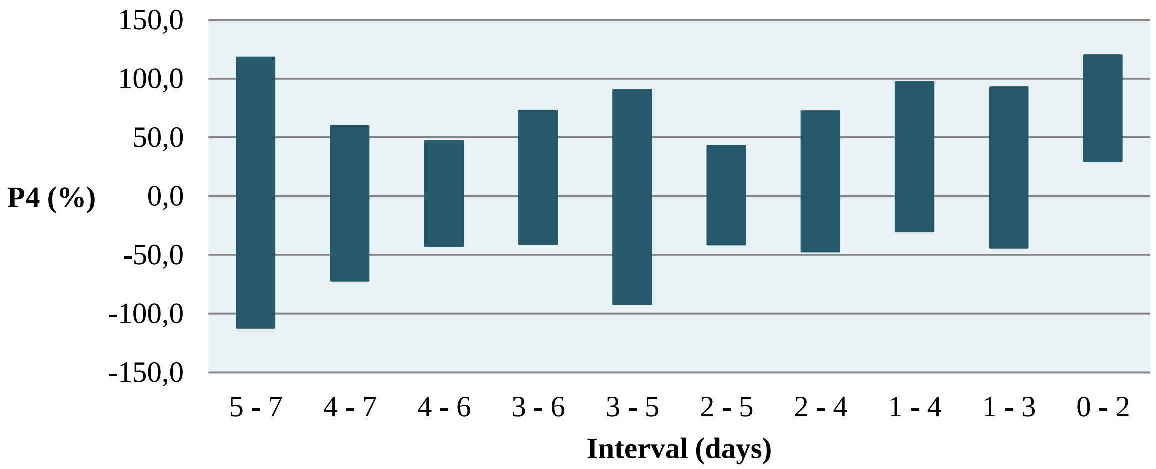


Fig 4.2 Variations in the decrease or increase in P4 concentrations (in percentages; mean ± 95% RI)



The H0 is rejected, because there is a significant decrease in P4 levels in the last 7 days of pregnancy. However, in answer to the question stated at the outset of this study, based on the mean P4 concentrations and the average decrease and increase in progesterone, P4 concentrations were found to not be a useful means to predict calving time in cattle, because of the high dispersion in concentrations.

In the future, more advanced statistical research can be done to investigate further the possibility of predicting the date of parturition using plasma-progesterone concentrations; however, this level of research is too complex for the scope of this master’s thesis.

5. References

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